# Evaluation of Non-clinical Requirements for Medicinal Products containing Monoclonal Antibodies as an Active Substance based on Review of EPARs during last 10 years (2010-2019)

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# List of Abbreviations

| | |
|---|---|
| 3R | Reduce/Refine/Replace |
| ADA | Anti-Drug Antibody |
| ADC | Antibody-Drug Conjugates |
| ADME | Absorption, Distribution, Metabolism and Excretion |
| APAAP | Alkaline Phosphatase Anti-Alkaline Phosphatase |
| ATA | Anti-Therapeutic Antibody |
| ATD | Anticipated Therapeutic Dose |
| AUC | Area Under the Curve |
| $C_{max}$ | Maximum Serum Concentration |
| CHMP | Committee for Medicinal Products for Human Use |
| CMC | Chemistry, Manufacturing, Control |
| CPMP | Committee for Proprietary Medicinal Products |
| CTD/eCTD | Common Technical Document/electronic CTD |
| CVMP | Committee for Medicinal Products for Veterinary Use |
| DNA | Deoxyribonucleic Acid |
| DART | Developmental and Reproductive Toxicity |
| DDI | Drug-Drug Interactions |
| EEA | European Economic Area |
| EEC | European Economic Community |
| EFD | Embryo-Foetal Development |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EMA | European Medicines Agency |
| EPAR | European Public Assessment Report |
| ePPND | Enhanced Prenatal and Postnatal Development |
| ERA | Environmental Risk Assessment |
| F | Bioavailability |
| FDA | Food and Drug Administration |
| FEED | Fertility and Early Embryonic Development |
| FIH | First In Human |
| GLP | Good Laboratory Praxis |
| ICH | International Council for Harmonisation |
| IL | Interleukin |

| | |
|---|---|
| JAS | Juvenile Animal Studies |
| mAb | Monoclonal antibody |
| M | Multidisciplinary Guidelines |
| MABEL | Minimal Anticipated Biological Effect Level |
| MMAE | Monomethyl auristatin E |
| NAb | Neutralizing Antibodies |
| NHP | Non-Human Primates |
| NOAEL | No Observed Adverse Effect Level |
| PAD | Pharmacologically Active Dose |
| PD | Pharmacodynamics |
| PDCO | Paediatric Committee |
| PEG | Polyethylene glycol |
| PK | Pharmacokinetics |
| PPND | Prenatal and Postnatal Development |
| RMP | Risk Management Plan |
| S | Safety Guidelines |
| SLAMF | Signalling Lymphocytic Activation Molecule Family |
| SmPC | Summary of Product Characteristics |
| SPR | Surface Plasmon Resonance |
| $T_{max}$ | Time take to reach $C_{max}$ |
| $T_{1/2}$ | Elimination half-life |
| TCR | Tissue Cross Reactivity Study |
| $V_d$ | Volume of distribution |
| $V_{ss}$ | Apparent volume of distribution at steady state |

# 1 Introduction

Biotechnology-derived pharmaceuticals (biopharmaceuticals) include a diverse range of products and are generally large, complex molecules, that require living systems such as bacteria, yeast, insect, plant, or mammalian cell lines for their production [1]. The active substances may be proteins or peptides and their derivatives and conjugates. Examples for biopharmaceuticals are cytokines, plasminogen activators, recombinant plasma factors, growth factors, fusion proteins, enzymes, receptors, hormones and monoclonal antibodies [2]. Monoclonal antibodies represent a major product class - around 38% of biotechnology-derived medicinal products in Europe - and it is expected that the increase in monoclonal antibodies will continue in the coming years [3].

## 1.1 Objective

The focus of the present master thesis is to characterize the non-clinical program of biopharmaceutical products containing monoclonal antibodies. The evaluation of the non-clinical program of each individual product is provided in the European Public Assessment Reports (EPARs) based on the data submitted by the applicants.

As the ICH-S6 guideline on preclinical safety evaluation of biotechnology-derived pharmaceuticals [2] does not set uniform standards but proposes to apply a case by case strategy, it can be expected that the non-clinical parts of the product dossiers vary. This work will also compare the scope and extent of non-clinical studies of products with a new active substance and biosimilars: for biosimilars many non-clinical as well as clinical properties are already known from the originator product and its pre-clinical program is expected to be considerably shortened, as the corresponding guideline [4] states.

Of particular interest are the questions of whether it was possible to follow the proposals of the various guidelines, how variable the extent of the non-clinical programs is and for which type of products which aspects were particularly important or negligible. These findings may have a practical significance for product developers in the future as the information is obtained through the EPARs and represents the perspective of the assessors.

The structure of this thesis follows the CTD document structure as required by the ICH-M4S guideline [5], which is also the basis for the document structure of EPARs.

## 1.2 European Public Assessment Report (EPAR)

Due to commitment to transparency the EMA publishes information on its website (www.ema.europa.eu) about its decisions relating to medicines evaluations, as referred in Article 13 (3) of Regulation 726/2004 [6]. This “European public assessment report” abbreviated as EPAR outlines the whole evaluation process including a scientific rationale on which a decision was made to approve or refuse an application. The EPARs are continuously updated to reflect the latest data throughout the life of the medicine.

EPARs provide latest data and recommendations on a medicinal product regarding its efficacy and safety. Structure and content of each EPAR follow the internationally agreed format, in which applicants are required to submit data on candidate medicine when

applying for a marketing authorisation [5]. EPARs describe the information regarding data limitations or uncertainties from regulators point of view. The evaluation of studies is usually presented after description of the methodology and the results are discussed in context with compliance to reasonable guidelines. Each EPAR complies with the predefined format, which is structured similar to CTD and provides a summarizing assessment of the corresponding sections of the CTD. The non-clinical aspects are described as standard in section 2.3 of the EPAR.

## 1.3 Antibody Structure and Function

Antibodies are essential molecular components of the adaptive immune system, which are produced and excreted by the B cells. Antibodies can recognize and neutralize the exogenous bodies such as pathogenic bacteria or viruses directly via high affinity binding or can recruit other players of the immune system (cells and molecules such as complement), both of which can promote an elimination of target cells, when tagged by antibodies.

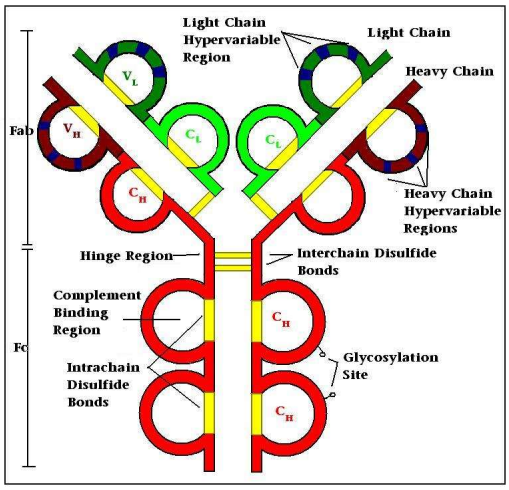


**Figure 1: Antibody Molecule Components**

Image by David Canner, accessed 01.01.2020;
available from: https://proteopedia.org/wiki/index.php/Image:New_Antibody2.JPG

Antibodies are Y-shaped molecules, of which the basic structural unit consists of four polypeptide chains, two identical light (L) chains and two identical heavy (H) chains. These four chains are held together by a combination of non-covalent and covalent (disulphide) bonds. The molecule is composed of two identical halves, each with the same antigen-binding site. Both light and heavy chains usually cooperate to form the antigen-binding surface with two identical antigen-binding sites.

Parts of both, the heavy and light chains, usually combine to form the antigen-binding sites (Fab-fragment antigen binding). The heavy chains also form the tail (Fc region) of the antibody, which determines what other proteins will bind to the antibody and therefore what biological property the antibody class has [7].

## 1.4 Historical Background

After the discovery of the principles of the immune system and especially of the antibody function, the therapeutic potential of antibodies became obvious. The researchers have

aimed to use their specificity and efficacy for a targeted treatment of diseases that are difficult to cure, such as neoplasia and autoimmune diseases.

With the introduction of the hybridoma technology in 1975 it was possible to produce a pre-defined mono-species of antibodies in larger quantities from a single clone of fused cells [8]. Later, the development of molecular biology and the use of recombinant DNA technologies made it possible to engineer and create stabile biological systems for the expression of a large number of antibodies with specific properties, *i.e.* chimeric, fully humanized or human antibodies, directed to a number of different physiological and pathophysiological processes [9; 10]. As antibodies are large protein molecules in nature their production is only possible using biotechnological methods, even if these methods differ from each other. Depending on the target disease the use of free antibodies or antibody fragments, antibody-drug conjugates, radioimmunoconjugates or bispecific molecules is possible [11].

The first mouse monoclonal antibody-containing drug in the world, which received regulatory approval, was Orthoclone OKT3 and was approved by the Food and Drug Administration (FDA) in the United States in 1986 followed with a subsequent approval in the European community [12]. As required by the Directive 87/22/EEA [13], valid at that time, this medicinal product was evaluated in Europe by the *Committee for Proprietary Medicinal Products* (CPMP, mandated in 75/319/EEA [14], Article 8), which was the prototype of the current *Committee for Medicinal Products for Human Use* (CHMP).
The requirement to evaluate and approve “high-technology medicinal products, particularly those derived from biotechnology“ exclusively via the centralized community authorization procedure was enhanced by the Council Regulation (EEC) No 2309/93 of 22 July 1993 [15], when the European Medicinal Agency (EMA) was established, and it still applies today: in the EU the evaluation and authorisation of Quality, Safety and Efficacy of a new biotechnology-derived medicine can only be carried out by EMA through a centralised procedure.

The rate of approved antibodies for the therapeutic use is rapidly increasing. As of today, 91 medicinal products containing monoclonal antibodies are available to patients in the EU, which are directed against a large number of very different antigens [16].

## 1.5 General Regulatory Framework

In the course of marketing authorization in EEA, both ICH and EMA scientific guidelines are applicable, which are addressed to the developers of new drugs and represent the current scientific and technical standards. The ICH guidelines are categorised in four groups: Quality Guidelines (Q) to achieve harmonised standards in quality and manufacturing processes; Efficacy Guidelines (E) on design, conduct, safety and reporting of clinical trials; Safety Guidelines (S) intended to detect potential risks such as carcinogenicity, genotoxicity and reproduction toxicity during the non-clinical testing phase; Multidisciplinary Guidelines (M) are applicable to all fields of drug development (ICH Homepage). The EMAs scientific guidelines clarify how to interpret and apply the requirements for the evidence of quality, safety and efficacy, which are set out in the Community directives. Although the guidelines are not legally binding, deviations from their rules need to be justified.

For the marketing authorisation application of each medicine to the EMA an internationally agreed data set is required, which must be presented in format of the Common Technical

Document (CTD or, nowadays eCTD). CTD should be compiled according to the ICH-M4S guideline [5] and should cover the information on product manufacture (CMC), non-clinical programme, and clinical trials. Therefore, the approval of a new drug depends on the successful planning and realization of a regulatory strategy, which coordinates these three aspects of development. The data provided by applicants should be obtained with compliance to the number of guidelines.

## 1.6 General Non-Clinical Requirements

The marketing authorization of biotechnology-derived pharmaceuticals, similar to all other therapeutics, requires a comprehensive pre-clinical data set, which should be presented in module 4 of CTD [5]. In general, non-clinical data provide the primary understanding of the product's mode of action, allow a prediction of the safety profile of the product, provide the support for all phases (I, II, III) of clinical development and, beyond that, are important basic tools in standardizing the manufacturing process or during a significant change of manufacturing or application mode.

The scope and objectives of the non-clinical studies are predetermined and referred in the ICH-M3 (R2) guideline on *non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals* [17]. According to this,

- pharmacology studies (primary, secondary and safety)
- pharmacokinetic (ADME) and toxicokinetic studies
- Toxicity studies (acute and repeated dose)
- Local tolerance studies
- Genotoxicity and Carcinogenicity studies
- Reproduction toxicity studies
- and other toxicity studies, when needed

should be performed to obtain the sufficient and reliable data until a drug is released for human use.

The timing of pre-clinical studies with regard to FIH application depends on the kind of study and its purpose: the pharmacology studies for investigation of the mode of action and effects of a substance in relation to its desired therapeutic target should be evaluated prior to human clinical studies. *In vitro* metabolic and plasma protein binding studies and the systemic exposure *in vivo* studies in the species generally used for repeated-dose toxicity should be conducted before human exposure as well [17; 18].

## 1.7 Non-clinical Requirements for Biotechnology-Derived Products

Due to unique and heterogeneous structural and biological properties of biopharmaceuticals, such as species specificity, immunogenicity and unpredicted pleiotropic activities, the conventional drug testing approaches may not be feasible or appropriate. Therefore, a “flexible, case-by-case, science-based approach” to non-clinical testing has been agreed for this product group. These important framework conditions are reflected in the ICH-S6 guideline *preclinical safety evaluation of biotechnology-derived pharmaceuticals* and its complementary addendum from 2011 [2]. This guideline recommends a specific

approach, which is needed to be considered for the evaluation of this particular product group. Nevertheless, it should be applied in combination with the relevant “over ranged” guidelines maintained above [5; 17; 18]. The details of this and other applicable guidelines will be referred in the corresponding topics.

By means of a non-clinical study program using these regulations, at least the following information should be obtained: the determination of an initial dose for FIH studies and dose adjustment frame for following human studies; the identification of potential target organs/tissues; the reversibility of the toxicity, if it is observed; the determination of the relevant safety parameters for clinical monitoring.

While the GLP standard is mandatory for the testing of small molecules, for biotech products this is mostly required if the application of the standards is feasible [18].

### 1.7.1 Specification of the Test Material

Due to the “live” origin of an active substance, additional risks of biotechnology-derived pharmaceuticals may be associated with host cell contaminants. These production-specific impurities may cause allergic reactions or other undesirable immunological effects. Further, the nucleic acid contamination bears the risk of viral infections or integration of foreign DNA sequences into the recipient genome. For mitigation of these risks the specification of the identity, purity, stability, and potency of test entity, which can be evaluated on the basis of biochemical and biological characterisation, is required [2].

The test materials used during the whole development programme *e.g.* in non-clinical and clinical studies and - beyond this - in clinical practice after approval of the product, have to be comparable in predefined specification parameters. In some cases, when the manufacturing process is changed/optimized, additional studies may be required in order to assure consistent quality. *The guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process: non-clinical and clinical issues* [19] outlines in detail the rationale for the additional obligations, if the manufacturing process was modified. The extent of the additional comparative studies (non-clinical and clinical) depends on whether the change in manufacturing is introduced before or after the confirmatory trials. In general, the comparability of all critical non-clinical endpoints should be demonstrated, *i.e.* the PD parameters with relevance to clinical application, the PK parameters, consistency of toxicological effects and the immune response, *e.g.* antibody titres, neutralising capacity, cross-reactivity. The adequate techniques and strategies should be sufficiently justified.

### 1.7.2 Pharmacodynamics for Biological Medicinal Products

#### 1.7.2.1 Primary and Secondary Pharmacodynamics

The aims of pharmacodynamics as a main branch of pharmacology are to demonstrate the effects and modes of action of drugs in the organism. Pharmacology studies can be divided into three categories: 1. studies on the mode of action and effects of a substance in relation to its desired therapeutic target (primary pharmacodynamic studies), 2. studies on the mode of action and effects of a substance not related to its desired therapeutic target (secondary pharmacodynamic studies) and 3. studies, which investigate the potential undesirable

pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above (safety pharmacology studies). These definitions are given in the ICH-S7A guideline [18].

The effects of biological activity of biotechnology derived products, both related and not related to the intended clinical use, may be assessed in the frame of primary and secondary pharmacodynamic studies. The effects on any sign of phenotype or metabolic status, *i.e.* expression or post-translational modification of proteins, proliferation or apoptosis of cells, can be determined using *in vitro* detection techniques with cell lines. For example, using the cell lines derived from various species (incl. human) may be quantitatively assessed and compared for determining the receptor occupancy, affinity and the activating or inhibition status of clinically relevant or irrelevant cell signalling pathways and their "down-stream" effects.

In particular, especially for antibodies, the binding with the target is crucial for the desired mode of action or even for the undesired effects, which the drug can cause. To assess both, on-target and off-target binding, pharmacological activity of the molecule and its immunogenic potential*, in vitro* and *ex vivo* tissue cross reactivity studies should be applied as advised by the ICH-S6 guideline [2]. As well as the results of primary and secondary pharmacodynamic (PD) studies, the immunological properties should be described in detail, "including antigenic specificity, complement binding, and any unintentional reactivity and/or cytotoxicity towards human tissues distinct from the intended target. Cross-reactivity studies should be carried out by appropriate immunohistochemical procedures using a range of human tissues" [2].

Tissue cross-reactivity studies in animal tissues are an important supportive tool for the selection of an appropriate pharmacologically relevant animal species for studies *in vivo*. Selection of relevant species is a key point for the predictability of the non-clinical toxicity testing because of the high species specificity of biotechnology-derived pharmaceuticals. *In vivo* characterisation of pharmacological activity by means of other appropriate test systems and determination of mechanisms of action, gives the rationale for potential clinical efficacy in human [2]. These experiments can be used to assist in the interpretation of any safety-related findings of the animal toxicology studies or the relevance of any observed binding in the human tissue panel.

#### 1.7.2.2 Safety Pharmacology

Safety pharmacology detects and investigates potential undesirable pharmacodynamic effects. Its findings indicate which endpoints should be particularly monitored in the context of toxicity and clinical studies. The aim of the *in vivo* safety pharmacology studies is to reveal any functional effects on the vital organ systems - cardiovascular, respiratory, renal and central nervous systems, called the core battery tests.

For biotechnology-derived products with high specific receptor targeting, such as antibodies, it is acceptable to incorporate the detection endpoints on vital functions in the design of other toxicology and/or pharmacodynamic studies. Whereas for those biotech products that do not have highly specific binding to targets, like for small molecules, an extensive safety pharmacology investigation should be performed as outlined in the ICH-S7A guideline [18].

### 1.7.3 Pharmacokinetics and Toxicokinetics

Because of large variety of biotechnology-derived products, it is not appropriate to predefine the same mandatory set of pharmacokinetic studies for all biotech products. Nevertheless, during the early development stage pharmacokinetic properties of the biotech substances need to be characterized with respect to drug availability. According to the ICH-S6 guideline:

- single dose pharmacokinetics
- multiple dose pharmacokinetics
- toxicokinetics
- tissue distribution studies in relevant species

should be performed. Because of the physicochemical features of biopharmaceuticals, the non-clinical testing strategy may be substantially different from that of small molecules. Toxicokinetics is an extension of pharmacokinetics and provides the kinetic patterns of higher doses of drugs.

The distribution, metabolism and elimination of biotech drugs with a polypeptide structure is more likely to result in endogenous proteins and is therefore easily predictable. The studies on interactions of biotech products with tissue components and the possible influence of binding proteins may be essential to understand the pharmacological behaviour of the drug molecule. The non-specific or target mediated (*i.e.* via internalisation drug-receptor complex) proteolysis is a common elimination pathway for protein-based products and the oxidative hepatic metabolism is not expected. Therefore, the drug interaction studies involving liver metabolic enzymes and biliary/renal excretion of metabolites do not usually need to be performed [20; 21].

Great differences in pharmacokinetics among various test animal species or immune-mediated clearance mechanisms may affect the kinetic profiles of the drug and may have a significant impact on the predictive value of studies. Therefore, the interpretation of the study result should be carried out carefully and all possible factors should be taken into account [2].

### 1.7.4 Route of Administration

The route and frequency of administration used in non-clinical animal studies should be as close as possible to the proposed clinical use. The use of routes of administration other than those used clinically should be justified [2].

### 1.7.5 Data Needed for FIH Dose Selection

Dosage levels should be selected and tested in relevant species to provide information on the dose-response relationship. For this, pharmacokinetic/pharmacodynamic (PK/PD) modelling is appropriate. The addendum to the ICH-S6 guideline 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”. The higher of these two doses should be chosen for the high dose group in non-clinical toxicity studies.

This (PK/PD) approach is suitable to find out a toxic dose and a no observed adverse effect level (NOAEL) as well (requirements according to the ICH-S6 guideline [2]. The NOAEL is a generally accepted reference for FIH safe starting dose determination. But, if there is any hypothetical factor influencing risk, the use of minimal anticipated biological effect level (MABEL), estimated pharmacologically active dose (PAD) and anticipated therapeutic dose range (ATD) approach are recommended. The calculation of MABEL should be performed using all *in vitro* and *in vivo* information available from PK/PD studies. The adjustment for anticipated exposure and duration as for inter-species differences in binding affinity to target should be performed [22].

The implementation of this cautious strategy - to start with the lowest active dose *vs.* to start with highest safe dose after the TGN1412 case – in the guideline is probably a matter of course [23]. However, the strategy for the calculation of the starting dose should be chosen on a case-by-case basis and should be supported by a robust scientific rationale. Selection of the start dose using a MABEL is particularly appropriate for biopharmaceuticals with immune agonistic properties as stated by the ICH-S9 guideline [24].

### 1.7.6 Toxicology

#### 1.7.6.1 Single and Repeated Dose Toxicity Studies

Single dose or acute toxicity studies are carried out to determine the relationship of dose (low, intermediate and high doses) to systemic and/or local toxicity in the representative animal species. The importance of species selection in toxicity studies will be discussed in the next section.

The treatment regimen for repeated dose toxicity studies should be specified on the basis of toxicokinetic findings, single dose toxicity studies and obtained pharmacodynamic data [2].

The duration of repeat dose studies, the route and the frequency of administration should cover the intended duration of clinical exposure and should be scientifically justified. According to the ICH-S6 guideline, for biopharmaceuticals intended for single or short-term use (under 7 days) repeat dose studies up to a two weeks duration have been considered sufficient to support marketing authorisation.

For products intended for long-term use (*i.e.* for chronic indications) repeat dose toxicity studies of 6 months duration are considered adequate. This duration may vary when appropriately justified. The ICH-S6 guideline refers to experience rather than instructions, and the duration for most biotech products has generally been 1-3 months.

As outlined in the *ICH-S9 Guideline on nonclinical evaluation for anticancer pharmaceuticals* [24], for most pharmaceuticals intended for the treatment of patients with advanced cancer, non-clinical studies of 3 months duration are considered sufficient to support marketing.

The toxicology testing strategy as specified in ICH-S4 guidance *Duration of Chronic Toxicity Testing in Animals* [25] does not apply to this product group. According to this guidance the studies in rodents should last 6 and in non-rodents 9 months.

A recovery monitoring period, which is important to demonstrate of reversibility or worsening of occurred events or revealing of delayed toxic effects, should generally be

included in study designs. For products inducing prolonged pharmacological/toxicological effects, recovery group animals should be observed until reversibility is demonstrated [2].

#### 1.7.6.2 Selection of Relevant Species

Due to the unique and disparate structural and biological properties, as species specificity, one of the most important challenges for biotechnology-derived products is to determine the immunogenicity or pleiotropic activity and the selection of appropriate animal species to allow for meaningful extrapolation of toxicity findings to humans. The use of two species (rodent and non-rodent) for toxicological assessment in short-term toxicology studies is mandated by regulatory guidance [17; 26; 24] for both small and large molecules. Long-term general toxicity studies (chronic toxicity studies) in one species are sufficient. Since the studies in non-relevant species may lead to false safety interpretation, the performance of such studies must be avoided. Indeed, the biotechnology-derived molecules are frequently pharmacologically active in only one species [Addendum of 2].

On the one hand, the reduction of the number of animals used for experiments in accordance with the 3Rs (reduce/refine/replace) principles [2] is desired. However, sample size should be selected with regard on the ability to detect toxicity [2]. No gender exclusion is proposed for in the development program.

A relevant species should be defined before FIH trials and the relevance should be assessed on the basis of the functional/pharmacological activity of the candidate molecule in species specific cell systems or *in vivo*. The modulation of a known biological response or of pharmacodynamic marker indicates the functional activity and therefore, the species relevance. Tissue cross reactivity (TCR) studies provide only a limited evidence for this, but when target binding is expected, TCR can be used as a reference for animal to human comparison in the choice species for toxicology studies. Especially for mAbs the relevant species are those, which express the target epitope and show a similar tissue cross-reactivity profile as for human tissues [2].

Summarized, the justification of species pharmacological relevance should be based on following comparison criteria to humans:

- sequence homology expressions pattern of the target
- *in vitro* binding affinity, receptor occupancy
- *in vitro* bioactivity
- *in vivo* pharmacological activity.

When no relevant species exist, the use of transgenic animals expressing the human receptor or the use of homologous proteins should be considered [2].

#### 1.7.6.3 Immunotoxicity and Immunogenicity/Antigenicity in Test Animals

Immunotoxicity include any unintended suppression or enhancement of the immune response. The ICH-S8 Guidance [27] provides general recommendations for non-clinical test approaches to reveal immunotoxic potential of medicinal products containing small molecules. The guideline does not apply to biotechnology-derived pharmaceutical products covered by the ICH-S6 guideline [2]. The classical immunotoxicity test (28 day study with consecutive daily dosing in rodents) or other standard testing batteries are not

recommended for biotechnology-derived pharmaceuticals.

The need for animal immunogenicity testing for biotechnology products should be carefully considered. According to the ICH-S6 guideline, one aspect of immunotoxicity testing for biotechnology-derived molecules is the assessment of its immunogenicity. In some cases, especially in the complete absence of knowledge on the biological functions of the endogenous protein intended to be used in humans and "if theoretical considerations are suggestive of a safety risk, animal immunization studies with the therapeutic protein or the animal homolog may be considered in order to gain information on the potential consequences of an unwanted immune response" [28]. Inflammation at the application site may be predictive for immune response of drug or the expression of surface antigens on target cells may be altered. Such toxic or autoimmune issues should be evaluated via appropriate monitoring strategies. It may be appropriate to incorporate the immunotoxic endpoints in the long-term studies such as repeat or developmental toxicity studies, especially to detect delayed immunotoxic effects.

Humans may also develop serum antibodies against endogenous human or humanised proteins. Nevertheless, animal models are not predictive of such safety risks.

The biotech products intended for human use, which are of human origin or fully or partly humanized, are expected to be immunogenic in animals per se. The extrapolation of non-clinical animal data for immunogenicity prediction in humans is not correct. But, drug associated antibody (also called anti-drug antibody-ADA or anti-therapeutic antibody-ATA) may reduce the circulating drug levels during the multiple dose application required for chronic, development or reproductive toxicity studies. This can lead to the safety-relevant misinterpretation of these studies. Therefore, the assessment of ADA levels is essential for the reliable interpretation of toxicity studies and it can reveal potential drug related toxic effects, that could be monitored in subsequent clinical trials [2]. When an altered PD activity is observed, or, in the absence of a PD marker, unexpected changes in exposure or any immune-mediated reaction occurs, the antibody response should be comprehensively characterized including:

- ADA-titre
- proportions of neutralizing and non-neutralizing antibodies (NAbs and non-NAbs)
- number of responding animals
- impact on pharmacokinetic/pharmacodynamic parameters
- incidence and/or severity of adverse effects
- complement activation
- the emergence of new toxic effects [2].

Measurement of anti-drug antibodies in animal studies may be performed as a part of repeat dose toxicity studies and supports their interpretation. Immunogenicity assessment may be relevant for single dose pharmacokinetic studies, whereas there is no need for this in single dose toxicity studies. When ADA measurement is not a part of the toxicity study protocols, the collection and conservation of blood samples is recommended for future evaluation of pharmacokinetic/toxicokinetic data, as stated in the *Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins* [28].

#### 1.7.6.4 Immunotoxicity and Immunogenicity in Humans

The induction of unwanted immune responses in humans by biopharmaceuticals, mAbs in particular, is not an unexpected effect that needs to be considered at both non-clinical and clinical levels. There can be several mechanisms of induction of immunogenicity: the extrinsic origin of biopharmaceuticals commonly causes immune response in host organism, both test animals and humans, depending on the test materials. But, humanized or fully human mAbs, *i.e.* intrinsic proteins have shown to induce anti-drug antibodies (ADAs) in clinical use. This may be caused by many product- and patient-related factors, including the presence of the product related impurities, sequence variation from the endogenous protein, post-translational modifications, or protein aggregation. In case of aggregation the structure of the protein changes completely. Such molecule aggregates are recognized as foreign and cause the immune response involving the same mechanisms as molecules of foreign origin [29]. The clinical impact of the immunogenicity of therapeutic proteins is very individual and is not easily predictable: severe adverse reactions have been reported, including IgE-mediated anaphylactic shock, neutralization of a critical endogenous protein, but in most cases, the clinical consequence of ADA development is impairment of treatment efficacy [30]. While pre-clinical animal studies are not predictive for immunogenicity in humans, the assessment of immunotoxic potential of biopharmaceuticals *in vivo* animal studies, is valuable supportive pre-clinical tool, particularly if pharmaceuticals are intended to modulate the immune system and may affect humoral or cell-mediated immunity [2].

### 1.7.7 Genotoxicity and Carcinogenicity Studies

Since the biotechnology-derived substances, particularly mAbs, do not interact directly with DNA, no genotoxic potential is expected. In some cases, *i.e.* the presence of an organic linker molecule in a conjugated protein product, genotoxic evaluation may be needed.

In general, according to the ICH-S1A guideline, carcinogenicity studies are required for any pharmaceutical whose expected clinical use is more than 6 months [31].

Products with proliferation stimulating properties or immunosuppressive agents may lead to neoplasias. Therefore, the ability to enforce growth of normal or malignant cells and target receptor expression pattern for therapeutic antibodies should be determined. The testing strategies may include both *in vitro* and *in vivo* studies. The respective data may be obtained from long term repeated dose toxicity studies [2].

Carcinogenicity studies are not recommended for therapeutics, which are intended for treatment of advanced cancer [24]. For biosimilars, studies regarding carcinogenicity are omitted [32].

### 1.7.8 Reproductive and Developmental Toxicity (DART) Studies

Assessment of potential developmental and reproductive toxicity of human pharmaceuticals is regulated by the ICH-S5 (R2) guideline [33].

Reproduction toxicity studies aim to identify the effects of drug use on mammalian reproduction. All treatment related findings and abnormalities should be adequately evaluated, allowing a reliable assessment of the risks to human fertility. As considered by the ICH-S5 guideline [33] all developmental stages should be covered through the testing set

and any treatment or observational gaps should be avoided:

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

For studies related to reproductive and developmental toxicity it is of great importance that the evaluation is conducted only in a pharmacologically relevant species. The specificities of identifying relevant species for biotech products have already been discussed above. If the active substance of the product is highly targeted, it is unlikely that more than one relevant species can be identified. If no relevant species can be identified, use of surrogate molecules or transgenic models can be justified. For products, directed against exogenous agent (bacteria, virus, other drugs), no reproductive toxicity studies are required [2]. In the ICH-S5 guideline, the study designs with different objectives with regard to reproduction characteristics have been presented as examples, which can serve as a basis for the planned studies and should be adapted as necessary [33].

#### 1.7.8.1 Fertility and Early Embryonic Development (FEED) Studies

The design and objective of fertility studies should be adequately address *i.e.*, to detect the impact of the drug on stages A-B (see 1.7.8). The Extrapolation of the impact of the drug on factors, such as male or female reproductive hormone level, oestrous cycle, sperm count, morphology and motility, mating behaviour, conceptions and implantations issues (*i.e.* early embryonic development) should be possible [33]. These evaluations may be part of repeat dose toxicity studies. In this case, the repeat dose study should be of at least 3 months duration and should include sexually mature animals.

#### 1.7.8.2 Embryo-Foetal Development (EFD) Studies

The Embryo-foetal development (EFD) studies should be addressed to detection the impact of the drug on pregnant female and development of the embryo and foetus in stage C - implantation to closure of the hard palate (see 1.7.8). Those studies are not critical for support of clinical trials with sexual immature participants. If the embryo-foetal exposure is expected to be low because of placental barrier, what is typical for large molecules, the EFD-study-reports can first be submitted with the marketing application [17]. Although monoclonal antibodies cannot diffuse passively across the placenta because of the size of molecules, they 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. Anyway, the studies aiming to detect any possible secondary effects on the maternal or placental toxicity should be performed [17].

The common and representative endpoints for the detection of unwanted impact of therapeutics are increased toxicity or abortion in pregnant females, any growth or structural alterations [33].

The choice of adequate dose level, number of animals (at minimum six) and treatment timing (during organogenesis) are crucial factors determining the significance of the studies performed [17]. GLP or, if this not possible, highest scientific conditions are required [17]. The adequate developmental stage scaling from test animal to human should be determined for the data extrapolation [2].

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

These studies should provide information the effect of drug exposure in the females from implantation through weaning and its effect on pregnant/lactating female and the offspring. To detect the delayed effects, the observations should be cover the period including sexual maturity of the offspring (stages C to F listed in 1.7.8). Following endpoints should be analysed in the PPND studies: enhanced toxicity, enhanced pre- and postnatal death of offspring, altered growth and development, or any functional deficits in offspring, including behaviour, maturation and reproduction.

As a combination of this study with EFD studies is not always feasible, *i.e.* if embryo foetal development or pregnancy is impacted by drug exposure, for products pharmacologically active only in NHPs, one well-designed study in NHPs which includes dosing from day 20 of gestation to birth, called enhanced PPND (ePPND), are appropriate. This Study combines the endpoints from both the EFD and PPND studies [2]. Developmental toxicity studies in NHPs did not allow the risk quantification, but can only provide hazard identification.

### 1.7.9 Juvenile Animal Studies (JAS)

For the inclusion of paediatric population in clinical program data on exposed juvenile animals is not mandatory. But, sometimes, human safety data and previous animal studies give insufficient information for a safety evaluation in the intended paediatric age group. In those cases, the conducting studies in juvenile animals may predict if the safety profiles differ between juveniles and adults. If such studies are considered necessary, they should be available before the initiation of paediatric clinical studies. The EMA-guideline *on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications* [34] addresses the details for justification, objectives and the study endpoints. The draft ICH-S11 guideline *nonclinical safety testing in support of development of paediatric medicines* is available since 18. Sept 2018 [35], which is expected to promote harmonisation of the non-clinical safety studies recommended to support the development of paediatric medicines. The other guidelines (ICH-M3, ICH-S5 and ICH-S9) refer to the need for JAS, as well.

### 1.7.10 Local Site Tolerance

The EMA guideline on non-clinical local tolerance testing of medicinal products [36] regulates the implementation of the local tolerance studies. This guideline itself does not specify whether it is equally valid for small molecules and biotechnologically manufactured products. But the guideline ICH-S6 highlights the need for an assessment of local tolerance for the biotech product group. The “stand-alone” studies on local tolerance are generally not recommended and whenever possible, the endpoints of the local tolerance testing should be incorporated in other toxicity studies as per the ICH-S6 guideline [2].

According to the guideline on non-clinical local tolerance testing, the tolerability of parenteral application is preferentially tested in rabbits or other suitable animals. However, ICH-S6 guideline does not specify, that the test species must be a pharmacologically active animal, as is the case with repeat dose toxicity studies, if the studies will be carried out as separate studies.

### 1.7.11 Other Toxicity Studies

According the ICH-M4S guideline on nonclinical overview and nonclinical summaries of CTD-module 2, if the studies on

- Antigenicity
- Immunotoxicity
- Mechanistic studies (if not reported elsewhere)
- Dependence
- Studies on metabolites
- Studies on impurities
- Other studies

have been performed, they should be summarised, providing the rationale for conducting of this studies. These summaries should be presented in CTD under 2.6.6.8 point named “Other Toxicity Studies”.

The antigenicity and immunotoxicity testing issues have been already discussed above (see 1.7.6.3 and 1.7.6.4). Studies on the impurities and metabolites (degradants) present in the drug substance are not applicable for biotechnology-derived products. That is specified in ICH-M4S guideline on the common technical document for the registration of pharmaceuticals for human use [5]. According to the ICH-S6 guideline, “It is preferable to rely on purification processes to remove impurities and contaminants rather than to establish a preclinical testing program for their qualification”.

ICH-S6 guideline did not contain any requirements and advices to conduct the pre-clinical studies on dependence (potential for abuse) or phototoxicity, therefore those studies are not required for biopharmaceuticals [2].

### 1.7.12 Environmental Risk Assessment (ERA)

According to *Guideline on the environmental risk assessment of medicinal products for human use* [37], drugs containing natural peptides or proteins are considered as readily degradable, and therefore no Environmental Risk Assessment is applicable for those products.

When a drug contains a non-natural protein/peptide, which is modified (*i.e.* to increase biostability), an additional screening step should be performed to demonstrate whether they would be quickly degraded in the environment. The environmental risk assessment is not applicable, when the non-natural peptide/protein is demonstrated to be excreted in amounts < 10% of the dose.

Protein-drug conjugates, including natural proteins do not belong to this group and would require standard assessment of the non-protein-moiety.

## 1.8 Similar Biological Medicinal Products

The guideline on similar biological medicinal products [38], defines a biosimilar as "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".

There are a number of complementarily applicable guidelines for biosimilars that address various issues such as quality, non-clinical and clinical aspects [39; 40]. The further product specific guideline to biosimilar mAbs focuses in detail important for monoclonals [4]. Other guidelines are addressed to specific issues (*i.e.* quality aspects to be consider, if manufacturing process was changed).

The aim of non-clinical, clinical or quality testing of biosimilars is to support comparability to the reference products.

The comparability testing of biosimilars to its reference product is based mainly on the *in vitro* binding properties to the target molecule, any unintended binding to the three isoforms of the relevant Fc gamma receptors, Fab- and Fc-associated cytotoxic or other safety related effects.

The *in vivo* studies should only be performed if the predictive power of these studies is given and if relevant species are also available. When the test animal model allows, the PK and PD of the similar biological medicinal product and the reference medicinal product should be quantitatively compared, including concentration-response assessment covering the therapeutic doses in humans.

Only if the need and the predictability of *in vivo* studies are well justified, some *in vivo* toxicity studies may be useful. So, for new manufacturing related excipients, immunotoxicity or local tolerance may need to be evaluated. The incorporation of different endpoints in one *in vivo* study should be a preferred strategy for biosimilars.

The studies regarding safety pharmacology, repeat dose and reproduction toxicology are in general omitted for biosimilar mAbs. Studies on local tolerance also are usually not required. If other *in vivo* studies are performed, evaluation of local tolerance may be part of the design of that study instead of the performance of separate local tolerance studies.

# 2 Results

Based on the analysis of the EPARs, it has been evaluated, which non-clinical studies/data were essential for the positive assessment of the application and the absence of which studies was not decisive for product authorization.

A quantitative evaluation of non-clinical studies was performed. As "performed" were recorded only those cases where EPARs provided a clear commitment to carry out the corresponding kind of studies. No interpretations were applied.

In order to minimise inaccuracies in the analysis, the EPARs were reviewed by author for the presence of studies at least twice with a time interval. The results of all evaluation actions were matched.

## 2.1 Products/EPARs to be analysed

Comprehensive information about all products which have been authorised through the centralised procedure is available on the dedicated EMA website https://www.ema.europa.eu/en/medicines/download-medicine-data. There are 1642 new authorised human and veterinary medicinal products in total, including refused or withdrawn authorisations (latest update at 29.10.2019). 118 products out of them are products containing monoclonal antibodies as an active substance. The downloaded list in excel format did not contain column which describes the type of active substance, therefor the products has been pulled out from the downloaded list column “active substance” by means of stem “*-mab”*, as this suffix clearly indicates monoclonals [41].

The review of the medicines list revealed, that, between 2009 and 2019, ninety medicinal products, containing a monoclonal antibody as an active substance, gained marketing authorisation by EMA (Annex 1). Nine of these products are currently withdrawn. Nevertheless, non-clinical data set of these products was included in this analysis, since these products have already received positive opinion by CHMP, what means, that the application submitted contained the valid and complete non-clinical data, which has fulfilled the requirements for authorisation. Since the EPAR of one medicinal product (Rituzena, previously Tuxella) was not available, a total of 89 EPARs were included in the analysis (Annex 1).

The structure and content of EPARs reviewed follows strongly consistent to ICH-M4 standard. Only EPARs of three products Blitzima, Zirabev and Fasenra contain the non-clinical data under chapter 2.2, 2.2 and 2.5, respectively, instead of chapter 2.3. Nevertheless, the information on the non-clinical aspects in these EPARs was complete and assessable.

The two largest therapeutic groups among the clinical indications of the antibodies are immunosuppressants (39 Products of 89) and antineoplastic agents (37 products of 89). Single products were indicated for treatment of other diseases: the products with vasomodulatory properties were qualified for prophylaxis or treatment of migraine (three products) and angioedema (one product). One product each is approved as an antithrombotic agent and as a coagulation-promoting agent. Two products with metabolic modulating properties are indicated to treat a hypercholesterolaemia. Two other products containing the same active

substance with osteoclast resorption-inhibiting properties are used to prevent bone fractures in postmenopausal women with osteoporosis or in adults with advanced bone malignancies. One product is intended to use as diagnostic agent for single use, and two products are directed against non-human antigens (B toxin of pathogen *Clostridium difficile* and antithrombotic drug molecule, Table 1).

**Table 1: Pharmacotherapeutic Groups of Medicinal Products containing Monoclonal Antibodies**

| Target or Pharmacotherapeutic Group | All 89 Products |
|---|---|
| Immunosuppressants / Immunomodulators | 39 |
| Advanced Malignancies | 37 |
| Vasomodulators | 4 |
| Antithrombotic Agents | 1 |
| Antihaemorrhagics | 1 |
| Metabolic Modulators | 2 |
| Diagnostic for Single Use | 1 |
| Bone Resorption Modulator | 2 |
| Non-human Antigen | 2 |

Among these 89 products there are 26 biosimilar products and 13 products designated as an orphan medicinal product.

## 2.2 Pharmacodynamics

### 2.2.1 Primary Pharmacodynamic Studies

The review of the EPARs showed that comprehensive primary pharmacodynamic studies were conducted for all 89 products (100%).

As already mentioned, 63 medicinal products of 89 contain the new active substance and 26 products are biosimilars.

In relation to the type of product, the scope of the studies for a new active substance was greater than for biosimilars. This result is presented in Table 2.

**Table 2: Primary Pharmacodynamic Studies**

| | | Primary Pharmacodynamic Studies | | | | | |
|---|---|---|---|---|---|---|---|
| | | *in vitro*+ *in vivo* | | *in vitro* only | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 76 | 85 | 12 | 13 | 1 | 1 |
| New Active Substance | 63 | 61 | 97 | 1 | 1,6 | 1 | 1,6 |
| Biosimilars | 26 | 15 | 58 | 11 | 42 | 0 | 0 |

For 61 of 63 products with the new active substance (97%), both *in vitro* and *in vivo* primary pharmacological studies were performed. For one product (Scintimun) only *in vitro* binding studies were performed. For the product Zinbryta, the kind of studies carried out was not clearly specified (*in vitro, in vivo*). *In vivo* studies were performed for each product in a suitable animal disease model system.

For all 26 biosimilars, comparative primary PD studies were performed. For 15 of them (58%), the comparability to the reference product was evidenced via both, *in vitro* and *in vivo* primary pharmacological studies.

Five biosimilar products (Blitzima, Ritemvia, Rixathon, Riximyo, Truxima) using the active NHP species for *in vivo* primary PD studies contained the same active substance rituximab.

### 2.2.2 Secondary Pharmacodynamic Studies

According to EPARs, for 38 products from 89, *i.e.* only in 43% of all cases secondary pharmacodynamic studies were performed. All these products contained a new active substance. That means, that applicants provided the extensive secondary pharmacodynamic data for 60 % of the products with a new active substance. For one product (Zinplava), no information has been provided regarding the conduct of secondary pharmacodynamic studies (Table 3).

**Table 3: Secondary Pharmacodynamic Studies**

| | | Secondary Pharmacodynamic Studies | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 38 | 43 | 46 | 52 | 5 | 6 |
| New Active Substance | 63 | 38 | 60 | 24 | 38 | 1 | 2 |
| Biosimilars | 26 | 0 | 0 | 22 | 85 | 4 | 15 |

For 24 of the products with a new active substance (38 %), the lack of secondary pharmacodynamic studies was considered acceptable. The omission of secondary PD studies for this product group was either not justified or supported by the following reasons: tight specificity, known pleiotropic activity, indications from other non-clinical studies, lack of adequate animal species or “the nature of active substance” (Annex 2).

In case of biosimilars, for 22 of 26 products the secondary pharmacodynamic data was not obtained intentionally. For four remaining biosimilar products (Cyltezo, Kromeya, Trazimera and Kanjinti) the EPARs contained no clear information on whether the studies were conducted or not.

### 2.2.3 Safety Pharmacology Programme

From all 89 analysed products, safety pharmacological data were obtained for the 66 products, for 20 products safety studies were not conducted and for three products (Kanjinti, Zinplava and Trazimera) appropriate information were not present.

For 59 of 63 products with a new active substance, safety pharmacology data were presented, for 3 products those studies were not performed and one EPAR (Zinplava) contains no information regarding this issue. The corresponding data for biosimilars are 10/14/2 products. So, the relation between presence and absence of safety studies in the products with a new active substance 94% *vs*. 5 %, in biosimilar products – 38 % *vs*. 54 % (Table 4).

**Table 4: Safety Pharmacology Studies**

| | | Safety Pharmacology Studies | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 69 | 78 | 17 | 19 | 3 | 3 |
| New Active Substance | 63 | 59 | 94 | 3 | 5 | 1 | 1 |
| Biosimilars | 26 | 10 | 38 | 14 | 54 | 2 | 8 |

The data on safety pharmacology were obtained either in dedicated stand alone studies (for 22 of 56 cases of new active substance) or the safety endpoints were incorporated in the repeat dose toxicity studies (in 37 of 56 cases of new active substance). For biosimilar products, in all 10 cases, the endpoints of safety pharmacology studies were assessed as part of the repeat dose toxicity studies (Table 5).

**Table 5: Proportion of Stand Alone Safety Pharmacology Studies**

| | | Safety Pharmacology Studies | | | |
|---|---|---|---|---|---|
| | | Stand alone | | Part of Repeat Dose Toxicity Study | |
| | | N | % | N | % |
| New Active Substance | 59 | 22 | 37 | 37 | 63 |
| Biosimilars | 10 | 0 | 0 | 10 | 100 |

### 2.2.4 Pharmacodynamic Drug-Drug Interactions

For 40 of 63 products with a new active substance (63%) and for 21 of 26 biosimilars (81%), no pharmacodynamic drug interaction studies were performed. The EPARs of five remaining biosimilars and six products containing the new active substance did not give any statement on the absence or presence of the pharmacodynamic drug-drug interaction data (Table 6).

Only for 17 of 63 (27%) products with a new active substance, the pharmacodynamic drug interaction studies were assessed in frame of non-clinical development.

**Table 6: Pharmacodynamic Drug-Drug Interaction Studies**

| | | Pharmacodynamic Drug Interactions | | | | | |
|---|---|---|---|---|---|---|---|
| | | Yes | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 17 | 19 | 62 | 70 | 10 | 11 |
| New Active Substance | 63 | 17 | 27 | 40 | 63 | 6 | 10 |
| Biosimilars | 26 | 0 | 0 | 21 | 81 | 5 | 19 |

The justification for the absence of the pharmacodynamic drug interaction data was not given in 16 cases. In other cases, the stated reasons were the high specificity and affinity to the target molecule (10 cases) or the mode of action, which did not have any potential to interfere with other drugs action during concomitant medication (5 cases). In some cases the justification for omission of studies are: intention to use the medicinal product as mono-therapeutics, absence of the pharmacokinetic interaction potential or the absence of a suitable animal model. There were individual cases with known potential, therefore the studies were not performed and the risks were adequately addressed in SmPC/RMP (Annex 4).

The lack of the PD drug interaction studies for biosimilars is in line with relevant guidelines [38; 40].

### 2.2.5 Approaches used for the Selection of Pharmacologically Relevant Species

The identification of relevant species in almost all cases was supported by a number of different assays. According to the EPARs, the methods used for the selection of relative species were: tissue cross reactivity studies, flow cytometry, time-resolved fluorometry, Biacore binding assay, ELISA, alkaline phosphatase anti-alkaline phosphatase (APAAP) technique, surface plasmon resonance, equilibrium binding assays, sequence homology analysis, epitope mapping, quantitative gene expression profiling, evaluation of Pharmacokinetic binding-linearity, evaluation of biological activity downstream effects *in vitro* and *in vivo* (Annex 3). There were only few cases (Cyramza, Empliciti, Kadcyla, Libtayo, Mylotarg, Praxbind, Removab, Unituxin), for which only one test (7x tissue cross reactivity study and 1x binding assay) was used to identify the relevant species (Annex 3).

### 2.2.6 Pharmacologically Relevant Species

According to the EPARs of 89 examined products, for 15 products, no animal species could be identified in which the active substance would show the required binding- and biological activity. Of these 15 products, 8 are biosimilars and 7 products contained a new active substance (Table 7).

**Table 7: Pharmacologically relevant Species**

| | | Pharmacologically Relevant Species | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No active Species | | 1 Species | | 2 Species | | 3 Species | | 4 or more Species | |
| | | N | % | N | % | N | % | N | % | N | % |
| All Products | 89 | 15 | 17 | 54 | 61 | 14 | 16 | 4 | 4 | 2 | 2 |
| New Active Substance | 63 | 7 | 11 | 36 | 57 | 14 | 22 | 4 | 6 | 2 | 3 |
| Biosimilars | 26 | 8 | 31 | 18 | 69 | 0 | 0 | 0 | 0 | 0 | 0 |

In nearly 2/3 of these products (54 cases), only one pharmacologically active species was identified. In 51 cases, the identified species was non-human primate (NHP) cynomolgus monkey (*Macaca fascicularis*), in four cases - chimpanzee (Pan troglodytes) and only in one case - marmoset monkey (*Callithrix jacchus*) (data displayed in Annex 3).

From the 14 cases with two active species, cynomolgus was an indispensable subject of the experiments in 11 cases. Chimpanzee and Rhesus were the representative of the NHPs in one case each (Simponi and Praxbind). In another case for the product (Zinplava), no NHP, but two different rodent species (mouse and hamster) were identified as active species. However, the species pair were NHP and non-NHP (rodent or not rodent) in 11 cases. In one already mentioned case - Zinplava - there were two rodents and in two cases (Simponi and Trogarzo) - two NHPs (cynomolgus/chimpanzee and cynomolgus/rhesus) have been identified.

In the rare cases (6 cases) with 3 or more identified active species, cynomolgus was an appropriate species for all products – for four products this NHP was combined with non-NHP species (rodent or non-rodent). In two cases all pharmacologically active species were NHPs only: for the product Cosentyx, there were identified three active NHPs - cynomolgus, rhesus

and marmoset; the product Sylvant cross reacts with 7(!) species, all of them are NHPs: cynomolgus, chimpanzee, baboon, pigtailed macaque, cotton-top tamarin, marmoset, and rhesus.

In all products with an active species, there was at least one NHP used. Cynomolgus was the active NHP in most cases. Only in one case Zinplava two rodents - mouse and hamster was identified as active species.

Only cynomolgus was a single active species for the biosimilars (17 cases of 26), in one case (Zessly), chimpanzee was considered as active species, but not used in toxicity studies, for eight products no active species was identified. This data is presented in Annex 3.

## 2.3 Pharmacokinetics

The review of pharmacokinetic data presented in EPARs show, that in 65 cases (73%) dedicated pharmacokinetic studies (at least one of absorption, distribution, metabolism or excretion studies) were carried out. For some products (22 products, see Annex 5) the PK data were additionally obtained in other non-clinical studies. For 25% of the products, the PK endpoints were integrated in other pre-clinical studies (*i.e.* primary pharmacodynamic and toxicology programme, see Annex 5). One product (Unituxin) refers to literature data (Table 8), and the EPAR of one biosimilar product (Ontruzant) contains no information, whether the PK (absorption) data was obtained.

**Table 8: Pharmacokinetic Data**

| | | Pharmacokinetic Data | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Yes | | Part of other Studies | | Literature Review only | | No Data/ not Performed | |
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 65 | 73 | 22 | 25 | 1 | 1 | 1 | 1 |
| New Active Substance | 63 | 50 | 79 | 12 | 19 | 1 | 2 | 0 | 0 |
| Biosimilars | 26 | 15 | 58 | 10 | 38 | 0 | 0 | 1 | 2 |

### 2.3.1 Studies/Data on Absorption

Although the basic pharmacokinetic data are available for nearly all products, data collection did not follow a classical approach - the structured series of tests covering absorption, distribution, metabolism and excretion (ADME), which is common for small molecules. In this evaluation, the presence of PK data was interpreted as evidence that absorption studies were performed, even if this data was obtained from of other non-clinical studies. Table 9 shows the presence of drug absorption related data for all analysed EPARs.

**Table 9: Studies on Absorption/Absorption Data**

| | | Studies on Absorption | | | |
|---|---|---|---|---|---|
| | | PK Absorption Data available | | No Data | |
| | | N | % | N | % |
| All Products | 89 | 88 | 99 | 1 | 1 |
| New Active Substance | 63 | 63 | 100 | 0 | 0 |
| Biosimilars | 26 | 25 | 96 | 1 | 4 |

The information on basic absorption characteristics was given for 88 of 89 products. There

was at least stated, that basic pharmacokinetic data was available (only three cases, Annex 5), but in most cases the comprehensive description of absorption studies including appropriate values (*i.e.* $T_{1/2}$, $C_{max}$, $T_{max}$, AUC, $V_d$, $V_{ss}$, F and other common PK parameters) were given. Only the EPAR of one biosimilar product Ontruzant does not mention whether the absorption data were obtained, but, besides of this the lack of studies on the distribution, elimination, excretion or pharmacokinetic drug interactions was commented as acceptable for this product as it was a biosimilar application.

### 2.3.2 Studies on Distribution

In the EPARs of only 28 products, it was clearly described that the distribution studies have been performed. In 13 products out of this 28, for the distribution studies, the radioactive labelled antibodies were used. Four of them were products containing mAb-drug conjugates (Annex 5). For the other 30 products it was reported that distribution studies were not carried out, and for 31 Products no information on the distribution studies was given (Table 10).

**Table 10: Studies on Distribution**

| | | Distribution | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 28 | 31 | 30 | 34 | 31 | 35 |
| New Active Substance | 63 | 28 | 44 | 16 | 25 | 19 | 30 |
| Biosimilars | 26 | 0 | 0 | 14 | 54 | 12 | 46 |

### 2.3.3 Studies on Metabolism

The studies on metabolism were performed for nine products only, as the corresponding evaluation of the EPARs showed. For 60 products in total, the metabolic studies were not performed, and for 20 products, the details of this issue were not provided (Table 11).

**Table 11: Studies on Metabolism**

| | | Metabolism | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 9 | 10 | 60 | 67 | 20 | 22 |
| New Active Substance | 63 | 9 | 14 | 45 | 71 | 9 | 14 |
| Biosimilars | 26 | 0 | 0 | 15 | 58 | 11 | 42 |

The justification for lack of metabolism studies in almost all cases was, that the metabolic pathways of antibodies - degradation to small peptides and individual amino acids - are generally understood and therefore, according to ICH-S6 guideline these data are not required.

The data on metabolism was available only for nine products with a new active substance. Six of them (Adcetris, Besponsa, Cimzia (PEG), Kadcyla, Mylotarg and Scintimun) contain the mAb-drug conjugate or radioactive labelled antibody as an active substance. For three products (Prolia, RoActemra and Xgeva) in the presented topic referred metabolism studies, the protective influence of the neonatal receptor FcRn from lysosomal degradation through binding to the Fc region of the antibody was discussed.

### 2.3.4 Studies on Excretion

The studies on excretion were carried out only for 12 products containing the new active substance. For 57 products excretion studies were not conducted, 42 of which are the products with the new active substance and 15 are biosimilars (Table 12).

**Table 12: Excretion Studies**

| | | Excretion | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 12 | 13 | 57 | 64 | 20 | 22 |
| New Active Substance | 63 | 12 | 19 | 42 | 67 | 9 | 14 |
| Biosimilars | 26 | 0 | 0 | 15 | 58 | 11 | 42 |

There were 9 products at all, for which all pharmacokinetic studies - absorption, distribution, metabolism and excretion - were performed. Five of them contain as active substance an antibody-drug conjugate. Therefore, for four therapeutic antibodies only (Prolia, RoActemra, Scintimun, Xgeva) the full set of ADME studies were conducted. For these cases the standard conditions of ADME studies, which are applicable for small molecule testing, were fulfilled: the use of two testing species - rodent and non-rodent and use of radiolabeled isotopes.

### 2.3.5 Immunogenicity/Antigenicity

The immunogenicity potential of the therapeutic antibodies were determined for 75 of 89 products as part of pharmacokinetic, acute, repeat dose or developmental toxicity pre-clinical studies (Table 13, Annex 7).

**Table 13: Immunogenicity (ADA Formation)**

| | | Immunogenicity | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 75 | 84 | 1 | 1 | 13 | 15 |
| New Active Substance | 63 | 56 | 89 | 1 | 2 | 6 | 10 |
| Biosimilars | 26 | 19 | 73 | 0 | 0 | 7 | 27 |

13 EPARs did not contain the information on ADA formation in test animals, only for one product Empliciti the lack of immunogenicity data was not considered to be relevant for the interpretation of the non-clinical result.

### 2.3.6 Pharmacokinetic Drug-Drug Interaction Studies

According to the analysed EPARs, the investigations on the pharmacokinetic drug-drug interactions were not conducted for 42 products (47%). For 41 products (46%) no information was provided whether those studies were carried out or not. For only six cases clear information about the availability of the data was presented in EPARs (Table 14).

**Table 14: Pharmacokinetic Drug Interactions**

| | | PK Drug Interactions | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 6 | 7 | 41 | 46 | 42 | 47 |
| New Active Substance | 63 | 6 | 10 | 29 | 46 | 28 | 44 |
| Biosimilars | 26 | 0 | 0 | 12 | 46 | 14 | 54 |

Three of these five products are products containing the mAb-drug conjugates Adcetris, Kadcyla and Mylotarg. Three products Cinqaero, Ilumetri and Perjeta contain monoclonal antibodies only.

### 2.3.7 Species used in Pharmacokinetic Studies

Species or test systems used for the collection of pharmacokinetic data were analysed (Table 15, Annex 6).

**Table 15: Species used in Pharmacokinetic Studies**

| | | PK Species Used | | | | | |
|---|---|---|---|---|---|---|---|
| | | Only Relevant Species (one or more) | | Relevant species + Non Relevant Species | | Non Relevant Species | |
| | | N | % | N | % | N | % |
| All Products | 89 | 40 | 45 | 33 | 37 | 16 | 18 |
| New Active Substance | 63 | 30 | 48 | 26 | 41 | 7 | 11 |
| Biosimilars | 26 | 10 | 38 | 7 | 27 | 9 | 35 |

The Pharmacokinetic data were obtained using exclusively the pharmacologically active species in 40 products (45%, Table 15). From these 40 cases, for 38 products the non-human primate cynomolgus as single species (30 cases) or in combination with other active species (1x rhesus, 2x rabbit, 2x rat, 1x mouse, 1x hamster and 1x guinea pig) were used. In one case (Praxbind) the NHP was rhesus in combination with rodent (rat). Hamster and guinea pig as a single species were used for two products Qarziba and Zinplava (Annex 6).

Only non-active animals were used for 16 products, 15 out of them were products, for which no relevant animals could be identified (see Table 7). For one product (Zessly) the active species was Chimpanzee, which was not used. The cases, for which only non-active species were used for obtaining pharmacokinetic/ toxicokinetic data, are listed in Table 16.

**Table 16: Products for which only Non-Active Species were used in PK Studies**

| Medicine Name | Active Substrate | Description | Rodent | Non Rodent |
|---|---|---|---|---|
| Besponsa | Inotuzumab ozogamicin | Conjug. | Mouse, Rat | Cynomolgus, Rabbit |
| Mylotarg | Gemtuzumab | Conjug. | Rat | Cynomolgus, Dog |
| Dupixent | Dupilumab | | Rat | Cynomolgus |
| Empliciti | Elotuzumab | | Mouse | Rhesus |
| Removab | Catumaxomab | | Mouse surrogate | |
| Ultomiris | Ravulizumab | | | Cynomolgus, Rabbit |
| Unituxin | Dinutuximab | | Mouse, Rat | Dog |
| Flixabi | Infliximab | Biosimilar | Rat, Mouse model | |
| Inflectra | Infliximab | Biosimilar | Rat | |
| Remsima | Infliximab | Biosimilar | Rat | |
| Zessly | Infliximab | Biosimilar | Rat | |
| Herzuma | Trastuzumab | Biosimilar | | Cynomolgus |
| Kanjinti | Trastuzumab | Biosimilar | | Cynomolgus |
| Ogivri | Trastuzumab | Biosimilar | | Cynomolgus |
| Ontruzant | Trastuzumab | Biosimilar | No Data | |
| Trazimera | Trastuzumab | Biosimilar | Mouse surrogate | |

In 33 cases, additional to the relevant species, non-relevant rodent and non-rodent species or animal model systems were used.

Therefore, in 45 cases for PK evaluation non-active species were used. The number of the pharmacokinetic studies with non-active species is given in Table 17 and Annex 6 (multiple counting is possible).

**Table 17: Number of Pharmacokinetic Studies with Non-Active Species**

| Non-Active Species used | Number of Products |
|---|---|
| Mouse | 20 |
| Rat | 22 |
| Mouse model | 4 |
| Dog | 2 |
| Rabbit | 7 |
| Cynomolgus | 8* |
| Cynomolgus surrogate AB | 1 |
| Rhesus | 1 |

*Three products out of 8 contain the same active substance trastuzumab

## 2.4 Toxicology

### 2.4.1 Single Dose/Acute Toxicity Studies

The proportion of products for which the applicants had submitted studies on acute (or single dose) toxicity was analysed. The results are shown in Table 18:

**Table 18: Single Dose Toxicity Studies**

| | | Single Dose /Acute Toxicity Studies | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Yes | | Part of Other Studies | | Not performed / No Data | | No Data | |
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 27 | 30 | 24 | 27 | 34 | 38 | 4 | 4 |
| New Active Substance | 63 | 27 | 43 | 22 | 35 | 11 | 17 | 3 | 5 |
| Biosimilars | 26 | 0 | 0 | 2 | 8 | 23 | 88 | 1 | 4 |

The dedicated single dose toxicity studies were performed for only for 30 % of the products.

Considering the products with the new active substance and biosimilars separately, the following can be observed: among new drugs the dedicated single dose toxicity studies were conducted in 43 % (27 products). The acute toxicity information was obtained from other non-clinical (one or more) studies in 35 % of the cases (22 products). For 17 % (11 products) no dedicated single dose toxicity studies were conducted, but it was not clearly specified, which non-clinical studies provide the information on acute toxicity. The EPARs of three products did not contain any information on single dose toxicity studies.

The acute toxicity data were obtained in most of the cases (15 products) in repeat dose toxicity studies after first dose administration. The single dose pharmacokinetic studies were a source of acute toxicity data in 9 cases. In single cases the acute toxicity data was obtained in primary pharmacodynamics, safety pharmacology and local tolerance studies.

For biosimilars no single dose toxicity studies were conducted in 88% of the products (23 products), in 8 % of the cases (2 products), the acute toxicity information was available from other non clinical studies, for 4% (1 product) no information on acute toxicity studies was given in the EPAR.

### 2.4.2 Repeat Dose Toxicity Studies

#### 2.4.2.1 Number of Repeat Dose Toxicity Studies

Table 19 summarizes the repeated-dose toxicity studies carried out for products containing mAbs as the active substance.

**Table 19: Repeat Dose toxicity Studies**

| | | Repeat Dose Toxicity Studies | | | | | |
|---|---|---|---|---|---|---|---|
| | | Performed | | Intentionally not performed | | No Data | |
| | | N | % | N | % | N | % |
| All Products | 89 | 86 | 97 | 3 | 3 | 0 | 0 |
| New Active Substance | 63 | 61 | 97 | 2 | 3 | 0 | 0 |
| Biosimilars | 26 | 25 | 96 | 1 | 4 | 0 | 0 |

For nearly all products (97 %), with just a few exceptions (3 cases in total), the extensive repeat dose toxicity studies were conducted. In 86 products, repeat dose toxicity studies at least in one species were conducted. For 3 products (Empliciti, Flixabi and Removab), the reason given for the lack of studies was: that no suitable species was found, in which the test substance would be pharmacologically active and no surrogate model was available as well. But, for products with a new active substance (Empliciti and Removab), single dose toxicity

study results in non-active species were available. For the biosimilar product Flixabi, no acute toxicity studies carried out.

#### 2.4.2.2 Duration of Repeat Dose Toxicity Studies

The duration of (longest) pivotal repeat dose toxicity studies was evaluated and the results are presented in Table 20.

**Table 20: Duration of the Longest Pivotal Repeat Dose Toxicity Studies**

| Study Duration | All Products | New Active Substance | Biosimilar | Species used in Study |
|---|---|---|---|---|
| | N=86 | N=61 | N=25 | |
| 2 weeks | 4 | 1 | 3 | Rat (3x), Rhesus (1x) |
| 3 weeks | 1 | 1 | 0 | Mouse (1x) |
| 4 weeks | 22 | 4 | 18 | Cynomolgus (20x), Rat (1x), Mouse (1x) |
| 5 weeks | 1 | 0 | 1 | Cynomolgus (1x) |
| 6 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| 7 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| 8 weeks | 3 | 0 | 3 | Cynomolgus (3x) |
| 12 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| 13 weeks | 5 | 5 | 0 | Cynomolgus (4x), Chimpanzee (1x) |
| 21 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| 24 weeks | 3 | 3 | 0 | Cynomolgus (3x) |
| 26 weeks | 33 | 33 | 0 | Cynomolgus (31x), Marmoset (1x) Murine surrogate (1x) |
| 30 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| 39 weeks | 7 | 7 | 0 | Cynomolgus (7x) |
| 40 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| 52 weeks | 1 | 1 | 0 | Cynomolgus (1x) |
| No Studies | 3 | 2 | 1 | |

For the products with new active substances, in more than half of the cases (33 of 60), the toxicity study duration was 26 weeks (corresponds 6 months). For the next group of seven products, the study duration was 39 weeks (corresponds 9 months). One study (for product Xgeva) had a duration of 52 weeks.

For the smaller group of 5 products, the duration of the longest repeat dose toxicity study was 13 weeks (3 months) and for only four products, it was 4 weeks. Isolated studies lasted one or to two weeks longer or shorter than the durations for the standard toxicity studies fixed in the guidelines (Table 20).

For the vast majority of biosimilars (18 of 24 cases), the duration of the repeat dose toxicity studies was 4 weeks, for one product 5 weeks, for three products 2 weeks only, and for other three products 8 weeks.

#### 2.4.2.3 Recovery Monitoring Period of Longest Repeat Dose Toxicity Studies

For the longest toxicity studies, it was analysed whether the recovery monitoring period was included in the study designs as required the ICH-S6 guideline. The results are presented in Table 21.

For 18 of 63 (29%) products with a new active substance, and for 23 biosimilars out of 26

(88%), there was no information on the status of recovery period of longest toxicity studies given in the EPARs. For about eight products with a new active substance the recovery period was included in designs of other toxicity studies of a shorter duration (data not shown). The recovery period was included in toxicity study designs of 43 products with a new active substance (68%) and two biosimilar products (8%). For two products with a new active substance and for one biosimilar, no repeat dose toxicity studies were performed.

**Table 21: Recovery Monitoring Period of Longest Pivotal Repeat Dose Toxicity Studies**

| | All Products | | New Active Substance | | Biosimilars | |
|---|---|---|---|---|---|---|
| Study duration | N | % | N | % | N | % |
| All Products | 89 | 100 | 63 | 100 | 26 | 100 |
| No Information | 41 | 46 | 18 | 29 | 23 | 88 |
| Recovery Period Included | 45 | 51 | 43 | 68 | 2 | 8 |
| 2 weeks | 1 | 1 | 1 | 2 | 0 | 0 |
| 3 weeks | 1 | 1 | 1 | 2 | 0 | 0 |
| 4 weeks | 6 | 7 | 6 | 10 | 0 | 0 |
| 6 weeks | 3 | 3 | 3 | 5 | 0 | 0 |
| 8 weeks | 6 | 7 | 6 | 10 | 0 | 0 |
| 9 weeks | 1 | 1 | 1 | 2 | 0 | 0 |
| 12 weeks | 5 | 6 | 5 | 8 | 0 | 0 |
| 13 weeks | 7 | 8 | 7 | 11 | 0 | 0 |
| 16 weeks | 2 | 2 | 2 | 3 | 0 | 0 |
| 26 weeks | 5 | 6 | 3 | 5 | 2 | 8 |
| 34 weeks | 1 | 1 | 1 | 2 | 0 | 0 |
| 37 weeks | 1 | 1 | 1 | 2 | 0 | 0 |
| duration unknown | 6 | 7 | 6 | 10 | 0 | 0 |
| No Repeat Dose Toxicity Studies | 3 | 3 | 2 | 3 | 1 | 4 |

The duration of the recovery period of repeat dose toxicity studies covered the duration range from 2 to 37 weeks. In this range the studies were distributed uniformly without forming a group of a certain duration with higher frequency.

### 2.4.3 Species used in Single and Repeat Dose Toxicity Studies

The test species utilisation in all *in vivo* toxicity studies (acute and repeat dose) were analysed and are shown in Table 22. An overall overview of the species used in toxicity studies can be seen in Annex 9.

Non-human primates (NHP) were used in 92% of the products for the toxicity testing. Cynomolgus was the most prominent non-rodent NHP species in toxicity studies used in 84% of cases in total. Other NHP species (chimpanzee, marmoset and rhesus) were presented only in isolated cases. Chimpanzees were used in 3 cases (Darzalex, Blincyto and Mylotarg) in combination with cynomolgus, rat, rabbit or mouse surrogate model. Other non-rodent species used in some individual cases were dog (Unituxin) and rabbit (Crysvita, Entyvio and Mylotarg).

**Table 22: Species used in Single and Repeat Dose Toxicity Studies.**
This figure does not indicate the number of individual studies, but represents the number of cases/products for which the species was used in at least one toxicity study. Multiple counting of cases/products is possible.

| | All Products | | New Active Substance | | Biosimilars | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Non-Human-Primates | 82 | 92 | 59 | 94 | 21 | 81 |
| Cynomolgus | 75 | 84 | 54 | 86 | 21 | 81 |
| Chimpanzee | 3 | 5 | 3 | 5 | 0 | 0 |
| Rhesus | 3 | 3 | 1 | 2 | 0 | 0 |
| Marmoset | 1 | 1 | 1 | 2 | 0 | 0 |
| Other Non-Rodents | | | | | | |
| Dog | 1 | 1 | 1 | 2 | 0 | 0 |
| Rabbit | 3 | 3 | 3 | 5 | 0 | 0 |
| Rodents | | | | | | |
| Rat | 17 | 19 | 13 | 21 | 3 | 12 |
| Hamster | 1 | 1 | 1 | 1 | 0 | 0 |
| Guinea Pig | 3 | 3 | 3 | 3 | 0 | 0 |
| Mouse | 5 | 6 | 4 | 6 | 1 | 4 |
| Mouse Surrogate | 5 | 6 | 5 | 8 | 0 | 0 |
| No Studies *in vivo* | 1 | 1 | 0 | 0 | 1 | 4 |

Rats were the most common rodent animal species, although these were only used in about one fifth of the cases (19%). Mouse, hamster and guinea pig were represented in 1-6% of the cases.

Not all of these test animals were pharmacologically relevant to the test molecules.
The Table 23 shows the proportion of cases with regard to using active or non-active species.

**Table 23: Species used for *in vivo* Single and Repeat Dose Toxicity Studies: Active or Non-Active Species**

| | | Active Species only (one or more) | | Active Species and Non-Active Species or Surrogate | | Non-Active Species or Surrogate only | | No *in vivo* Studies | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 64 | 72 | 10 | 11 | 14 | 16 | 1 | 1 |
| New Active Substance | 63 | 47 | 75 | 9 | 14 | 7 | 11 | 0 | 0 |
| Biosimilars | 26 | 17 | 65 | 1 | 4 | 7 | 0 | 1 | 4 |

For 64 cases, exclusively active species were used in *in vivo* toxicity studies. In 10 cases, an additional non-active animal or animal model was used, although one or two active species were available for these products. For 14 out of 15 products, for which no active animal species could be identified (see chapter “Pharmacologically Relevant Species”), only non-active species or surrogate antibodies were used. The example for the use of a surrogate model is the product Dupixent using Cynomolgus surrogate mAb since test antibody is biologically not active in other species except in humans. For one product (Flixabi), no *in vivo* toxicity studies were performed.

The frequency of use of 2 species for in-vivo toxicity was also analysed (rodent and non-rodent), in line with the guidelines for toxicological studies. The results are shown in Table 24.

**Table 24: Number of Species (Rodent and Non-Rodent) used *in vivo* Single and Repeat Dose Toxicity Studies**

| | | One Species only | | One Rodent and One non-Rodent (at least) | | Two Species other then Rodent and non-Rodent | | No *in vivo* Studies | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 64 | 72 | 20 | 22 | 4 | 4 | 1 | 1 |
| New Active Substance | 63 | 40 | 63 | 19 | 30 | 4 | 6 | 0 | 0 |
| Biosimilars | 26 | 24 | 92 | 1 | 4 | 0 | 0 | 1 | 4 |

The results suggest that in majority of the cases, there was only one species for toxicology studies used in both product groups (64 cases, 72 %). In 55 cases out of 64, the single species used was cynomolgus (Annex 9, extracted data not shown).

Taking into account the product group with the new active substance only, there were 40 products with the new active substance (63%), for which the toxicity studies were performed in one species only. Two species (rodent and non-rodent) were used in 19 cases, but only in eight cases of them the both used species were active for the test products (Annex 9, extracted data are not shown). In four additional cases, two species were either two NHPs (Darzalex: cynomolgus and chimpanzee; Trogarzo: cynomolgus and rhesus) or NHP and non-rodent (Crysvita and Entyvio: cynomolgus and rabbit).

## 2.5 Genotoxicity and Carcinogenicity Studies

### 2.5.1 Genotoxicity Studies

The presence of the genotoxicity studies were assessed and the results are shown in Table 25 and Annex 10.

**Table 25: Genotoxicity Studies**

| | | Performed | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| All Products | 89 | 7 | 8 | 79 | 89 | 3 | 7 |
| New Active Substance | 63 | 7 | 11 | 56 | 89 | 0 | 7 |
| Biosimilars | 26 | 0 | 0 | 23 | 88 | 3 | 0 |

For 79 products, no dedicated non-clinical genotoxicity studies were performed and presented by the applicants: of which, 56 were products with the new active substance and 23 products were biosimilars.

The EPARs of 3 biosimilar products (Cyltezo, Idacio and Kromeya) contain no information whether the studies were conducted.

Only for 7 products, the evaluation of genotoxicity was carried out. 5 of these products were Antibody-Drug Conjugates (ADC) consisting of three components: the antibody, the linker and the conjugated molecule. In 2 of these ADC products (Besponsa and Mylotarg), the conjugate possesses intended genotoxic properties (double-strand DNA breaks). In other 2 products

(Adcetris and Kadcyla), the conjugate is intended to disrupt the mitotic spindle and in one product (Cimzia), the presence of conjugate (PEG) is intended to extend plasma half-life. The product Scintimun contains a radioactive Technetium (99mTc)-labelled monoclonal antibody besilesomab as an active substance. The product Cinqaero contains the anti-IL-5 mAb monoclonal antibody without any conjugates.

The kind and extent of the corresponding genotoxicity studies are shown in the Table 26.

**Table 26: Products and Assays used for Genotoxicity Testing**

| | *In vitro*<br>Gene Mutations in Bacteria | *In vitro*<br>Chromosomal Aberrations | *In vivo*<br>Micronucleus Assay in Bone Marrow |
|---|---|---|---|
| Adcetris | *Salmonella* strains<br>*Escherichia coli* strains | Mouse lymphoma | Rat |
| Besponsa | *Salmonella* strains<br>*Escherichia coli* strains | Micronucleus in TK6 cells | Mouse |
| Cimzia | *Salmonella* strains<br>*Escherichia coli* strains | Human lymphocytes | CD-1 mouse |
| Cinqaero | *Salmonella* strains<br>*Escherichia coli* strains | Human peripheral blood lymphocytes | no |
| Kadcyla | *Salmonella* strains | no | Rat<br>Cynomolgus Monkey |
| Mylotarg | *Salmonella* strains<br>*Escherichia coli* strains | Micronucleus in TK6 cells | Mouse |
| Scintimun | Not specified: *"a number of genotoxicity studies have been conducted"*. | | |

### 2.5.2 Carcinogenicity Studies

The effort of the applicants to determine the carcinogenic potential of the medicinal products was analysed on the basis of the corresponding data in EPARs. The Table 27 presents the results of this evaluation.

**Table 27: Carcinogenicity Studies**

| | | Carcinogenicity | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dedicated | | Part of other Studies | | No | | No Data | |
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 1 | 1 | 1 | 1 | 83 | 93 | 4 | 4 |
| New Active Substance | 63 | 1 | 2 | 1 | 2 | 60 | 95 | 1 | 4 |
| Biosimilars | 26 | 0 | 0 | 0 | 0 | 23 | 88 | 3 | 4 |

For the product Cinqaero, which contains the new active substance, it was reported that the conventional stand-alone carcinogenicity risk assessment study had been carried out.
For 1 product (Repatha), the endpoints of the carcinogenicity study were incorporated in the classical repeat-dose toxicity studies of 6 months duration. The EPARs of 4 products (Cyltezo, Idacio, Kromeya and RoActemra) contains no information whether the studies were conducted.

In the remaining 83 EPARs, it was clearly stated that no conventional carcinogenicity risk assessment studies were performed for these products.

The *Weight of the Evidence Approach* for the justification of the lack of carcinogenicity studies was also analysed. Table 28 shows the common arguments stated for the omission of

carcinogenicity studies for products with a new active substance (63 products). The absence of the carcinogenicity studies for all 26 biosimilar products was justified with the lack of such requirements in the appropriate guideline [4].

**Table 28: Pro and Contra Criteria for the Requirement of Carcinogenicity Studies**

| Common Criteria Supporting Concerns of Carcinogenicity Potential | N |
|---|---|
| Long Term Treatment | 32 |
| Immunomodulators | 25 |
| High Risk Evidence | 1 |
| Not Certain Evidence | 4 |
| Common Criteria for Omission | N |
| Advanced Cancer | 24 |
| Low Risk Evidence (literature or other studies) | 27 |
| Directed Against Foreign Antigens | 2 |
| Not Feasible | 15 |
| Short term treatment or Single Diagnostic Use | 6 |
| Known Risk | 3 |
| Unpredictable/Pointless | 4 |

Several arguments pro and contra were raised on need of the carcinogenicity study for each product and the decision was taken on a case-by-case basis. Medicines intended for the treatment of advanced cancer were exempted from the obligation to conduct carcinogenicity studies in line with the ICH-S9 specific guideline (24 products except biosimilars). This also applied to products directed against foreign antigens (2 products Praxbind and Zinplava) or diagnostics for one-time use (Scintimun). For high risk carcinogenicity products, *i.e.* products intended for long term use and/or with immunomodulatory potential, in some cases it was not feasible to conduct the carcinogenicity studies due to technical reasons as the standard tests with rodents were not suitable due to lack of antibody reactivity. On the other hand, the NHPs, in which the test molecules show the activity, are not suitable for carcinogenicity studies.

The concerns on the carcinogenic potential were adequately addressed in the Risk Management Plan and/or SmPC.

## 2.6 Reproductive and Developmental Toxicity

### 2.6.1 Fertility and Early Embryonic Development FEED

The number of products, for which fertility and early embryonic development (FEED) studies were carried out, was evaluated. The results are presented in Table 29.

**Table 29: Fertility and Early Embryonic Development (FEED) Studies**

| | | Performed | | Part of Repeat Dose Toxicity Studies | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 22 | 25 | 28 | 31 | 33 | 37 | 6 | 7 |
| New Active Substance | 63 | 22 | 35 | 25 | 40 | 11 | 17 | 5 | 8 |
| Biosimilars | 26 | 0 | 0 | 3 | 12 | 22 | 85 | 1 | 4 |

For 22 products containing a new active substance (35%), dedicated FEED studies were performed. For other 25 products of this product group (40%), the endpoints of the fertility (male or female) or early embryonic development issues were incorporated in the repeat dose toxicity studies. In 11 cases (17 %), there were data on fertility and early development intentionally omitted. In five EPARs (8%), it was not clearly described whether the FEED studies were carried out for the products. In three cases (Ilaris, Prolia, Zinbryta), the FEED-relevant data were obtained from repeat dose toxicity studies (Annex 12), in addition to stand-alone studies.

For products containing a new active substance, it was analysed whether the test species were used for dedicated FEED studies. These studies were conducted in pharmacologically relevant species only in 10 out of cases. In 12 cases (three cases of them without identified active species), mouse or rat surrogate models were used. This data is shown in Annex 12.

For a vast majority of biosimilar products (85%), the FEED studies were not performed. For 1 product (Idacio), the appropriate data was not available in the EPAR. In all cases it was stated, that such studies are not required for a similar biological product (EMEA/CHMP/BMWP/42832/2005). However, for 3 products (Blitzima, Ritemvia, Truxima) some fertility relevant endpoints were evaluated in repeat dose toxicity studies.

### 2.6.2 Embryo-Foetal Development Studies

The scope of EFD studies in the non-clinical development program of monoclonal antibodies was analysed as well. The results are shown in Table 30.

In 30 cases (48%) for the products containing a new active substance, dedicated embryo-foetal development (EFD) studies were performed. In 2 cases (Praluent, Simponi) extended pre- and postnatal development (ePPND) studies were conducted in addition to the dedicated EFD studies. In 15 cases (24%) the FED studies were fully integrated in the ePPND. For 18 products, the applicants provided no EFD relevant data. For 1 biosimilar product (Idacio), no information was given on the availability of FED data. In 1 case (Yervoy) the ePPND study was ongoing.

**Table 30: Embryo-Foetal Development (FEED) Studies**

| | | Dedicated | | ePPND | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 30 | 34 | 15 | 17 | 43 | 48 | 1 | 1 |
| New Active Substance | 63 | 30 | 48 | 15 | 24 | 18 | 29 | 0 | 0 |
| Biosimilars | 26 | 0 | 0 | 0 | 0 | 25 | 96 | 1 | 4 |

In all studies (EFD+ePPND) for the products containing new active substance (46 products), there were pharmacologically active species used in 35 cases. In 10 cases, the studies were carried out in non-active species. Only for 4 products (Dupixent, Besponsa, Mylotarg and Ultomiris) there was no active species identified.

In the EPARs of 25 biosimilar products it was clearly stated that no FED studies were carried out and it was also explained that these studies are not required for biosimilars. For 1 product (Idacio), no information was given about the availability of FED data.

### 2.6.3 Pre- and Postnatal Development (PPND) Studies

The EPARs were evaluated regarding the availability of the pre- and postnatal development (PPND) studies. The results are presented in the Table 31.

**Table 31: Pre- and Postnatal Development (PPND) Studies**

| | | Dedicated | | ePPND | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 19 | 21 | 16 | 18 | 48 | 54 | 6 | 7 |
| New Active Substance | 63 | 19 | 30 | 16 | 25 | 23 | 37 | 5 | 8 |
| Biosimilars | 26 | 0 | 0 | 0 | 0 | 25 | 96 | 1 | 4 |

In 19 cases (30%) for the products containing a new active substance, the dedicated pre- and postnatal development (PPND) studies were performed. In 1 case (Simponi), the data from an additionally performed extended pre- and postnatal development (ePPND) study was used to obtain more information (Annex 13). In 16 cases (25%) the PPND were fully integrated in the ePPND. For 23 products the applicants provided no data on pre- and postnatal development. For 5 products, the EPARs contain no information about the availability of PPND study. In 1 case (Yervoy) the ePPND study was ongoing.

In the majority of cases pre- and postnatal development studies were conducted using active species (29 cases out of 35 (19 PPND +16 ePPND)). In 6 cases mouse surrogate model (4x), rat (1x) or cynomolgus (1x) were used although there was no active species for only 2 products (Dupixent and Ultomiris).

In the EPARs of 25 biosimilar products it was clearly stated that no PPND studies were carried out and it was also explained that these studies are not required for biosimilars. For 1 product (Idacio) no information was given about the availability of PPND relevant data.

## 2.7 Juvenile Animal Studies (JAS)

The presence of the juvenile animal studies (JAS) was also evaluated for all products containing monoclonals as an active substance. The results are presented in Table 32.

**Table 32: Juvenile Animal Studies**

| | | Dedicated | | Part of Other Studies | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 9 | 10 | 9 | 10 | 39 | 44 | 32 | 36 |
| New Active Substance | 63 | 9 | 14 | 9 | 14 | 15 | 24 | 30 | 48 |
| Biosimilars | 26 | 0 | 0 | 0 | 0 | 24 | 92 | 2 | 8 |

In the EPARs of products with a new active substance (63 products), the Information on juvenile animal studies was not present in 30 cases. Only for 9 products, there were specific juvenile animal studies conducted (in 1 case the study was ongoing). The data on juvenile toxicity was obtained as part of other studies in 9 cases. At the time point of application the JAS were intentionally not conducted for 15 products.

The status of PIP-completeness at the time point of application (as stated in EPARs) were analysed as well. Only for 17 cases out of 63 the PIP was completed at the time of application.

In all of these cases, PDCO agreed class- / product specific waiver or no need for non-clinical juvenile data. These 17 products correspond to the 14 products "no data" and the 3 products "not performed" Table 32.

For 5 products (Cimzia, Removab, RoActemra, Scintimun and Simponi,) no information on PIP was available in EPARs. All these products were authorised in 2009.

For the remaining 41 products, the PIP was not yet completed at the time of assessment.

For all biosimilar products, the juvenile animal studies or - in 2 cases - the information on the studies was not available.

## 2.8 Local Tolerance

The Table 33 represents the results of the evaluation if local tolerance studies were conducted for the products containing mAbs as an active substance.

**Table 33: Studies on Local Tolerance**

| | | Dedicated | | Part of Other Studies | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 22 | 25 | 58 | 65 | 4 | 4 | 5 | 6 |
| New Active Substance | 63 | 19 | 30 | 40 | 63 | 1 | 2 | 3 | 4 |
| Biosimilars | 26 | 3 | 12 | 18 | 69 | 3 | 12 | 2 | 4 |

The results show, that the information on the tolerability of the drug with the new substance at the injection site were obtained either in dedicated studies (30%), or as part of repeat dose toxicity studies (63%). Additional data were obtained in individual cases from pharmacokinetic, single dose toxicity and PPND/ePPND studies. In one case (Sylvant) EPAR state that the study was not submitted, but no explanation was given as to whether the relevant data were collected in other studies. In 3 cases (Praluent, RoActemra, Zinplava) it was not clearly stated whether those studies were performed.

For most biosimilars (69%), the data on local tolerability were derived from other toxicity studies. Only for three products (Halimatoz, Hefiya, Hyrimoz), stand-alone local tolerance studies in rabbit were performed. For 3 products (Flixabi, Ontruzant, Trazimera) the local tolerance studies were not submitted. For 2 products (Idacio, Kromeya) no information on local tolerance studies was provided.

In the Table 34 below, the list of species used in dedicated local tolerance studies are presented. Rabbit was used in local tolerance assessment studies in 15 out of 22 cases, followed by cynomolgus (4 studies) and rat (2 studies). For 2 products, the local tolerance data were obtained using 2 species (Cinqaero: rabbit and rat; Ultomiris: rabbit and cynomolgus).

**Table 34: Species used in Local Tolerance Studies**

| | All Products | | New Active Substance | | Biosimilars | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Rabbit * | 15 | 68 | 12 | 63 | 3 | 100 |
| Cynomolgus * | 4 | 18 | 4 | 21 | 0 | 0 |
| Rat * | 2 | 9 | 2 | 11 | 0 | 0 |
| Not Specified | 3 | 14 | 3 | 16 | 0 | 0 |

*when used two species, double counting of products is possible

Rabbit was used in local tolerance assessment studies in 15 cases; only in 3 cases of them rabbit was a cross-reactive species. For further 3 products there were no active species available. In 9 cases, rabbit was used as test species, although other species but not rabbit was cross-reactive for given the respective biopharmaceutical (Annex 15). In two cases (Cimzia, Cinqaero), rat was used, although the test biopharmaceuticals did not cross-react with the rat target (Annex 15).

## 2.9 Other Toxicity Studies

### 2.9.1 Immunotoxicity Studies

The data on immunotoxicity was presented in 27 EPARs (Table 35, Annex 15). Only 3 of them were obtained in dedicated studies. For 24 products the immunotoxicity relevant endpoints were incorporated in repeat-dose toxicity or in developmental toxicity studies.

**Table 35: Immunotoxicity Studies**

| | | Dedicated | | Part of Other Studies | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| All Products | 89 | 3 | 3 | 24 | 27 | 4 | 4 | 58 | 65 |
| New Active Substance | 63 | 3 | 5 | 21 | 33 | 3 | 5 | 36 | 57 |
| Biosimilars | 26 | 0 | 0 | 3 | 12 | 1 | 4 | 22 | 85 |

For 4 products it was specified that no immunotoxicity investigations were performed.
The EPARs of 58 products contain no information on the immunotoxicity studies.

### 2.9.2 Human Tissue Cross-Reactivity Studies

The tissue cross-reactivity studies directed to the human tissues were performed in 63 out of 89 products. For the remaining 26 products the EPARs provide no information whether these studies were carried out. The data is presented in Table 36 and more comprehensive in Annex 15.

**Table 36: Human Tissue Cross-Reactivity Studies**

| | | Performed | | No Data | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| All Products | 89 | 63 | 71 | 26 | 29 |
| New Active Substance | 63 | 51 | 81 | 12 | 19 |
| Biosimilars | 26 | 12 | 46 | 14 | 54 |

### 2.9.3 Studies on Impurities

In context of impurity toxicity studies, 5 products were counted (Cyltezo, Herzuma, Poteligeo, Scintimun, Stelara) for which the evaluation of toxic potential of impurities was carried out, what was clearly outlined in the EPARs under topic “other toxicity studies” (Table 37).

**Table 37: Studies on Impurities**

| | | Performed | | Intentionally not performed | | No Data | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| All Products | 89 | 5 | 6 | 10 | 11 | 74 | 83 |
| New Active Substance | 63 | 3 | 5 | 7 | 11 | 53 | 84 |
| Biosimilars | 26 | 2 | 8 | 3 | 12 | 21 | 0 |

In the non-clinical part of the EPARs for 10 products (Cinqaero, Ocrevus, Removab, Repatha, Skyrizi, Taltz, Xgeva - products with a new active substance; Inflectra, Remsima, Zessly - biosimilars), the information on the absence of non-clinical data is given.

A majority of the EPARs (53 products with a new active substance and 21 biosimilars) do not contain information in their non-clinical sections on whether the toxicological potential of the impurities has been investigated. In single products (Adcetris, Taltz - products with a new active substance; Hulio, Kromeya, Zirabev -biosimilars) the presence of appropriate non-clinical studies/assessment of the impact of products and process related impurities on safety are reported in quality related sections of the EPARs.

The extensive specifications of the test material (including investigations of the product and process related impurities), which is also required by ICH-S6 guideline, are placed in the quality section of the EPARs.

### 2.9.4 Studies not Analysed

The presence of dependence, metabolites and phototoxicity studies was not evaluated.

## 2.10 Ecotoxicity/Environmental Risk Assessment

The scope of an environmental risk assessment submitted for medicinal products containing monoclonal antibodies as an active substance has been evaluated. The results are presented in Table 38.

**Table 38: Environmental Risk Assessment**

| | | Yes | | No | | No data | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| All Products | 89 | 6 | 7 | 83 | 93 | 0 | 0 |
| New Active Substance | 63 | 6 | 10 | 57 | 90 | 0 | 0 |
| Biosimilars | 26 | 0 | 0 | 26 | 100 | 0 | 0 |

The specific ecotoxicity studies were performed for 6 products containing a new active substance. 5 of them were antibody-drug conjugates (ADC) consisting the antibody, the linker and the conjugated molecule. In 2 of these ADC products (Besponsa and Mylotarg) the conjugate possesses intended genotoxic properties (double-strand DNA breaks). In the other 2 products (Adcetris and Kadcyla), the conjugate can disrupt the mitotic spindle and in one product (Cimzia) the presence of conjugate (PEG) should extend plasma half-life. The product Scintimun contains the radioactive Technetium (99mTc)-labelled monoclonal antibody besilesomab as an active substance.

For all other products (with a new active substance and with biosimilars), no environmental risk assessment was performed.

# 3 Discussion

## 3.1 Pharmacodynamics

### 3.1.1 Primary pharmacodynamic studies

The primary pharmacodynamic studies provide a basic understanding of the mode of action of the products and the information whether the desired effect is achieved. Such studies are crucial for all products. It was therefore expected, that for all examined products within the scope of this analysis, without exception the comprehensive primary pharmacodynamic studies were conducted and presented.

For nearly all products (for 61 out of 63) with the new active substance the understanding of the mode of action was gained through both *in vitro* and *in vivo* studies. As the mode of action of new active substances is largely unknown in general, it is expected that more comprehensive primary pharmacodynamic studies will be conducted for this product group.

For one product Scintimun, which is a diagnostic for single use, only *in vitro* binding studies were performed. In order to understand its mechanism of action and to show that it achieves the desired effect - a highly selective binding to certain blood cells (granulocytes) - *in vitro* binding assays with different animal and human tissues was considered sufficient, which is in line with case by case science-based approach facilitated by the ICH-S6 guideline. For one product Zinbryta the used methods are not specified.

*In vivo* primary PD studies for products with the new active substance were conducted in suitable *in vivo* disease model systems. This approach is scientifically valid since the well-adjusted experiment in model system has a high scientific power for detection of pharmacological effects on the one hand. On the other hand, this makes it possible to avoid the unnecessary use of higher animal species such as non-human primates. Relevant species were used in primary pharmacodynamics studies mainly for the purpose of validating the reactivity of this species.

Although *in vivo* studies on primary pharmacodynamic issues are not mandatory for biosimilar products, for more than half of the products (58%) the *in vivo* studies were conducted. Five biosimilar products using the active NHP species (cynomolgus) for *in vivo* primary PD studies contain the same active substance rituximab. Therefore, the using of the relevant species in *in vivo* PD Studies for biosimilars could be considered an exception.

### 3.1.2 Secondary pharmacodynamic studies

For 46 products from 89, according to EPARs, no secondary pharmacodynamic studies were performed so far.

For more than half of the products with the new active substance (60%), secondary PD data were obtained. Different justifications were provided for omission of these studies in 38% of the reviewed products. In some cases, the reason was the given nature of the active substance. Probably the authors meant that the given strong specificity of the antibodies to targets, which indicates the low potential for unintended activities of the substance. In some single cases, the reasons for omission of secondary PD studies were: the absence of

adequate test system, findings from other non-clinical studies or already existing knowledge of the pleiotropic activities of the active substance.

For 22 biosimilars, secondary PD studies were not performed intentionally. For the remaining four biosimilars no information has been provided regarding the conduction of these studies. The lack of the secondary PD studies is in EPARs justified as consistent with the Biosimilar guideline.

### 3.1.3 Safety pharmacology programme

From all analysed 89 products, safety pharmacology data were obtained for 66 products in total. For 17 products, safety studies were not performed; three of them were products containing the new active substance and 14 biosimilars. For three products (Kanjinti, Zinplava and Trazimera) appropriate data were not present.

There were only three products (Darzalex, Empliciti, Ultomiris) containing the new active substance, for which intentionally no safety pharmacology data were obtained. Only for the product Empliciti the justification was given, that "because elotuzumab does not recognize non-human forms of SLAMF7 protein, *in vivo* safety data from animal studies are irrelevant." Indeed, for this product there was no active species identified. But this rule is not consistent with other products without active species, for which the safety pharmacology data was obtained: for the product Dupixent as part of repeat dose toxicity studies and for the products Besponsa, Mylotarg, Removab, Unituxin - as part of stand alone safety pharmacology studies. Both, Besponsa and Mylotarg contain the mAb-drug conjugate as an active substance. Therefore the safety pharmacology applies to the cytotoxic conjugate ozogamicin, but Removab and Unituxin contain only monoclonal antibodies.

Safety pharmacology data were determined for 69 products in total (59 products containing the new active substance and 10 biosimilars). For all biosimilars safety pharmacology data were integrated in repeat dose toxicity studies. In products containing the new active substance the corresponding part was 63% of the products. For 22 products dedicated stand-alone studies were conducted, for 4 products additionally safety pharmacology data was obtained from repeat dose studies.

The incorporation of safety pharmacology endpoints in repeat dose toxicity studies seems to be a common practice, applicable to the majority of products, which is in line with 3Rs imperative of appropriate guidelines (*i.e.* ICH-S6) since that leads to minimising use of non-human primates.

### 3.1.4 Pharmacodynamic drug-drug interaction studies

For 63% of the products with a new active substance and 81% of biosimilars no pharmacodynamic drug-drug interaction (DDI) studies were performed. Some EPARs (10% of the new active substance and 19% of biosimilars) did not contain any information on this issue.

For biosimilars, no requirements for PD drug-drug interactions studies have been detailed in relevant guideline [4]. With caution it can be assumed, that the lack of information is due to the fact that there have been no DDI studies carried out. This 100% lack of PD drug-drug interaction studies for biosimilars would still comply with the guideline.

The justification for the absence of the pharmacodynamic drug interaction data was not given in 16 cases. In other cases, the stated reasons were the high specificity and affinity to the target molecule (10 cases) or the mode of action, which did not have any potential to interfere with other drugs action while concomitant medication (5 cases). Intention to use the medicinal product as mono-therapeutics, absence of the pharmacokinetic interaction potential or absence of a suitable animal model were also enlisted as reasons in some cases. There were individual cases with known potential, therefore the studies were not performed and the risks were adequately addressed in SmPC/RMP.

In 27 cases where DDI studies were conducted, the trigger was the intended clinical protocol to treat the disease in combination with other drugs. This data is shown more detailed in Annex 4.

This individual justification for or against conducting the pharmacodynamic drug-drug interaction studies was to be expected in accordance with the ICH-S6 requirements as it prescribes the procedure based on the case by case strategy.

### 3.1.5 Approaches used for the selection of pharmacologically relevant species

According to the ICH-S6 guideline, the selection of the relevant animal species is the most important step for toxicity testing. The combination of multiple testing only leads to a reliable identification of the pharmacologically active species.

Cytoplasmic binding only does not indicate biological activity *in vivo* and these findings should be considered in context with toxicity studies. Nevertheless, there were some cases identified (Cyramza, Empliciti, Kadcyla, Libtayo, Mylotarg, Praxbind, Removab, Unituxin), in which active species were determined using only one test method (7x tissue cross reactivity study (TCR) and 1x binding assay, see Annex 3). As for example, in EPAR of product Unituxin it is stated, that “GLP tissue cross-reactivity studies were conducted with rat, rabbit and human tissues: staining with ch14.18 (specific Ab) was generally consistent with reported sites of GD2 expression. This information confirms the relevance of rat and rabbit as relevant species in accordance with ICH-S6”. Another example is the product Qarziba: “Results from *in vitro* cross-reactivity studies using cynomolgus monkey tissue (as well as guinea pig and human tissue) served to validate the cynomolgus monkey as a relevant toxicology species”.

In EPARs there is no mention of any other efforts made by applicants for identification of relevant species apart from TCR (or in case of Libtayo apart from Biacore binding assay). But, it cannot be excluded, that EPARs did not discuss all issues containing application dossier, or EPAR contains information on additional testing methods (or its results), which are not highlighted as relevant for active species screening, and it was not possible to interpret the given information adequately in this work.

However, for a vast majority of the products a combination of different testing methods were used including functional analysis *in vitro* and *in vivo*, a wide range of binding assays, sequence homology, epitope mapping and classical immunohistochemistry (TCR).

### 3.1.6 Pharmacologically Relevant Species

Among 89, there were 15 products, for which no animal species could be identified, eight of them are biosimilars and seven products contain the new active substance (Annex 3).

In all remaining products there were at least one NHP identified as an active species. Cynomolgus was the active NHP in most cases. Only in one case Zinplava two rodents - mouse and hamster - was identified as active species.

Only cynomolgus was a single active species for the biosimilars (17 cases of 26), in one case (Zessly), chimpanzee was considered as active species, but not used in toxicity studies, for eight products no active species was identified. This data is presented in Annex 3.

## 3.2 Pharmacokinetics

The review of pharmacokinetic data presented in EPARs show, that the basic pharmacokinetic data are available for nearly all products. Nevertheless, data collection structure did not follow a classical approach - the series of tests covering absorption, distribution, metabolism and excretion (ADME), which is common for small molecules.

The application of the 3Rs principles led here to the reduction of stand-alone studies for the collection of pharmacokinetic data: in 19 % of the products with new active substances and in 38 % of biosimilars, the endpoints of PK/TK studies were integrated in other non-clinical studies: in majority of cases there were repeat dose toxicity studies, and in single cases – acute and pre- and postnatal developmental (PPND) toxicity studies.

### 3.2.1 Studies on Absorption

The availability of the corresponding absorption data (*i.e.* basic pharmacokinetic data) was reviewed, regardless of whether they were collected as stand alone studies or as part of other non-clinical projects. For 88 of 89 products the absorption related data was available. For all products containing the new active substance, the absorption data was presented.

A generic drug is considered bioequivalent to an innovator, if it exhibits the same rate and extent of absorption of the active substance. For 25 biosimilars out of 26 the comparability of the PK data to the originator was the subject of the EPARs. For one biosimilar Ontruzant, no information was given in EPAR. Reflecting the importance of PK data for biosimilars, the reason may not really be the general lack of PK data in the applicant's dossier, but rather the disregard for the issue by the EPAR rapporteurs. This assumption can be verified by reviewing the application documents, which is outside the scope of this thesis.

The information on bioavailability and /or $T_{max}$ is required if the route of administration is other then intravenous. In the subcutaneous (or intramuscular) application route, it would be expected that in addition to the pharmacokinetic basic data, the data on bioavailability and $T_{max}$ would also be collected [20]. The extent of the PK data regarding the intended intravenous or subcutaneous route of administration was also analysed. There was no clear correlation between the collection of these parameters and the route of administration. There were several products with the IV route, for which the corresponding PK data was also collected in the subcutaneous application among other things. This may be data collected aiming to proof the similarity of various formulations, but this was not clearly defined in all cases. In addition, there were cases with the intended SC application route, for which no information was given whether bioavailability and/or $T_{max}$ would be investigated. The EPARs do not always contain clearly assigned descriptions of the details, but are rather general. This is probably due to the evaluative character of this document: EPAR is not a document

like a dossier, which is a primary source of information on the drug product and does not necessarily contain the detailed information about the medicinal product. For this reason, the evaluation of these correlations would not lead to plausible conclusions. It may be concluded, that the EPARs do not carry all information for this kind of analysis and here is needed more detailed and structured data source for a comprehensive data analysis.

### 3.2.2 Studies on Distribution

Distribution studies were conducted only in 28 products. These were products containing a new active substance, *i.e.* no drug distribution related data were collected for biosimilar products.

Only for 13 of these products, study was conducted with a radiolabeled active substance, which is common for distribution studies on small molecules. Four products contained drug conjugates. Thus, the use of this method could be related to the conjugate. There were also only nine active substances, which clearly used the radiolabeled mAbs for distribution studies. In principle, also for other products, for which *in vivo* distribution has been tested, the use of the radioactive labelled active substances cannot be excluded, as there is no clear statement in EPARs.

In one quarter of the EPARS of the new active substances and in one half of the EPARS of the biosimilars, it was clearly indicated that the distribution studies were intentionally not performed.

The proportion of EPARs, in which no information is given about the availability of distribution studies, is high in both product groups: 30 % for the new molecules and 46 % for generic antibodies.

### 3.2.3 Studies on Metabolism

The expected metabolic pathway of biotechnology-derived pharmaceuticals, particularly for monoclonal antibodies, is degradation to small peptides and individual amino acids. Therefore, the classical biotransformation studies as performed for pharmaceuticals are not required.

The evaluation of 89 EPARs containing mAbs as an active substance shows that for the majority of products (71% of a new active substance and 58% of biosimilars), the studies on metabolism were omitted. The proportion of EPARs, that did not address this issue, was also considerable (particularly for biosimilars - 42 %, and 14 % for new active substances).

The availability of data on metabolism was rather an exception: there were only nine such products with a new active substance. Six of them (Adcetris, Besponsa, Cimzia (PEG), Kadcyla, Mylotarg and Scintimun) contain the mAb-conjugate or a radioactive labelled antibody as an active substance, therefore the presence of studies on metabolism for these products would also be expected.

For three products (Prolia, RoActemra and Xgeva), in the presented metabolism studies, the protective influence of the neonatal receptor FcRn from lysosomal degradation through binding to the Fc region of the antibody was evaluated. These studies were performed for other products (Gazyvaro, Hemlibra, Nivolumab BMS, Opdivo, Poteligeo, Praluent) as well. But, in these cases the studies are not declared as metabolism studies and they were

presented in different parts of EPARs. For the products Cablivi, Dupixent and Imfinzi the engineered mutations of Fc regions made this evaluation redundant. For other product sparse or no information was given.

EPARs do not always follow an uniform order of information presentation, as in this case. Therefore, the quantitative evaluation of presence or absence of the studies only on the basis of the mention in EPARs without additional content-related assessment may result in a not representative picture.

In summary, it may be stated that no metabolism studies were performed for only mAb containing products as those are not required according to the ICH-S6 guideline.

### 3.2.4 Studies on Excretion

The lack of excretion studies is in accordance with ICH-S6 guideline in general. Therefore, the excretion studies were infrequent: only for 12 products containing the new active substance the excretion studies were carried out. Five of them (Adcetris, Besponsa, Cimzia (PEG), Kadcyla and Mylotarg) contain the mAb-conjugate and one product Scintimun contains a radioactive labelled antibody as an active substance. Because of its “small molecule or radioactive compartments”, which have a great potential to ”off-target” toxicity, the presence of the assessment of excretion for these products would also be expected.

According to EPARs of the products Blincyto, Cosentyx, Praxbind, Prolia, RoActemra, Xgeva (6 products containing only mAb), the excretion studies were conducted for these products.

For monoclonal antibodies the renal excretion is negligible due to its molecular size. But, for Antibodies of lower molecular weight the renal excretory route can be relevant. As for the products Blincyto (molecular weight 54.1) and Praxbind (molecular weight < 60kDa) the role of renal elimination was investigated using bilaterally nephrectomised mice and rats.

Other products have molecular weight between 147-150 kDa, which is common for monoclonal antibodies (see EPARs of Cosentyx, Prolia, RoActemra, Xgeva). The results of studies presented in EPARs conform the expected elimination behaviour of IgG, which undergo little or no renal or biliary elimination, but are mainly catabolised by proteolysis via the reticuloendothelial system. These data appear to be redundant and therefore the conduct of these studies could be omitted. However, the rapporteurs made no respective objections as no additional resources (animals) were used to obtain the data. But the data were collected as an additional parameter of the PK study, which was conducted anyway.

### 3.2.5 Studies on Immunogenicity/ Antigenicity

The humanized or human antibodies are immunogenic in animals per se and cause the formation of drug-associated antibodies (also called anti-drug antibody - ADA or anti-therapeutic antibody - ATA) that are able to reduce the circulating drug levels during the multiple dose application. The presence of ADA should be taken into account as factor influencing the pharmacokinetic and toxicokinetic parameters.

In view of the importance of the ADA assessment, the proportion of the therapeutic antibodies, for which immunogenicity potential has been investigated, is high: the immunogenicity was determined for 75 of 89 products as part of pharmacokinetic, acute, repeat dose or developmental toxicity pre-clinical studies (Table 13, Annex 7). 13 EPARs did

not contain the information on ADA formation in test animals: six products with a new active substance and seven biosimilars, whereas among biosimilars, only two active substances were represented (dalimumab and trastuzumab).

Only for one product Empliciti, it was stated that the lack of immunogenicity data was not to be relevant for the interpretation of the non-clinical results: there were no explanation for this provided in EPAR. Taking into account, that there were no cross reactive species for Empliciti found, and as a consequence of this, the repeat dose toxicity studies were omitted, it may be concluded, the data on ADA formation is obsolete for this product.

### 3.2.6 Pharmacokinetic Drug-Drug Interaction Studies

It is generally assumed that the monoclonal antibodies are primarily metabolised through catabolic pathways and do not involve cytochrome P450 (CYP450)-mediated metabolism or interaction with cell membrane transporters. The pharmacokinetic interaction potential with small molecule drugs is limited.

Some drugs with cytotoxic or immunomodulatory properties may have an effect on expression of hepatic CYP450 enzymes or on transporters through cytokine dependent modulation. According to reviewed EPARs these concerns did not lead to the need for a non-clinical evaluation, but were followed up in further clinical pharmacokinetic studies or addressed in SmPC/RMP (Example cases: Ajovy, Arzerra, Dupixent, Kevzara, Keytruda, Skyrizi, RoActemra, Sylvant).

Therefore, in a number of cases (46%), no non-clinical *in vivo* drug-drug interaction studies were conducted. The justification of omission was, as mentioned before, a low potential of interference with the CYP-system. For the product Repatha the evidence from the tissue-cross reactivity study was additionally given as a reason to waive the PK drug-drug interaction studies. The proportion of EPARs, which did not give any information on efforts regarding PK drug-drug interaction, is remarkably large in both – new active substance and biosimilar product groups. One explanation of this may be that the lack of studies was acceptable for reviewers and therefore, no comments were made by CHMP.

However, for 6 products PK drug-drug interaction was assessed. Three of them are products containing mAb-drug conjugates (Adcetris, Kadcyla, Mylotarg), for which, probably, the expectation of conjugate metabolisms justifies the necessity of these studies. For two further products containing mAb-drug conjugates (Cimzia and Besponsa), the EPARs do not contain any specifications regarding PK-DDI. For the product Cimzia with PEG conjugate the data on drug-drug interaction are not crucial since the nature of PEG metabolism is well understood and it is not expected to interfere with other possible CYP metabolised drugs. But, for the product Besponsa, the conjugate is expected to undergo metabolism in the liver. The lacking information on drug-drug interaction should be questioned as part of the assessment.

For the products Cinqaero and Ilumetri, *in vitro* studies in human hepatocytes to investigate potential direct cytotoxicity and the effect on expression of selected CYP450 enzymes were conducted. For the product Perjeta pharmacokinetic drug-drug interactions with a possible concomitant monoclonal antibody (but not possible concomitant small molecular drugs) were investigated in a single-dose pharmacokinetic study in SD rats.

### 3.2.7 Species used in Pharmacokinetic Studies

The use of relevant species seems to be essential for the evaluation of pharmacokinetic issues such as toxicology. In all cases whenever the relevant species was identified, they were used for obtaining pharmacokinetic data, except of one case Zessly containing biosimilar infliximab. For this product, it was declared that the Chimpanzee is the only relevant species, but for PK data evaluation, rats were used. This is in line with 3Rs principles, according to this, the aim should be to reduce the use of animals, in particular NHPs, whenever it is possible. In this active substance, literature evidence suggested, that, for the characterization of the FcRn mediated non-target related clearance, *in vivo* studies in rat were suitable.

In 33 cases, additional to the relevant species, non-relevant rodent and non-rodent species or animal model systems were used. The non-relevant species used in pharmacokinetic studies were in vast majority of cases rodents (mice and rats, Table 15, Annex 6).
The supplemental use of less elaborate rodent species, which replace the active NHP, if it doesn't affect the significance of the study, is compliant with the 3Rs principles.

There were differences in the testing strategy of the products containing the same biosimilar active substance trastuzumab: for the products Herzuma and Kanjinti the PK was assessed in a multiple-dose toxicology study in cynomolgus monkey, for the product Ogivri in a single-dose study in cynomolgus, for the product Trazimera in male CD-1 mice and the EPAR of the product Ontruzant did not contain any data on PK studies. This indicates, that the use of case by case principle is applicable during the development of a dossier even if the previous experiences with same biosimilar active substance are available.

## 3.3 Toxicology

### 3.3.1 Single Dose Toxicity Studies

According to the ICH-S6 guideline, single dose toxicity studies are useful to generate information on dose-response relationships, but, when the acute toxicity information is available from any study, separate single-dose studies are not required [17].

The results of the EPAR-analysis were consistent with the requirements of the guidelines: there were several products (43%) for which the separate single dose toxicity study was performed. But there were also number of products, for which the single dose toxicity studies were not carried out and the acute toxicity data was obtained from other non-clinical studies. The EPARs of 11 products were not evaluable in this respect. Mostly only the information, that single dose toxicity studies were not performed, was given without any additional explanation. However, in case of the product Kevzara, the lack of these studies was explained by the fact, that "findings of acute toxicity are rare for monoclonal antibodies”. But this explanation is not applicable to other products.

For biosimilars the focus is on the evidence of similarity to the originators and not on exploring the unknown properties of the drug. Since the dose-response relationships of the active substance is known for the originator, it is not surprising, that the acute toxicity studies were not applicable for the vast majority (88%) of products.

### 3.3.2 Repeat Dose Toxicity Studies

For nearly all products (97 %) with just a few exceptions (3 cases in total), the extensive repeat dose toxicity studies were conducted implying that for 86 products, repeat dose toxicity studies at least in one species were conducted. For 3 products (Empliciti, Flixabi and Removab) the reason given for the lack of studies was, that no suitable species was found, in which the test substance would be pharmacologically active and no surrogate model was available as well. For products with a new active substance (Empliciti and Removab), single dose toxicity study results were available at least, even if in a non-active species.
For biosimilar Flixabi, there were no acute toxicity studies carried out. But there were 15 products, which were lacking an active species (the discussion how these species were replaced see in 3.3.5).

### 3.3.3 Duration of longest Repeat Dose Toxicity Studies

The duration of the longest pivotal repeat dose toxicity studies was evaluated. Results are presented in Table 20 and Annex 8.

The evaluation shows, that the majority of the longest pivotal chronic toxicity studies with products containing a new active substance were 26 weeks in duration (37 cases out of 61 including some borderline cases with study duration from 21 to 30 weeks). Less frequently studies were 39 weeks in duration (9 cases including one borderline case with study duration of 40 weeks). Only one study (product Xgeva) lasts 52 weeks. The ICH-S6 guideline generally recommends 6-month studies but does not exclude a deviating duration. However, the most important imperative of this guideline is, that the duration of long-term toxicity studies should be scientifically justified. EPARs do not contain such justifications for the determination of the specific duration of studies. One possible explanation for the extension of toxicity study-duration to 9 month or longer is, that the treatment regimen in non-clinical toxicity studies was adapted to the intended treatment regimen in humans.

In some cases, the duration of the longest repeat dose toxicity studies was 13 weeks (5 cases including one borderline case with study duration of 12 weeks). All these products (Bavencio, Blincyto, Imfinzi, Mylotarg, Nivolumab BMS, Opdivo) are antineoplastic agents intended to treat advanced cancer. According to ICH-S9 guideline, for most pharmaceuticals intended for the treatment of patients with advanced cancer, non-clinical studies of 3 months duration are considered sufficient to support marketing. The results are in line with this statement.

For the product Qarziba, the longest repeat dose toxicity study lasted 7 weeks. Its administration scheme mimicked exactly the clinical administration scheme. Qarziba is an orphan medicinal product addressing the medical need of high-risk neuroblastoma patients. The uncertainties related to the efficacy and safety of the product will be further studied with the conduct of a drug exposure registry as outlined in EPAR of this product.

In one product, Darzalex, the longest toxicity study lasted only 6 weeks. This is an antineoplastic agent, for which orphan designations and accelerated assessment procedure were applied.

For the products Aimovig, Lemtrada, Scintimun and Unituxin the duration of repeat dose toxicity studies was 4 weeks. The explanation for the exceptionally short study duration of

the products with a new active substance was as follows: For the product Aimovig the theoretical safety concerns were given particularly in cases of ischemia. Nevertheless, further non-clinical studies were seen as unnecessary. The product Lemtrada is an immunosuppressant and the treatment is associated with the risk of severe infections. Long-term application would only be feasible under co-administration of antibiotics, which may be interfering with the evaluation of the medicine's toxicology profile. Therefore, the long-term studies would not be useful here. For the product Unituxin, there were no active species identified. The 4-week toxicity study conducted in rats was not sufficient especially for the complete understanding of adverse effects on peripheral nerves. To clarify this issue, further non-clinical investigations after authorisation of the product are planned. The applicant's proposal to conduct the juvenile toxicity study in monkeys of 5 months duration was not supported by CHMP, but the performance of one additional study in the same species along with monitoring in clinical use. Scintimun is intended for single diagnostic use. Therefore, long-term repeat dose toxicity studies were considered unnecessary.

For medicinal products containing antibody directed against non-human antigens (Praxbind - 2 weeks, Zinplava - 3 weeks), the findings of repeat dose toxicity studies are not crucial. More particularly for the product Zinplava, since there was no species in which target-mediated toxicity can be investigated. Therefore, the short duration of toxicity studies for these products was probably well founded.

For biosimilar products the toxicity studies of 4-week duration was common (19 products out of 25 including one product with 5 weeks). Studies of two weeks duration were performed in three cases (Inflectra, Remsima and Zessly), which all contain the same active substance infliximab. It should be mentioned here, that for 1 further biosimilar product Flixabi containing infliximab, there were no toxicity studies carried out. This fact indicates, that the case by case approach will be applied even in case of the same active substance.

### 3.3.4 Recovery Monitoring Period of Longest Repeat Dose Toxicity Studies

A recovery monitoring period to demonstrate reversibility undesired toxic effects were included in study designs of 43 products with a new active substance (68%) and two biosimilar products (8%). The duration of the recovery period of repeat dose toxicity studies was chosen individually for each product and covered the range from 2 to 37 weeks. In this range, the studies were distributed uniformly without forming a group of a certain duration with higher frequency.

For 18 products with a new active substance out of 63 (29%) and for 23 biosimilars out of 26 (88%) there was no information given in EPARs on the status of recovery period of the longest toxicity studies. For about eight products with a new active substance the recovery period was included in designs of other toxicity studies of shorter duration.

### 3.3.5 Species Used in Single and Repeat Dose Toxicity Studies

The use of pharmacologically reactive species in the toxicological studies is crucial for the validity of the study. This requirement of the ICH-S6 guideline is well reflected in the results of EPAR assessments regarding species used in toxicity studies: in all cases, when the relevant species was identified, they were used in toxicity studies. Such cases were 56 products out of 63 with the new active substance and 18 biosimilar products (Table 22).

Non-active species or surrogate systems additionally to the active species were used in 9 products with the new active substance and 1 biosimilar. For 15 products (7 with the new active substance and 8 biosimilars) there were no active species identified. Therefore, in *in vivo* toxicity studies only non-relevant species or surrogate test models were used, apart of the product Flixabi, for which no *in vivo* toxicity studies were performed.

Usually the toxicity evaluation is performed in two species - rodents and non-rodents. However, if two relevant species are not available, according to the ICH-S6 guideline, the use of only one species is acceptable. The analysis of the EPARs showed, that for the majority of products (64 cases, 72 %), it was not possible to use more than one species for the toxicology studies. In 55 from 64 cases, the single specie used was cynomolgus (Table 24).

If taking into account only the product group with the new active substance, there were 40 products with the new active substance (63%), for which the toxicity studies were performed only in one species. Two species (rodent and non-rodent) in line with guidelines for toxicity testing were used in 19 cases (Table 24), of which, only for 8 cases, both species used were active for the test products (Annex 9, extracted data are not shown).
In 4 additional cases two species were either two NHPs (Darzalex: cynomolgus and chimpanzee; Trogarzo: cynomolgus and rhesus) or NHP and non-rodent (Crysvita and Entyvio: cynomolgus and rabbit).

The proportion of non-human primates used in toxicity studies was high, particularly for products containing new active substances – almost all products except 2 products (Ultomiris and Unituxin) included studies with NHPs in the toxicity program, even if these species were not relevant. The products Besponsa, Dupixent, Empliciti, Mylotarg and Removab are such cases, in which the used NHPs were not pharmacologically active. Since the products Besponsa and Mylotarg contain the mAb-drug conjugates, the use of cynomolgus and chimpanzee as well as the use of other rodent and non-rodent species was reasonable for target specific toxicity testing. In the product Dupixent, a species-specific surrogate mAb was used for toxicity testing in cynomolgus. For the product Empliciti (in rhesus) and for the product Removab (in cynomolgus), single dose off-target toxicity studies were performed.

The cynomolgus was used for toxicity testing in 94 % of products with a new active substance, while chimpanzee, rhesus and marmoset were used only in isolated cases.
The other used non-rodents (3x rabbit and 1x dog) obviously took over a subordinate role for testing the toxicity of therapeutic antibodies. Rats were the most used rodents in toxicity studies and were used for 17 products (as active species in 5 products). Rat was followed by mouse (10x), guinea pig (3x) and hamster (1x, see Table 22).

The use of NHPs for toxicological testing in biosimilars was remarkably high in general: for 21 products out of 26 the repeat-dose toxicity studies were performed in cynomolgus - in 17 cases of them cynomolgus was an active species. Rats were used in 3 cases (Inflectra, Remsima, Zessly) and mouse in 1 case (Trazimera); this case will be discussed below.

It has already been discussed in numerous publications that *in vivo* studies in general are not essential for the proof of biosimilarity and it is possible to develop a biosimilar with *in vitro* data alone [42; 43; 44]. As advised in the *Guideline on similar biological medicinal products containing monoclonal antibodies* [40], the conduct of repeated dose toxicity studies in NHPs

is usually not recommended, if an *in vivo* study is necessary. There were only 4 EPARs of the biosimilar products Amgevita, Hulio, Idacio and Kromeya assessing the redundancy of cynomolgus study for toxicity testing, stating, that the toxicity studies should not have been performed, because non-human primate toxicity studies are considered of limited value for biosimilarity evaluation and such studies are not a formal request by EMA. This statement was obviously not applied for other products, because as it was found in this review, for 21 out of 26 biosimilar products the *in vivo* studies in NHP cynomolgus were conducted. It may be, that these studies were performed for global registration purposes. However, the appropriate highlighting for these studies in EPARs is missing. For the product Flixabi, no toxicity studies were performed. The justification for this given in the EPAR was not the requirements of guidelines, as it would be expected, but the lack of the relevant animal models.

The biosimilar products, for which no active species could be identified, have been observed more closely, as noted in EPARs. 3 biosimilars (Flixabi, Inflectra, Remsima) lacking an active species contained the same active substance infliximab. An originator product is Remicade, for which one active species chimpanzee was identified and used in toxicity studies. There is also a biosimilar product Zessly that contains infliximab as an active substance, for which it is stated in EPAR, that the binding to the chimpanzee tissues has been detected. For Flixabi, no toxicity studies were conducted. For Inflectra and Remsima, the rat as non-relevant species was used in single- and repeat- dose toxicity studies. Toxicity (single and repeat dose) studies for Zessly were conducted in rats as well. Rat is not a relevant animal for infliximab, but, according to reported literatures (EPAR of Flixabi) the clearance mechanism of infliximab in rat is relevant and rat is a suitable species for the comparison of off-target toxicity profiles of two infliximab versions. But the “classical” target directed toxicity testing in relevant species (Chimpanzee) was omitted for infliximab containing products, which is in line with the 3Rs principles.

For 5 biosimilar products (Herzuma, Kanjinti, Ogivri, Ontruzant and Trazimera) containing trastuzumab as an active substance, no relevant species was reported in the EPARs as well. For the originator Herceptin two relevant species, rhesus and mouse were reported. For Trazimera, the repeat dose toxicity studies were conducted in mouse. But the species used in toxicology studies was cynomolgus for the products Herzuma, Kanjinti, Ogivri and Ontruzant. There is no explanation for this decision given in EPARs. It is not possible to assess whether this information is available in the applicant’s dossier. However, the use of NHP species for toxicity assessment of biosimilars is questionable, even if this species was not clearly biologically active. The information contained in the EPAR is therefore is incomplete with respect to this aspect.

## 3.4 Genotoxicity and Carcinogenicity Studies

### 3.4.1 Genotoxicity Studies

For 79 products, no dedicated non-clinical genotoxicity studies were performed and presented by the applicants: 56 of them were products with the new active substance and 23 products were biosimilars. From a high proportion of products lacking genotoxicity studies it can be concluded that the evaluation of the genotoxic potential was not

substantial for market authorisation for both product groups, which is in line with the ICH-S6 guideline.

The EPARs of 3 biosimilar products (Cyltezo, Idacio and Kromeya) contains no information, whether the studies were conducted. These products contain the same active substance adalimumab. The reference product Humira is approved since 2003 and 7 other biosimilar products with the same active substance have been approved during the last 10 years, which means, that the genotoxic risk of this substance is well characterized. For these 7 other products, the applicants did not perform any genotoxic studies. It can therefore be concluded that for these products, neither genotoxic studies were necessary nor these studies were performed.

Genotoxicity studies were carried out for products with potential for interaction with DNA or other chromosomal material: 5 products containing antibody-drug conjugates (Adcetris, Besponsa, Cimzia, Kadcyla and Mylotarg) and one (Scintimun) containing the radioactive Technetium (99mTc)-labelled monoclonal antibody besilesomab as an active substance.

Only one product Cinqaero contains the anti IL-5 mAb monoclonal antibody without any conjugates and additional properties. The Ames test and chromosomal aberration assay did not give any useful information for this product or for monoclonal antibodies in general, as reported by EPAR itself. These tests were considered redundant by the CHMP.

### 3.4.2 Carcinogenicity Studies

The analysis of the EPARs shows, that only for the product Cinqaero, containing the new active substance, it was reported, that the dedicated carcinogenicity risk assessment study had been carried out. For the product Repatha, the endpoints of the carcinogenicity study were incorporated in the classical repeat-dose toxicity studies of 6 months duration. The EPARs of four products (Cyltezo, Idacio, Kromeya and RoActemra) contains no information, whether the studies were conducted.

For 83 other products, which are the vast majority (93%), there were no carcinogenicity studies conducted.

According to reviewed EPARs the carcinogenicity study need was determined on the basis of the *Weight of the Evidence Approach* (Table 28, Annex 11). This approach means the carcinogenicity potential assessment based on all known criteria, which can influence this risk: mode of action, intended drug target and it's downstream pathway, it's pleiotropic effects or intended duration of treatment, as the use of medicine longer than 6 months carry a certain carcinogenicity risk *per se* [31]. But even in such cases the studies were not always carried out, if the assumed risk was classified as very low on the basis of literature review specifically addressing tumour promotion potential or on the basis of findings from other non-clinical studies (especially long term repeat dose toxicology studies). The target disease (*i.e.* advanced cancer), the already known risk or it's unpredictability were given as further possible justifications of absence of the studies as well. Even if an assessment of carcinogenicity was necessary when the product met certain conditions, the standard carcinogenicity assays in mice and rats were not feasible for most active substances, because they did not have biologic activity in rodents.

The absence of the carcinogenicity studies for all 26 biosimilar products was based on the

requirements of the appropriate guideline.

Cinqaero is the only product with a carcinogenicity study. According to EPAR the literature data suggested the relevance of IL-5 and eosinophils in tumour immune surveillance. This is relevant for this product since the active substance of Cinqaero - reslizumab - binds to IL-5 thereby preventing its binding to the IL-5 receptor and consequently reduces circulating and tissue eosinophils. A 6-month carcinogenicity study with reslizumab was performed in transgenic rasH2 mouse model for carcinogenicity testing, that carries the human prototype c-Ha-ras gene, as advised by FDA. The CHMP agreed with the adequacy of the design. It should be noted that mouse is not an active species for this product, but cynomolgus and rabbit. As stated in EPARs, the test was performed with reslizumab and not with a surrogate antibody that would be active in mouse.

In order to compare the arguments given in two different assessment procedures, in the following a citation from the EPAR of the product Aimovig is presented. This product is an analgesic intended for use in chronic disease – migraine - and the expectation of continuous use of the product for > 6 months is given. "*For small molecule therapeutics, carcinogenicity assays in mice and rats would be the standard approach to assess the risk of carcinogenesis. These assays are not feasible for {erenumab} because it is a monoclonal antibody that does not have biologic activity in rodents"* (EPAR Aimovig). The active species for Aimovig was cynomolgus.

The well-founded reasons are not given in EPARs, why in one case a non-active animal was suitable as a test system (Cinqaero, rasH2 mouse) and - in another case is not (Aimovig).

If no carcinogenicity studies were performed, the concerns on the carcinogenic potential were adequately addressed in the Risk Management Plan and/or SmPC.

## 3.5 Reproductive and Developmental Toxicity

### 3.5.1 Fertility and Early Embryonic Development (FEED) Studies

A number of products, for which fertility and early embryonic Development (FEED) studies were carried out, was evaluated.

For the products containing a new active substance dedicated FEED studies were performed for approximately one third of the products (35%), for another third of this product group (40%) the endpoints of the fertility (male or female) or early embryonic development issues were incorporated in the repeat dose toxicity studies. In 11 cases (17 %) data on fertility and early development were intentionally omitted. In 5 cases (8%) it was not clearly specified, if the FEED studies were carried out for the products. In 3 cases (Ilaris, Prolia, Zinbryta) the FEED-relevant data were obtained from repeat dose toxicity studies in addition to stand-alone studies (Annex 12).

For products containing a new active substance it was analysed whether the test species were used for dedicated FEED studies. These studies were only in a part of cases (10 cases of 22) conducted in pharmacologically relevant species. In 12 cases (three cases of them without identified active species) mouse or rat surrogate model were used. The data is shown in Annex 12. This is in line with the 3Rs principles, which aim to reduce the use of NHP in drug development.

The lack of FEED studies in 11 cases with a new active substances was justified as follows: for 5 medicinal products (Keytruda, Lartruvo, Nivolumab BMS, Portrazza, Unituxin) intended to treat patients with advanced cancer this is acceptable [24]; in 2 cases (Cyramza, Kadcyla) the risk to have potential impact on fertility was expected. For further 4 cases (Empliciti, Darzalex, Arzerra, Trogarzo) the low expected risk and target population beyond reproductive age were given as a reason for the omission of FEED studies. One medicinal product (Hemlibra) was intended to be co-administered in the clinical setting with chlorambucil, which has induced sterility in men and women. Therefore, the properties of the Hemlibra´s active substance would be irrelevant.

For a vast majority of biosimilar products (85%) the FEED studies were not performed. For 1 product (Idacio) the appropriate information was not available in the EPAR. In all cases it was stated that such studies are not required for a similar biological product [40]. However, there were 3 biosimilar products (Blitzima, Ritemvia and Truxima) with the same active substance rituximab, for which some fertility relevant endpoints were evaluated in 8 weeks repeat-dose toxicity studies.

### 3.5.2 Embryo-foetal Development Studies

Fc fragment of IgG, on the one hand extend the plasma half-life of therapeutic mAbs, but, on the other hand is responsible for FcRn-mediated placental transfer, resulting in foetal exposure to the active substance. Therefore, the conduct of the pre-clinical embryo-foetal and pre- and postnatal development studies are reasonable according to the ICH-S6 guideline.

In 30 cases (48%) for products containing a new active substance, dedicated embryo-foetal development (EFD) studies were performed. In 2 cases (Praluent and Simponi), the additionally performed extended pre- and postnatal development (ePPND) studies contributed further information on the embryo-foetal impact of the product (the data is presented in Annex 13). In 15 cases (24%) the FED studies were fully integrated in the ePPND. For 18 products the applicants provided no EFD relevant data. For 1 product (biosimilar Idacio), no information was given on the availability of FED data. In 1 case (Yervoy) the ePPND study was not yet presented, but was ongoing.

Considering all studies (EFD+ePPND) for the products containing a new active substance (45 products) pharmacologically active species were used in 35 cases (78%). Cynomolgus was used in 29 cases out of them. In 4 cases, mouse/mouse homolog, rabbit or rat were used in addition to cynomolgus. In 1 case Ilaris, marmoset as an active species and additionally mouse surrogate mAb were used. Rabbit were used in 5 cases alone or in addition to cynomolgus (Kyntheum) and rat in 2 cases. In 10 cases the studies were carried out in non-active species. Only for 3 products of them (Dupixent, Besponsa and Ultomiris) there was no active species identified. In 3 cases (Blincyto, Cimzia and Mylotarg), the active species were replaced by rats. For 4 products (Blincyto, Cosentyx, Lartruvo and Lemtrada) mouse and homologous murine antibodies were used.

The lack of EFD studies in 29 % of products containing a new active substance (18 cases) is high. In most cases studies were not required when the mode of action was associated with known (high or low) developmental risk. In other cases, the reasons were intended clinical

setting with a potential teratogen, absence of relevant species, age or sex of target population (male, children under 10, females beyond reproductive age). The intended indication advanced cancer was accounted for most of the cases. In some individual cases, no justification was given. In 2 cases (Yervoy and Zinplava) the ePPND study was ongoing.

In the EPARs of 25 biosimilar products it was clearly stated that no FED studies were carried out and it was also explained that these studies are not required for biosimilars. For one product (Idacio) no information was given about the availability of FED data.

### 3.5.3 Pre- and Postnatal Development (PPND) Studies

In 19 cases (30%) of products containing a new active substance the dedicated pre- and postnatal development (PPND) studies were performed. In one case (Simponi) the data from an additionally performed extended pre- and postnatal development (ePPND) study was used to obtain more information (Annex 13). In 16 cases (25%) the PPND were fully integrated in the ePPND. For 23 products the applicants provided no data on pre- and postnatal development. For five products the EPARs contain no information on the availability of a PPND study. In one case (Yervoy) the ePPND study was ongoing.

In the majority of cases (29 out of 35 cases in total including 19 PPND and 16 ePPND) pre- and postnatal development studies were conducted using active species. In 4 cases (Cimzia, Cosentyx, Ilaris and Lemtrada) the active NHP species (cynomolgus and marmoset) were replaced by mouse surrogate model (3x) and rat (1x). This is in line with the 3Rs principles. Despite that no active species was available for 2 products the studies were performed in cynomolgus (Dupixent) and in mouse homolog model (Ultomiris).

The justification for omission of PPND studies was individual for each product. In some cases (9 out of 23 cases) the risk for foetus was already identified. Therefore, the studies would be pointless. For four products (Arzerra, Darzalex, Hemlibra and Scintimun) the risk on foetus was negligible since the target population was either post-reproductive age women or male (haemophilia A patients). The intended clinical medication setting with other teratogen or contraindicated therapeutic or advanced cancer was the explanation for absence of PPND studies in some further cases (Empliciti, Qarziba beta Apeiron, Perjeta, Poteligeo). In some cases (Cablivi, Lartruvo, Praxbind, Removab, Scintimun) no information on the PPND data was given in EPAR. But, for example, the cases Praxbind and Scintimun do not raise questions, because they are directed at a foreign target and no reproductive toxicity studies are expected for such products according to the ICH-S6 guideline.

In the EPARs of 25 biosimilar products it was clearly stated that no PPND studies were carried out and it was also explained that these studies are not required for biosimilars. For one product (Idacio) no information was given about the availability of PPND relevant data. FEED and EFD studies are also missing for this product.

## 3.6 Juvenile Animal Studies (JAS)

Juvenile animal studies are not mandatory for the product authorisation. But, if such studies are considered necessary, they should be available before the initiation of paediatric clinical studies. The corresponding data in EPARs were not complete for all products probably for this reason: only for 9 out of 63 products containing a new active substance specific juvenile

animal studies were conducted (in one case Ocrevus the study was planned). In three cases (Blincyto, Ilaris and Kevzara) the active species NHP (chimpanzee, marmoset, cynomolgus) were replaced by mouse surrogate model. In other cases, the cynomolgus and rat as an active species alone or in combination with other testing systems were used (Annex 14). The data on juvenile toxicity was obtained as part of other studies in nine cases. These studies were ePPND, EFPD, repeat dose toxicity studies, primary PD or safety pharmacology studies (Annex 14).

At the time of application the JAS were intentionally not conducted for 15 products. The Information on juvenile animal studies was not present in 30 EPARs cases.

Since the obligation to conduct the juvenile animal study is defined in the paediatric investigational plan (PIP) by PDCO the status of PIP-completeness at the time point of application (as stated in EPAR) was also analysed. For five products (Cimzia, Removab, RoActemra, Scintimun and Simponi) no information on PIP was available in EPARs. All these products were authorised in 2009.

Only for 17 cases out of 63 the PIP was completed at the time point of application and in all of these cases PDCO agreed class- / product specific waiver or no need for non-clinical juvenile data. These 17 products correspond to the 14 "no data" products and 3 "not performed" products in Table 32.

For the remaining 41 products the PIP was not yet completed at the time point of applications assessment. For 16 of these products the paediatric waiver was requested, as stated in EPARs. Two products (Taltz and Trogarzo) were not intended for use in paediatric population. For two other products (Nivolumab BMS and Xgeva) the class waiver should be applicable. For three products paediatric subset was covered by waiver. But this does not indicate that these products are exempt from the requirement to perform juvenile animal studies. For about 17 products it would be expected that the JUS studies would be required after PDCO decision. The analysis of the corresponding PDCO decisions available at a later date was included in the scope of this analysis.

For biosimilar products the requirements for paediatric population and therefore juvenile animal studies are not applicable. The juvenile animal studies or - in two cases - the information on the studies) are absent in all EPARs of this product group.

## 3.7 Local Tolerance

The ICH-S6 guideline points out the needs of local tolerance data for biotechnological drugs. In accordance with this requirement the corresponding data were not available in a few cases only: in four EPARs of products with a new active substance (total number 63) and 5 EPARs of biosimilars (total number 26). The complete lack of data/studies in the application dossier would be recognized and not tolerated by CHMP. Therefore, this might be rather due to incomplete data presentation in EPARs. The limited presentation of the data in the EPARs is further illustrated by the fact, that for individual cases (Gazyvaro, Kadcyla, Qarziba, Taltz, Unituxin, Dupixent, Emgality and Takhzyro) the corresponding information on local tolerance was not clearly given. However, it should be concluded from the brief mention that the injection site was observed.

In most cases (63% of products with a new active substance and 69% of biosimilars) the

injection site was observed in repeat dose or other toxicity studies. Nevertheless, the proportion of dedicated studies was high (30 %) in products with the new active substance.

The use of rabbit (or in individual cases of rat) for local tolerance studies seems to be a suitable for the evaluation of the irritant potential of the test molecule even if the biopharmaceutical may not cross-react with this species: from 15 cases, which used rabbit as test animal it was not pharmacologically relevant in 12 cases. In two cases (Cimzia and Cinqaero) rat was used, although the test biopharmaceuticals did not cross-react with the rat target.

## 3.8 Other Toxicity Studies

### 3.8.1 Immunotoxicity Studies

The data on the immunotoxic potential of biopharmaceuticals containing monoclonal antibodies as an active substance were presented in 27 EPARs. Only three of them were obtained in dedicated studies. For 24 products, the immunotoxicity relevant endpoints were incorporated in repeat-dose toxicity or in developmental toxicity studies.

The approaches of immunogenicity testing were individual for each product and within the scope of the ICH-S8 guideline, although this guideline is not directly binding for monoclonals. The following approaches were used: peripheral blood immunophenotyping, haematology, organ weight and histopathology for lymphoid tissue (thymus, spleen and lymph node), serum cytokine or plasma complement levels and infection risk assessment (data not shown). Even if the effects of antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) alter immunological resistance to infectious diseases, these types of tests are not considered immunotoxicity studies.

For four products only it was specified that the immunotoxicity investigations were intentionally not performed. The EPARs of 58 products contain no information and it is not possible to interpret, if the immunotoxicity data were intentionally not obtained or if it is just an inaccurate representation of the data in EPAR, which is available in the application dossier.

### 3.8.2 Human Tissue Cross-Reactivity Studies

The importance of the cross-reactivity studies using a range of human tissues, which are predictive for any unintentional reactivity and/or cytotoxicity towards human tissues distinct from the intended target, is described in the ICH-S6 guideline.

The analysis of EPARs of biopharmaceuticals containing monoclonal antibodies has shown that the tissue cross reactivity studies directed to the human tissues were performed in 63 out of 89 products. For the remaining 26 products, the EPARs provide no information whether these studies were carried out.

The proportion of cross-reactivity studies in products containing new active substances was higher than for biosimilars (81% *vs*. 46%). These studies have overlapping areas with several pre-clinical studies and provide evidence of the off-target effects, possibly with toxic outcomes. This was probably a reason why in the EPARs, which were supposed to have a uniform structure, these studies were assigned to different chapters: primary and secondary

PD, safety pharmacology or in most cases to the so-called "other toxicity studies" (The data is provided in Annex 15).

### 3.8.3 Studies on Impurities

According to the ICH-S6 and ICH-M4 guidelines for biotechnology-derived products, non-clinical toxicology assessments are not required, but the description of the purification processes to remove impurities should be provided. The extensive specifications of the test material (including investigations of the product and process related impurities) are provided in the quality section of the EPARs of all products. This is in line with the ICH-S6 guideline and complemented the lack of information on impurity studies in appropriate non-clinical sections. However, only for 10 products, the information on the lack of studies was provided and for the rest 74 products, it was not. Interestingly, for the products Cinqaero and Removab, the reason for omission of the impurities studies was the lack of the animal model, which allows toxicological testing of the pharmacological activity.

Nevertheless, for 5 products, the assessment of toxic potential of impurities was carried out. The studies on impurities are not described in detail in the EPARs: in 2 cases (Cyltezo and Herzuma), the toxicological risk assessment on process related impurities were performed, in 1 case (Poteligeo) comparability between the drug substance lots and the drug product lots was verified. In 1 case (Scintimun), the test for potentially genotoxic impurities was carried out. In the case of Stelara, it was only stated “there are no toxicological issues arising from the impurities”.

In single products (Adcetris, Taltz -products with a new active substance; Hulio, Kromeya, Zirabev -biosimilars), the presence of appropriate non-clinical studies/assessment of the impact of products and process related impurities on safety are reported in the quality sections of the EPARs. For the product Taltz, it is stated in the quality section of its EPAR, “*a comprehensive toxicological evaluation … was provided*”. This information is conflicting with the information given in the section of non-clinical assessment: “*the Applicant considered that specific studies to assess … impurities were not warranted, which was considered acceptable by the CHMP*”. In other cases, the non-clinical section did not contain any information on impurity studies.

### 3.8.4 Studies not Analysed

The studies on Dependence, Metabolites and Phototoxicity were not evaluated since these studies are irrelevant for the products containing monoclonals as an active substance [5] and the presence of these studies in a pre-clinical program is not expected.

## 3.9 Ecotoxicity/Environmental Risk Assessment

For all products containing protein-drug conjugates (Besponsa, Mylotarg, Adcetris, Kadcyla and Cimzia), and for the product Scintimun containing the radioactive Technetium (99mTc)-labelled monoclonal antibody besilesomab, standard ecotoxicity studies were performed. For all other products (with a new active substance and with biosimilars), no environmental risk assessment was performed. This is in line with the specific *Guideline on the environmental risk assessment of medicinal products for human use* [37].

# 4 Conclusion and Summary

Although the non-clinical testing program of monoclonal antibodies takes into consideration all sections of the pre-clinical development program for conventional small molecules, the non-clinical testing program needs to be specifically adapted for mAb therapeutics. This flexibility is also recommended by the ICH-S6 guideline, applying a case by case, science-based assessment approach.

The establishment of relevant animal models to assess pre-clinical safety and to establish FIH dosing range is a key element for the preclinical programme of the monoclonals. The identification of relevant species was supported by a number of different assays in almost all cases. There are some mAb therapeutics, for which no or more than one cross-reactive animal species exist. But more common are products with only one pharmacologically active species (about 2/3 of cases). The non-human primate (NHP) cynomolgus monkey (*Macaca fascicularis*) was an active species in almost all cases, with only few exceptions.

The comprehensive primary pharmacodynamic *in vivo and/or in vitro* studies were conducted for all reviewed products. *In vivo* studies for the detection of pharmacological effects in products containing a new active substance were conducted in well-adjusted model systems. Active species were used for the purpose of validating the reactivity of these species only.

Although *in vivo* studies on primary pharmacodynamic issues are not mandatory for biosimilar products, for more than half of the products (58%) the *in vivo* studies were conducted.

Secondary pharmacodynamic studies were performed for more than half (60%) of the products with the new active substance. The common reasons for omission include strong specificity of the antibodies to the respective targets, which indicates the low potential for unintended activity, the absence of an adequate test system or already existing knowledge of the pleiotropic activities of the antibody.

Although dedicated safety pharmacology studies were conducted in part of the cases, the incorporation of safety pharmacology endpoints in repeat dose toxicity studies was common for the majority of products, particularly for biosimilars, which is in line with the 3Rs principles.

Basic pharmacokinetic data were available for all products. Nevertheless, PK data do not comprise the tests covering absorption, distribution, metabolism and excretion (ADME), which would be common for small molecules. The data on distribution, metabolism and excretion are presented in 31%, 10% and 13 % of the products, respectively. There is a high percentage of EPARs not containing any information on the availability of these studies (35%, 22%, 22% respectively), making these interpretations less significant.

The use of relevant species was essential for the evaluation of pharmacokinetic issues. In all cases, whenever the relevant species was identified, they were used for obtaining pharmacokinetic data (except one biosimilar product).

A large proportion of the products, for which anti-drug-antibodies (ADA) status has been

evaluated (84%), underline its importance for the interpretation of pharmacokinetic and other pre-clinical data.

Only for a part of the products containing a new active substance (43%), the separate single dose toxicity studies were performed as proposed by the ICH-S6 guideline. For biosimilars dedicated single dose toxicity studies were not conducted in 88% of the products.

For nearly all products (97 %) with just a few exceptions (3 cases) the extensive repeat dose toxicity studies were conducted. The omission of the repeated dose toxicity study was justified by the lack of active species. The majority of the longest pivotal chronic toxicity studies with products containing a new active substance were 26 weeks in duration (61%). Less frequently, studies were 39 weeks in duration (15%). If the repeat dose/chronic toxicity studies were of shorter duration (13 weeks, or in some cases 4 weeks), this was scientifically well justified.

The use of pharmacologically active species in the toxicological studies is crucial for the validity of the study: in all cases, when the relevant species was identified, they were used in toxicity studies. Such cases were 56 products out of 63 with the new active substance and 18 out of 26 biosimilars.

For 40 cases (63%) of the products with the new active substance the toxicity studies were performed only in one species. Two species (rodent and non-rodent) for toxicity testing were used in 19 cases (30%) in line with the ICH-S4 guideline, but only in 8 cases of them the both used species were active for the test products.

The proportion of non-human primates used in toxicity studies was high particularly for products containing new active species. The cynomolgus was used for toxicity testing in 94 % of products with a new active substance, while chimpanzee, rhesus and marmoset were used only in isolated cases. Rats were the most used rodents in toxicity studies and were used for 17 products, in five cases of them as an active species. Rat was followed by mouse (10x), guinea pig (3x) and hamster (1x).

From a high proportion of products (89 %) lacking genotoxicity studies it can be concluded, that the evaluation of the genotoxic potential was not substantial for authorisation, which is in line with the ICH-S6 guideline. The studies were conducted only for products containing antibody-drug conjugates or a radioactive labelled active substance.

For a vast majority (93%) of the products reviewed in this thesis, no carcinogenicity studies were conducted, which was from case to case well justified, using the *Weight of the Evidence Approach.*

For the products containing a new active substance dedicated fertility and early embryonic Development (FEED) studies were performed for 35% of the products, for another 40% the endpoints of FEED were incorporated in the repeat dose toxicity studies. For a vast majority of biosimilar products (85%) the FEED studies were not performed.

The dedicated embryo-foetal development (EFD) studies were performed in 48% of the products containing a new active substance. In 24% of the products the FED studies were fully integrated in the ePPND. Considering all studies (EFD+ePPND) for the products containing a new active substance (45 products) pharmacologically active species were used

in 35 cases (78%). Cynomolgus was used in 29 cases out of them.

In 30% of products containing a new active substance the dedicated pre- and postnatal development (PPND) studies were performed. In further 25% the PPND were fully integrated in the ePPND. In the majority of cases (29 out of 35) PPND studies were conducted using active species.

In the EPARs of 25 biosimilar products it was clearly stated that no FED and PPND studies were carried out and it was also explained that these studies are not required for biosimilars.

Only for 9 out of 63 products containing a new active substance specific juvenile animal studies (JAS) were conducted. The data on juvenile toxicity was obtained as part of other studies in further 9 cases.

The proportion of dedicated local tolerance studies was high (30 %) in products with the new active substance. In most cases (63% of products with a new active substance and 69% of biosimilars) the injection site was observed in repeat dose or other toxicity studies. Rabbit was a prominent test species in local tolerance studies (used in 15 of 22 cases, 68%) even if it was not a cross-reactive species.

The data of immunotoxic potential of biopharmaceuticals containing monoclonal antibodies as an active substance were presented in 30% of the products, whereas for a vast majority of these cases the immunotoxicity relevant endpoints were incorporated in other toxicity studies.

Tissue cross-reactivity studies directed to the human tissues were performed in 63 out of 89 products. For the remaining 26 products, the EPARs provided no information whether these studies were carried out. The proportion of cross-reactivity studies for products containing new active substances was higher than for biosimilars (81% *vs.* 46%).

For biotechnology-derived products a non-clinical toxicology assessment of process or product related impurities is not required according to the ICH-S6 and ICH-M4 guidelines. Only the description of the purification processes to remove impurities should be provided. In line with this rule the extensive specification of the test material (including investigations of the product and process related impurities) were provided in the quality section of the EPARs of all products. Only for five products the assessment of toxic potential of impurities was carried out.

The studies on Dependence, Metabolites and Phototoxicity were not evaluated since these studies are irrelevant for the products containing monoclonals as an active substance.

For all products containing protein-drug conjugates or radioactive labelled mAb, standard environmental risk assessments (ERA) were carried out. For all other products no ERA was performed. This is in line with the specific guideline (EMEA/CHMP/SWP/4447/00).

EPARs are not a primary source of information on the drug product and do not always contain the complete detailed information about the medicinal product. In fact, the proportion of missing data ranged from individual cases to more than half of the products, which made the assessment challenging and at times made it difficult to arrive at the conclusion for the products in question.

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# 6 Annexes

**Annex 1:** Medicinal products containing monoclonal antibody as an active substance, gained marketing authorisation by EMA between 2009 and 2019

| Medicine name | Active substance | Authorisation status | Marketing authorisation date | Bio similar | Orphan | Indication | Human pharmacotherapeutic group (EMA Wording) | Target or pharmaco-therapeutic group (own naming) |
|---|---|---|---|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | yes | 24.10.2012 | no | yes | relapsed or refractory HL and sALCL | Antineoplastic agents | Antineoplastic agents |
| Aimovig | Erenumab | yes | 25.07.2018 | no | no | prophylaxis of migraine | Analgesics | Vasomodulator |
| Ajovy | Fremanezumab | yes | 27.03.2019 | no | no | treatment of episodic and chronic migraine in adults | Analgesics | Vasomodulator |
| Amgevita | Adalimumab | yes | 20.03.2017 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Arzerra | Ofatumumab | Withdr. | 18.04.2010 | no | no | chronic lymphocytic leukaemia | Monoclonal antibodies | Antineoplastic agents |
| Bavencio | Avelumab | yes | 17.09.2017 | no | no | metastatic Merkel cell carcinoma | Antineoplastic agents | Antineoplastic agents |
| Benlysta | Belimumab | yes | 12.07.2011 | no | no | systemic lupus erythematosus | Immunosuppressants | Immunosuppressants |
| Besponsa | Inotuzumab ozogamicin | yes | 27.06.2017 | no | yes | relapsed or refractory B-cell precursor acute lymphoblastic leukaemia (ALL) | Antineoplastic agents | Antineoplastic agents |
| Blincyto | Blinatumomab | yes | 22.11.2015 | no | yes | acute lymphoblastic leukaemia | Antineoplastic agents | Antineoplastic agents |
| Blitzima | Rituximab | yes | 12.07.2017 | yes | no | NHL, CLL, granulomatosis, microscopic polyangiitis | Antineoplastic agents | Antineoplastic agents |
| Cablivi | Caplacizumab | yes | 29.08.2018 | no | yes | episode of acquired thrombotic thrombocytopenic purpura (aTTP) | Antithrombotic agents | Antithrombotic agents |
| Cimzia | Certolizumab pegol | yes | 30.09.2009 | no | no | active rheumatoid arthritis | Immunosuppressants | Immunosuppressants |
| Cinqaero | Reslizumab | yes | 14.08.2016 | no | no | reduction exacerbations, relieve symptoms and improve lung function | Other systemic drugs for obstructive airway diseases | Immunomodulators |
| Cosentyx | Secukinumab | yes | 13.01.2015 | no | no | severe plaque psoriasis | Immunosuppressants | Immunosuppressants |

| Medicine name | Active substance | Authorisation status | Marketing authorisation date | Bio similar | Orphan | Indication | Human pharmacotherapeutic group (EMA Wording) | Target or pharmaco-therapeutic group (own naming) |
|---|---|---|---|---|---|---|---|---|
| Crysvita | Burosumab | yes | 18.02.2018 | no | yes | X-linked hypophosphataemia | Drugs for treatment of bone diseases | Immunomodulators |
| Cyltezo | Adalimumab | Withdr. | 9.11.2017 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Cyramza | Ramucirumab | yes | 18.12.2014 | no | no | gastric cancer | Antineoplastic agents | Antineoplastic agents |
| Darzalex | Daratumumab | yes | 27.04.2017 | no | yes | plasma cell myeloma, relapsed and refractory multiple myeloma | Antineoplastic agents | Antineoplastic agents |
| Dupixent | Dupilumab | yes | 26.09.2017 | no | no | atopic dermatitis in adult patients who are candidates for systemic therapy | Immunosuppressants | Immunosuppressants |
| Emgality | Galcanezumab | yes | 13.11.2018 | no | no | prophylaxis of migraine | Analgesics | Vasomodulator |
| Empliciti | Elotuzumab | yes | 10.05.2016 | no | no | multiple myeloma in adult patients who have received one or more prior therapies | Antineoplastic agents | Antineoplastic agents |
| Entyvio | Vedolizumab | yes | 21.05.2014 | no | no | active ulcerative colitis | Selective immunosuppressants | Immunosuppressants |
| Fasenra | Benralizumab | yes | 7.01.2018 | no | no | severe asthma with an eosinophilic phenotype | Drugs for obstructive airway diseases | Immunomodulators |
| Flixabi | Infliximab | yes | 25.05.2016 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Gazyvaro | Obinutuzumab | yes | 21.07.2014 | no | yes | co-treatment with chlorambucil, which is a human carcinogen | Antineoplastic agents | Antineoplastic agents |
| Halimatoz | Adalimumab | yes | 25.07.2018 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Uveitis | Immunosuppressants | Immunosuppressants |
| Hefiya | Adalimumab | yes | 25.07.2018 | yes | no | Arthritis, Psoriasis, Uveitis | Immunosuppressants | Immunosuppressants |
| Hemlibra | Emicizumab | yes | 22.02.2018 | no | no | prevent bleeding or reduce the frequency of bleeding in patients with haemophilia A | Antihemorrhagics | Antihemorrhagics |
| Herzuma | Trastuzumab | yes | 7.02.2018 | yes | no | Metastatic, Early Breast Cancer, Metastatic Gastric Cancer | Antineoplastic agents | Antineoplastic agents |
| Hulio | Adalimumab | yes | 15.09.2018 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants, TNFα inhibitors | Immunosuppressants |

| Medicine name | Active substance | Authorisation status | Marketing authorisation date | Bio similar | Orphan | Indication | Human pharmacotherapeutic group (EMA Wording) | Target or pharmaco-therapeutic group (own naming) |
|---|---|---|---|---|---|---|---|---|
| Hyrimoz | Adalimumab | yes | 25.07.2018 | yes | no | Arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Idacio | Adalimumab | yes | 1.04.2019 | yes | no | Rheumatoid, Juvenile idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Ilaris | Canakinumab | yes | 22.10.2009 | no | no | Treatment of Cryopyrin-Associated Periodic Syndromes | Interleukin inhibitors | Immunomodulators |
| Ilumetri | Tildrakizumab | yes | 16.09.2018 | no | no | psoriasis who are candidates for systemic therapy | Immunosuppressants, IL inhibitors | Immunosuppressants |
| Imfinzi | Durvalumab | yes | 20.09.2018 | no | no | locally advanced, unresectable non-small cell lung cancer (NSCLC) whose disease has not progressed following platinum-based chemoradiation therapy | Antineoplastic agents | Antineoplastic agents |
| Imraldi | Adalimumab | yes | 23.08.2017 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Inflectra | Infliximab | yes | 8.09.2013 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants, TNFα inhibitors | Immunosuppressants |
| Kadcyla | Trastuzumab emtansine | yes | 14.11.2013 | no | no | unresectable recurrent locally advanced or metastatic breast cancer | Antineoplastic agents | Antineoplastic agents |
| Kanjinti | Trastuzumab | yes | 15.05.2018 | yes | no | Metastatic, Early Breast Cancer, Metastatic Gastric Cancer | Antineoplastic agents | Antineoplastic agents |
| Kevzara | Sarilumab | yes | 22.06.2017 | no | no | active rheumatoid arthritis (RA) | Immunosuppressants | Immunosuppressants |
| Keytruda | Pembrolizumab | yes | 16.07.2015 | no | no | unresectable or metastatic melanoma | Antineoplastic agents | Antineoplastic agents |
| Kromeya | Adalimumab | yes | 1.04.2019 | yes | no | Rheumatoid, Juvenile idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Uveitis | Immunosuppressants | Immunosuppressants |
| Kyntheum | Brodalumab | yes | 16.07.2017 | no | no | moderate to severe plaque psoriasis in adult patients who are candidates for systemic therapy | Immunosuppressants | Immunosuppressants |
| Lartruvo | Olaratumab | Withdr. | 8.11.2016 | no | yes | advanced soft tissue sarcoma | Antineoplastic agents | Antineoplastic agents |

| Medicine name | Active substance | Authorisation status | Marketing authorisation date | Bio similar | Orphan | Indication | Human pharmacotherapeutic group (EMA Wording) | Target or pharmaco-therapeutic group (own naming) |
|---|---|---|---|---|---|---|---|---|
| Lemtrada | Alemtuzumab | yes | 11.09.2013 | no | no | relapsing remitting multiple sclerosis | Selective immunosuppressants | Immunosuppressants |
| Libtayo | Cemiplimab | yes | 27.06.2019 | no | no | unresectable metastatic cutaneous squamous cell carcinoma or patients with locally advanced cutaneous squamous cell carcinoma | Antineoplastic agents | Antineoplastic agents |
| Mvasi | Bevacizumab | yes | 14.01.2018 | yes | no | metastatic carcinoma of the colon or rectum, metastatic breast cancer, non-small cell lung cancer, metastatic renal cell cancer, epithelial ovarian, fallopian tube, or primary peritoneal cancer, carcinoma of the cervix | Antineoplastic agents | Antineoplastic agents |
| Mylotarg | Gemtuzumab ozogamicin | yes | 18.04.2018 | no | yes | previously untreated, de novo acute myeloid leukaemia (AML). | Antineoplastic agents | Antineoplastic agents |
| Nivolumab BMS | Nivolumab | Withdr. | 19.07.2015 | no | no | advanced or metastatic non-small cell lung cancer | Antineoplastic and immunmodulating agents | Antineoplastic agents |
| Nucala | Mepolizumab | yes | 30.11.2015 | no | no | severe eosinophilic asthma in adult patients | Drugs for obstructive airway diseases | Immunomodulators |
| Ocrevus | Ocrelizumab | yes | 7.01.2018 | no | no | relapsing remitting multiple sclerosis | Immunosuppressants | Immunosuppressants |
| Ogivri | Trastuzumab | yes | 11.12.2018 | yes | no | Metastatic, Early Breast Cancer, Metastatic Gastric Cancer | Antineoplastic agents | Antineoplastic agents |
| Ontruzant | Trastuzumab | yes | 14.11.2017 | yes | no | Metastatic, Early Breast Cancer, Metastatic Gastric Cancer | Antineoplastic agents | Antineoplastic agents |
| Opdivo | Nivolumab | yes | 18.06.2015 | no | no | advanced (unresectable or metastatic) melanoma in adults | Antineoplastic agents | Antineoplastic agents |
| Perjeta | Pertuzumab | yes | 3.03.2013 | no | no | HER2-positive metastatic or locally recurrent unresectable breast cancer | Antineoplastic agents | Antineoplastic agents |
| Portrazza | Necitumumab | yes | 14.02.2016 | no | no | locally advanced or metastatic squamous non-small cell lung cancer | Antineoplastic agents | Antineoplastic agents |
| Poteligeo | Mogamulizumab | yes | 21.11.2018 | no | yes | cutaneous T-cell lymphoma (CTCL) in adults who have received at least one prior systemic therapy | Antineoplastic agents | Antineoplastic agents |
| Praluent | Alirocumab | yes | 22.09.2015 | no | no | long-term use in adult patients with primary hypercholesterolaemia | Lipid modifying agents | Metabolic modifier |

| Medicine name | Active substance | Authorisation status | Marketing authorisation date | Bio similar | Orphan | Indication | Human pharmacotherapeutic group (EMA Wording) | Target or pharmaco-therapeutic group (own naming) |
|---|---|---|---|---|---|---|---|---|
| Praxbind | Idarucizumab | yes | 19.11.2015 | no | no | anti - dabigatran | All other therapeutic products | Non-human antigen |
| Prolia | Denosumab | yes | 25.05.2010 | no | no | osteoporosis in postmenopausal women at increased risk of fractures | Drugs for treatment of bone diseases | bone resorption |
| Qarziba (previously Dinutuximab beta EUSA and Dinutuximab beta Apeiron) | Dinutuximab beta | yes | 7.05.2017 | no | yes | neuroblastoma | Antineoplastic agents | Antineoplastic agents |
| Removab | Catumaxomab | Withdr. | 19.04.2009 | no | no | malignant ascites in patients with epithelial cancers | Other antineoplastic agents | Antineoplastic agents |
| Remsima | Infliximab | yes | 9.09.2013 | yes | no | Rheumatoid, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease | Immunosuppressants, TNFα inhibitors | Immunosuppressants |
| Repatha | Evolocumab | yes | 16.07.2015 | no | no | Hypercholesterolaemia and mixed dyslipidaemia: | Lipid modifying agents | Metabolic modifier |
| Ritemvia | Rituximab | yes | 12.07.2017 | yes | no | NHL, granulomatosis with microscopic polyangiitis | Antineoplastic agents | Antineoplastic agents |
| Rixathon | Rituximab | yes | 14.06.2017 | yes | no | NHL, CLL, granulomatosis, microscopic polyangiitis, Rheumatoid arthritis | Antineoplastic agents | Antineoplastic agents |
| Riximyo | Rituximab | yes | 14.06.2017 | yes | no | NHL, granulomatosis, microscopic polyangiitis, Rheumatoid arthritis | Antineoplastic agents | Antineoplastic agents |
| RoActemra | Tocilizumab | yes | 14.01.2009 | no | no | moderate to severe active rheumatoid arthritis in adult patients | Immunosuppressants | Immunosuppressants |
| Scintimun | Besilesomab | yes | 10.01.2010 | no | no | a diagnostic containing an antibody for a single use | Diagnostic radiopharmaceuticals | Diagnostic for single use |
| Simponi | Golimumab | yes | 30.09.2009 | no | no | Rheumatoid arthritis (RA) | Immunosuppressants | Immunosuppressants |
| Skyrizi | Risankizumab | yes | 25.04.2019 | no | no | moderate to severe plaque psoriasis | Immunosuppressants | Immunosuppressants |
| Solymbic | Adalimumab | Withdr. | 21.03.2017 | yes | no | Rheumatoid, Juvenile, Idiopathic, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease | Immunosuppressants | Immunosuppressants |
| Stelara | Ustekinumab | yes | 14.01.2009 | no | no | psoriasis | Immunosuppressants | Immunosuppressants |

| Medicine name | Active substance | Authorisation status | Marketing authorisation date | Bio similar | Orphan | Indication | Human pharmacotherapeutic group (EMA Wording) | Target or pharmaco-therapeutic group (own naming) |
|---|---|---|---|---|---|---|---|---|
| Sylvant | Siltuximab | yes | 21.05.2014 | no | yes | multicentric Castleman's disease angiofollicular lymph node hyperplasia | Immunosuppressants, Interleukin inhibitors | Immunosuppressants |
| Takhzyro | Lanadelumab | yes | 21.11.2018 | no | yes | angioedema | Other hematological agents | Vasomodulator |
| Taltz | Ixekizumab | yes | 24.04.2016 | no | no | moderate to severe plaque psoriasis | Immunosuppressants | Immunosuppressants |
| Tecentriq | Atezolizumab | yes | 19.09.2017 | no | no | advanced or metastatic urothelial carcinoma | Antineoplastic agents | Antineoplastic agents |
| Trazimera | Trastuzumab | yes | 25.07.2018 | yes | no | Metastatic, Early Breast Cancer, Metastatic Gastric Cancer | Antineoplastic agents | Antineoplastic agents |
| Tremfya | Guselkumab | yes | 9.11.2017 | no | no | moderate to severe plaque psoriasis | Immunosuppressants | Immunosuppressants |
| Trogarzo | Ibalizumab | yes | 25.09.2019 | no | no | adults infected with HIV-1 resistant to at least 1 agent in 3 different classes, in combination with other antiretroviral medicinal products | Antivirals for systemic use | Immunomodulators |
| Truxima | Rituximab | yes | 16.02.2017 | yes | no | NHL, CLL, granulomatosis, microscopic polyangiitis, Rheumatoid arthritis | Antineoplastic agents | Antineoplastic agents |
| Ultomiris | Ravulizumab | yes | 1.07.2019 | no | no | paroxysmal nocturnal haemoglobinuria (PNH) | Selective immunosuppressants | Immunosuppressants |
| Unituxin | Dinutuximab | Withdr. | 13.08.2015 | no | no | neuroblastoma | Antineoplastic agents | Antineoplastic agents |
| Xgeva | Denosumab | yes | 12.07.2011 | no | no | Prevention of skeletal related events in adults with advanced malignancies involving bone | Drugs for treatment of bone diseases | bone resorption |
| Yervoy | Ipilimumab | yes | 11.07.2011 | no | no | advanced melanoma in adults who have received prior therapy | Antineoplastic agents | Antineoplastic agents |
| Zessly | Infliximab | yes | 17.05.2018 | yes | no | Rheumatoid, Psoriatic arthritis, Psoriasis, Colitis ulcerosa, Crohn's disease, Ankylosing spondylitis | Immunosuppressants | Immunosuppressants |
| Zinbryta | Daclizumab | Withdr. | 30.06.2016 | no | no | relapsing forms of MS | Immunosuppressants | Immunosuppressants |
| Zinplava | Bezlotoxumab | yes | 17.01.2017 | no | no | prevention of Clostridium difficile infection (CDI) recurrence | Immune sera and immunoglobulins | Non-human antigen |
| Zirabev | Bevacizumab | yes | 13.02.2019 | yes | no | metastatic carcinoma of the colon or rectum, metastatic breast cancer, non-small cell lung cancer, metastatic renal cell cancer, carcinoma of the cervix | Antineoplastic agents | Antineoplastic agents |

**Annex 2:** Primary Pharmacodynamic Studies

| Medicine Name | Active Substance | Biosimilar | Primary PD Studies | Primary Pharmacology | Primary Pharmacology *in vivo* Study Species | Secondary PD Studies | Secondary PD: Justification of Absence | Safety Pharmacology Programme |
|---|---|---|---|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | no | yes | *in vitro, in vivo* | immunocompromised mouse | yes | | yes |
| Aimovig | Erenumab | no | yes | *in vitro, in vivo* | cynomolgus model capsaicin ind. increase in dermal blood flow | yes | | yes |
| Ajovy | Fremanezumab | no | yes | *in vitro, in vivo* | several animal models | no | pleyotropic activity allready known | yes |
| Amgevita | Adalimumab | yes | comparative | *in vitro, ex vivo* | | no | | no |
| Arzerra | Ofatumumab | no | yes | *in vitro, in vivo* | immunocompromised mouse | yes | | part of repeat dose |
| Bavencio | Avelumab | no | yes | *in vitro, in vivo* | Immunocompromised mouse tumor model | yes | | part of repeat dose |
| Benlysta | Belimumab | no | yes | *in vitro, in vivo* | cynomolgus, mouse, | yes | | part of repeat dose |
| Besponsa | Inotuzumab ozogamicin | no | yes | *in vitro, in vivo* | Immunocompromised mouse tumor model | no | target-specificity | yes |
| Blincyto | Blinatumomab | no | yes | *in vitro, in vivo* | Immunocompromised mouse tumor model | yes | | yes |
| Blitzima | Rituximab | yes | comparative | *in vitro, in vivo* | cynomolgus | no | | part of repeat dose |
| Cablivi | Caplacizumab | no | yes | *in vitro, ex vivo, in vivo* | Baboon Folts model | yes | | part of repeat dose |
| Cimzia | Certolizumab pegol | no | yes | *in vitro, in vivo* | mice and rabbits | yes | | part of repeat dose |
| Cinqaero | Reslizumab | no | yes | *in vitro, in vivo* | cynomolgus, mouse, guinea pig, rabbit | yes | | yes |
| Cosentyx | Secukinumab | no | yes | *in vitro, in vivo* | inflammatory mouse models | yes | | yes |
| Crysvita | Burosumab | no | part of repeat dose | *in vitro, in vivo* | cynomolgus, murine homologue model | yes | | part of repeat dose |
| Cyltezo | Adalimumab | yes | comparative | *in vitro* | | no data | | no |
| Cyramza | Ramucirumab | no | yes | *in vitro, in vivo* | cancer xenograft models in mice | no | but CDC / ADCC abcence was evaluated | part of repeat dose |
| Darzalex | Daratumumab | no | yes | *in vitro, in vivo* | mouse tumor model | yes | | no |
| Dupixent | Dupilumab | no | yes | *in vitro, in vivo* | transgenic mouse, wt mouse surrogate ab | no | all other non-clinical studies indicates absence of off-target effect | part of repeat dose |

| Medicine Name | Active Substance | Biosimilar | Primary PD Studies | Primary Pharmacology | Primary Pharmacology *in vivo* Study Species | Secondary PD Studies | Secondary PD: Justification of Absence | Safety Pharmacology Programme |
|---|---|---|---|---|---|---|---|---|
| Emgality | Galcanezumab | no | yes | *in vitro, in vivo* | cynomolgus model capsaicin ind. increase in dermal blood flow, rat | yes | | part of repeat dose |
| Empliciti | Elotuzumab | no | yes | *in vitro, in vivo* | cancer xenograft models, transgenic mouse models | no | absence of adequate animal models. | no |
| Entyvio | Vedolizumab | no | yes | *in vitro, in vivo* | rhesus encephalomyelitis (EAE) model, cotton-top tamarin | yes | | yes |
| Fasenra | Benralizumab | no | yes | *in vitro, in vivo* | cynomolgus | no | no justification | part of repeat dose |
| Flixabi | Infliximab | yes | comparative | *in vitro, in vivo* | mouse transgenic model | no | | no |
| Gazyvaro | Obinutuzumab | no | yes | *in vitro, in vivo* | several xenograft mouse models | no | TCR indicates absence of off-target effect | part of repeat dose |
| Halimatoz | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse transgenic model | no | | part of repeat dose |
| Hefiya | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse transgenic model | no | | part of repeat dose |
| Hemlibra | Emicizumab | no | yes | *in vitro, in vivo* | cynomolgus chronic haemophilia A model | yes | | part of repeat dose |
| Herzuma | Trastuzumab | yes | comparative | *in vitro* | | no | | part of repeat dose |
| Hulio | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse transgenic model | no | | no |
| Hyrimoz | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse model | no | | part of repeat dose |
| Idacio | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse arthritis model | no | | no |
| Ilaris | Canakinumab | no | yes | *in vitro, in vivo* | mouse inflammation model | yes | | part of repeat dose |
| Ilumetri | Tildrakizumab | no | yes | *in vitro, in vivo* | mouse surrogate antibody | yes | | part of repeat dose |
| Imfinzi | Durvalumab | no | yes | *in vitro, in vivo* | cancer xenograft models in mice | no | no justification | part of repeat dose |
| Imraldi | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse transgenic model | no | | no |
| Inflectra | Infliximab | yes | comparative | *in vitro* | | no | | no |
| Kadcyla | Trastuzumab emtansine | no | yes | *in vitro, in vivo* | mouse tumor model | no | no justification | yes |
| Kanjinti | Trastuzumab | yes | comparative | *in vitro* | | no data | | no data |
| Kevzara | Sarilumab | no | yes | *in vitro, in vivo* | mouse transgenic model | yes | | part of repeat dose |
| Keytruda | Pembrolizumab | no | yes | *in vitro, in vivo* | mouse tumor model | yes? | | no |
| Kromeya | Adalimumab | yes | comparative | *in vitro, in vivo* | mouse transgenic model | no data | | part of repeat dose |
| Kyntheum | Brodalumab | no | yes | *in vitro, in vivo* | mouse surrogate antibody | part of repeat dose | | part of repeat dose |
| Lartruvo | Olaratumab | no | yes | *in vitro, in vivo* | mouse surrogate antibody | yes | | no |

| Medicine Name | Active Substance | Biosimilar | Primary PD Studies | Primary Pharmacology | Primary Pharmacology *in vivo* Study Species | Secondary PD Studies | Secondary PD: Justification of Absence | Safety Pharmacology Programme |
|---|---|---|---|---|---|---|---|---|
| Lemtrada | Alemtuzumab | no | yes | *in vitro, in vivo* | mouse transgenic model | yes | | part of single and repeat dose |
| Libtayo | Cemiplimab | no | yes | *in vitro, in vivo* | mouse transgenic model | no | As no PD effects other than those already described are expected for this class of agents | part of repeat dose |
| Mvasi | Bevacizumab | yes | comparative | *in vitro, in vivo* | mouse tumor xenograft models | no | | no |
| Mylotarg | Gemtuzumab ozogamicin | no | yes | *in vitro, in vivo* | mouse tumor xenograft models | yes | | yes |
| Nivolumab BMS-Opdivo | Nivolumab | no | yes | *in vitro, in vivo* | mouse tumor model | no | anticancer/monoclonals? lack of studies for secondary pharmacodynamics are acceptable | yes |
| Nucala | Mepolizumab | no | yes, part of repeat dose | *in vitro, in vivo* | cynomolgus, tox | no | acceptable given the nature mepolizumab ? | yes |
| Ocrevus | Ocrelizumab | no | yes | *in vitro, in vivo* | cynomolgus | no | target-specificity | part of repeat dose |
| Ogivri | Trastuzumab | yes | comparative | *in vitro* | | no | | part of repeat dose |
| Ontruzant | Trastuzumab | yes | comparative | *in vitro, in vivo* | mouse tumor xenograft models | no | | no |
| Opdivo Nivolumab BMS | Nivolumab | no | yes | *in vitro, in vivo* | mouse tumor xenograft models | no | anticancer/monoclonals? lack of studies for secondary pharmacodynamics are acceptable | yes |
| Perjeta | Pertuzumab | no | yes | *in vitro, in vivo* | mouse tumor model | yes | | part of repeat dose |
| Portrazza | Necitumumab | no | yes | *in vitro, in vivo* | mouse tumor xenograft models | no | no justification | part of repeat dose |
| Poteligeo | Mogamulizumab | no | yes | *in vitro, in vivo* | cynomolgus, murine xenograft model | yes | | part of single and repeat dose |
| Praluent | Alirocumab | no | yes | *in vitro, in vivo* | cynomolgus (pk/PD), Hamster | no | no justification | part of single and repeat dose |
| Praxbind | Idarucizumab | no | yes | *in vitro, in vivo* | rat, pig model of liver trauma, intercranial bleeding mice | no | non-human target-specificity | yes + part of repeat dose |
| Prolia | Denosumab | no | yes | *in vitro, in vivo* | cynomolgus ovariectomized model, transgenic mouse model | yes | | yes |

| Medicine Name | Active Substance | Biosimilar | Primary PD Studies | Primary Pharmacology | Primary Pharmacology *in vivo* Study Species | Secondary PD Studies | Secondary PD: Justification of Absence | Safety Pharmacology Programme |
|---|---|---|---|---|---|---|---|---|
| Qarziba (beta Apeiron) | Dinutuximab beta | no | yes | *in vitro, in vivo* | syngeneic tumour mouse model | no | no justification | no |
| Removab | Catumaxomab | no | yes | *in vitro, in vivo* | syngeneic tumour mouse model | yes | | yes |
| Remsima | Infliximab | yes | yes | *in vitro* | | no | | no |
| Repatha | Evolocumab | no | yes | *in vitro, in vivo* | cynomolgus, hamster | yes | | part of repeat dose |
| Ritemvia | Rituximab | yes | comparative | *in vitro, in vivo* | cynomolgus | no | | part of repeat dose |
| Rixathon | Rituximab | yes | comparative | *in vitro, in vivo* | cynomolgus, murine xenograft model | no | | no |
| Riximyo | Rituximab | yes | comparative | *in vitro, in vivo* | cynomolgus, murine xenograft model | no | | no |
| RoActemra | Tocilizumab | no | yes | *in vitro, in vivo* | cynomolgus model of collagen-induced arthritis | yes | | yes + part of repeat dose |
| Scintimun | Besilesomab | no | yes | *in vitro* | | no | no justification | yes |
| Simponi | Golimumab | no | yes | *in vitro, in vivo* | mouse model of arthritis | no | no justification | part of repeat dose |
| Skyrizi | Risankizumab | no | yes | *in vitro, in vivo* | mouse ear thickening model | no | target-specificity | part of repeat dose |
| Solymbic | Adalimumab | yes | comparative | *in vitro* | | no | | no |
| Stelara | Ustekinumab | no | yes | *in vitro, in vivo* | several animal models | yes | | part of repeat dose |
| Sylvant | Siltuximab | no | yes | *in vitro, in vivo* | mouse surrogate antibody | yes | | part of repeat dose |
| Takhzyro | Lanadelumab | no | yes | *in vitro, in vivo* | rat carrageenan paw oedema model | yes | | part of repeat dose |
| Taltz | Ixekizumab | no | yes | *in vitro, in vivo* | mouse homologe model | yes | | part of repeat dose |
| Tecentriq | Atezolizumab | no | yes | *in vitro, in vivo* | syngeneic tumour mouse model | no | no justification | part of repeat dose |
| Trazimera | Trastuzumab | yes | comparative | *in vitro* | | no data | | no data |
| Tremfya | Guselkumab | no | yes | *in vitro, in vivo* | mouse model | cross-reactivity studies | | yes + part of repeat dose |
| Trogarzo | Ibalizumab | no | yes | *in vitro, in vivo* | rhesus | yes | | part of repeat dose |
| Truxima | Rituximab | yes | comparative | *in vitro, in vivo* | cynomolgus | no | | part of repeat dose |
| Ultomiris | Ravulizumab | no | yes | *in vitro, in vivo* | Immunocompromised mouse model | yes | | no |
| Unituxin | Dinutuximab | no | yes | *in vitro, in vivo* | mouse tumor xenograft models | yes | | yes |
| Xgeva | Denosumab | no | yes | *in vitro, in vivo* | several mouse models of bone metastasis | yes | | yes + part of repeat dose |

| Medicine Name | Active Substance | Biosimilar | Primary PD Studies | Primary Pharmacology | Primary Pharmacology *in vivo* Study Species | Secondary PD Studies | Secondary PD: Justification of Absence | Safety Pharmacology Programme |
|---|---|---|---|---|---|---|---|---|
| Yervoy | Ipilimumab | no | yes | *in vitro, in vivo* | cynomolgus, transgenic mouse model | no | no justification | part of repeat dose |
| Zessly | Infliximab | yes | yes | *in vitro* | | no | | no |
| Zinbryta | Daclizumab | no | yes | no data | | no | target-specificity | yes |
| Zinplava | Bezlotoxumab | no | yes | *in vitro, in vivo* | mouse and hamster models of CDI | no data | | no data |
| Zirabev | Bevacizumab | yes | yes | *in vitro* | | no | | part of repeat dose |

**Annex 3:** Pharmacologically Relevant Species and Approaches used for its Selection

| Medicine Name | Active substance | Biosimilar Comparator | Number relevant species | Active Species 1 | Active Species 2 | Active Species 3, 4, 5, 6, 7 | Used Testing Methods for Relevant Species | Human Tissue Directed TCR |
|---|---|---|---|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | | 1 | cynomolgus | | | Flow cytometry; time-resolved fluorometry | yes |
| Aimovig | Erenumab | | 1 | cynomolgus | | | binding assay, not specified, *in vivo* cynomolgus functional | no data |
| Ajovy | Fremanezumab | | 3 | cynomolgus | rat | rabbit | TCR, sequence homology, binding assay biacore | yes |
| Amgevita | Adalimumab | Humira | 1 | cynomolgus | | | no data | no data |
| Arzerra | Ofatumumab | | 1 | cynomolgus | | | TCR and sequence analysis | yes |
| Bavencio | Avelumab | | 2 | cynomolgus | rat | | binding assay, functional activity (cytocine release) | no data |
| Benlysta | Belimumab | | 2 | cynomolgus | mouse | | TCR, *in vivo* cynomolgus | yes |
| besponsa | Inotuzumab ozogamicin | | 0 | | | | TCR, binding assays | yes |
| Blincyto | Blinatumomab | | 1 | chimpanzee | | | variouse binding assays, Flow cytometry functional activity (cytocine release) | yes |
| Blitzima | Rituximab | MabThera | 1 | cynomolgus | | | no | yes |
| Cablivi | Caplacizumab | | 2 | cynomolgus | guinea pig | | binding assay, pharmacology profile | yes |
| cimzia | Certolizumab pegol | | 1 | cynomolgus | | | binding assay, functional activity | yes |
| Cinqaero | Reslizumab | | 2 | cynomolgus | rabbit | | TCR, *in vivo* cynomolgus | yes |
| Cosentyx | Secukinumab | | 3 | cynomolgus | rhesus | marmoset | TCR, binding, functional activity | yes |
| Crysvita | Burosumab | | 2 | cynomolgus | rabbit | | binding assay biacore, TCR was not usefull, *in vivo* cynomolgus | yes |
| Cyltezo | Adalimumab | Humira | 1 | cynomolgus | | | no | yes |
| Cyramza | Ramucirumab | | 1 | cynomolgus | | | TCR | yes |
| Darzalex | Daratumumab | | 1 | chimpanzee | | | TCR binding via flow cytometry, ELISA and ICH | yes |
| Dupixent | Dupilumab | | 0 | no | | | PK characteristics, binding, biological effects | yes |
| Emgality | Galcanezumab | | 3 | cynomolgus | rabbit | rat | *in vitro* activity rabbit, *in vivo* rat and cynomolgus | no data |

| Medicine Name | Active substance | Biosimilar Comparator | Number relevant species | Active Species 1 | Active Species 2 | Active Species 3, 4, 5, 6, 7 | Used Testing Methods for Relevant Species | Human Tissue Directed TCR |
|---|---|---|---|---|---|---|---|---|
| Empliciti | Elotuzumab | | 0 | no | | | TCR | yes |
| Entyvio | Vedolizumab | | 3 | cynomolgus | rhesus | rabbit | TCR, binding assay | yes |
| Fasenra | Benralizumab | | 1 | cynomolgus | | | sequence homology and binding assay, *in vivo* cynomolgus | yes |
| Flixabi | Infliximab | Remicade | 0 | no | | | Although the rat is not a relevant species for infliximab, it was reported from the literatures (Wallace and Rees 1980, McCarthy, Yoong et al. 2000) that the clearance mechanism of infliximab in rat is relevant. | no data |
| Gazyvaro | Obinutuzumab | | 1 | cynomolgus | | | TCR, binding assay | yes |
| Halimatoz | Adalimumab | Humira | 1 | cynomolgus | | | no | yes |
| Hefiya | Adalimumab | Humira | 1 | cynomolgus | | | no | yes |
| Hemlibra | Emicizumab | | 1 | cynomolgus | | | TCR, binding assay | yes |
| Herzuma | Trastuzumab | Herceptin | 0 | | | | no data | no data |
| Hulio | Adalimumab | Humira | 1 | cynomolgus | | | no data | no data |
| Hyrimoz | Adalimumab | Humira | 1 | cynomolgus | | | no | yes |
| Idacio | Adalimumab | Humira | 1 | cynomolgus | | | no data | no data |
| Ilaris | Canakinumab | | 1 | marmoset | | | TCR, binding assay | yes |
| Ilumetri | Tildrakizumab | | 1 | cynomolgus | | | TCR, sequence homology and binding assay biacore, *in vitro* biological activity, quantitative gene expression anatomical profiling for different species | yes |
| Imfinzi | Durvalumab | | 1 | cynomolgus | | | TCR, sequence homology, binding assay | yes |
| Imraldi | Adalimumab | Humira | 1 | cynomolgus | | | no data | no data |
| Inflectra | Infliximab | Remicade | 0 | no | rat (not active but was used) | | no | yes |
| Kadcyla | Trastuzumab emtansine | | 1 | cynomolgus | | | TCR | yes |
| Kanjinti | Trastuzumab | Herceptin | 0 | | | | no | yes |
| Kevzara | Sarilumab | | 1 | cynomolgus | | | Flow cytometry, binding assay biacore | no data |
| Keytruda | Pembrolizumab | | 1 | cynomolgus | | | binding assay, biological activity *in vitro* | yes |
| Kromeya | Adalimumab | Humira | 1 | cynomolgus | | | no data | no data |
| Kyntheum | Brodalumab | | 1 | cynomolgus | Rabbit bad quality | | TCR, binding, biological activity | yes |

| Medicine Name | Active substance | Biosimilar Comparator | Number relevant species | Active Species 1 | Active Species 2 | Active Species 3, 4, 5, 6, 7 | Used Testing Methods for Relevant Species | Human Tissue Directed TCR |
|---|---|---|---|---|---|---|---|---|
| Lartruvo | Olaratumab | | 1 | cynomolgus | | | TCR, ELISA, surface plasmon resonance | yes |
| Lemtrada | Alemtuzumab | | 1 | cynomolgus | | | TCR, biological activity (lymphocyte) depletion, *in vivo* cynomolgus, | yes |
| Libtayo | Cemiplimab | | 1 | cynomolgus | | | binding assay biacore | no data |
| Mvasi | Bevacizumab | Avastin | 1 | cynomolgus | | | no data | no data |
| Mylotarg | Gemtuzumab ozogamicin | | 0 | no | | | TCR | yes |
| Nivolumab BMS | Nivolumab | | 1 | cynomolgus | | | TCR, sequence homology and binding assay biacore, biological activity | yes |
| Nucala | Mepolizumab | | 1 | cynomolgus | | | TCR, binding biacore, pharmacologyca activity in toxicology study | yes |
| Ocrevus | Ocrelizumab | | 1 | cynomolgus | | | TCR, binding assay | yes |
| Ogivri | Trastuzumab | Herceptin | 0 | | | | no data | no data |
| Ontruzant | Trastuzumab | Herceptin | 0 | | | | no data | no data |
| Opdivo | Nivolumab | | 1 | cynomolgus | | | TCR, binding assay biacore | yes |
| Perjeta | Pertuzumab | | 1 | cynomolgus | | | TCR, PK linearity, binding affinity | yes |
| Portrazza | Necitumumab | | 1 | cynomolgus | Rabbit bad quality | | TCR, binding assay | yes |
| Poteligeo | Mogamulizumab | | 1 | cynomolgus | | | binding assay, not specified, functional activity, sequence comparison | no data |
| Praluent | Alirocumab | | 4 | cynomolgus | rat | 3) mouse bad quality 4) hamster bad quality | sequence homology and binding assay | yes |
| Praxbind | Idarucizumab | | 2 | rhesus | rat | | TCR (non human drug target) | yes |
| Prolia | Denosumab | | 1 | cynomolgus | | | PK characteristics, sequence homology and biological effects | no data |
| Qarziba | Dinutuximab beta | | 2 | cynomolgus | guinea pig | | TCR, PK data | yes |
| Removab | Catumaxomab | | 0 | no | | | TCR | yes |
| Remsima | Infliximab | Remicade | 0 | no | rat (not active but was used) | | surface plasmon resonance SPR | yes |
| Repatha | Evolocumab | | 2 | cynomolgus | hamster | | TCR, binding assay biacore, *in vivo* cynomolgus | yes |

| Medicine Name | Active substance | Biosimilar Comparator | Number relevant species | Active Species 1 | Active Species 2 | Active Species 3, 4, 5, 6, 7 | Used Testing Methods for Relevant Species | Human Tissue Directed TCR |
|---|---|---|---|---|---|---|---|---|
| Ritemvia | Rituximab | MabThera | 1 | cynomolgus | | | no | yes |
| Rixathon | Rituximab | MabThera | 1 | cynomolgus | | | no | yes |
| Riximyo | Rituximab | MabThera | 1 | cynomolgus | | | no | yes |
| RoActemra | Tocilizumab | | 1 | cynomolgus | | | TCR, binding assay, *in vivo* and *in vitro* | no data |
| Scintimun | Besilesomab | | 1 | cynomolgus | | | binding assay, alkaline phosphatase anti-alkaline phosphatase (APAAP) technique | yes |
| Simponi | Golimumab | | 2 | cynomolgus | chimpanzee | | binding and functional assay, not specified | yes |
| Skyrizi | Risankizumab | | 1 | cynomolgus | | | binding assay surface platon resonance, biological activity | yes |
| Solymbic | Adalimumab | Humira | 1 | cynomolgus | | | no data | no data |
| Stelara | Ustekinumab | | 1 | cynomolgus | | | TCR, binding assay | yes |
| Sylvant | Siltuximab | | 7 | cynomolgus | chimpanzee | 3) baboon<br>4) pigtailed macaque<br>5) cotton-top tamarin<br>6) marmoset<br>7) rhesus | TCR, functional assay | yes |
| Takhzyro | Lanadelumab | | 2 | cynomolgus | rat | mouse no tox | Surface plasmon resonance (SPR), ELISA, fluorescence based assays,<br>*in vitro, ex vivo* bioactivity assay | yes |
| Taltz | Ixekizumab | | 1 | cynomolgus | | | TCR, binding assay surface plasmon resonance (SPR) | yes |
| Tecentriq | Atezolizumab | | 2 | cynomolgus | mouse | | TCR, equilibrium binding assays | yes |
| Trazimera | Trastuzumab | Herceptin | 0 | | Mouse (used) | | no data | no data |
| Tremfya | Guselkumab | | 2 | cynomolgus | guinea pig | | TCR, functional assay | yes |
| Trogarzo | Ibalizumab | | 2 | cynomolgus | rhesus | | TCR, PK characteristics | yes |
| Truxima | Rituximab | MabThera | 1 | cynomolgus | | | no | yes |
| Ultomiris | Ravulizumab | | 0 | no | | | TCR, pharmacological activity screened in 10 NHP and 13 non-primate mammals | yes |
| Unituxin | Dinutuximab | | 0 | no | | | TCR | yes |
| Xgeva | Denosumab | | 1 | cynomolgus | | | TCR, biological activity (inactivation) | yes |

| Medicine Name | Active substance | Biosimilar Comparator | Number relevant species | Active Species 1 | Active Species 2 | Active Species 3, 4, 5, 6, 7 | Used Testing Methods for Relevant Species | Human Tissue Directed TCR |
|---|---|---|---|---|---|---|---|---|
| Yervoy | Ipilimumab | | 1 | cynomolgus | | | TCR, *in vivo* cynomolgus | yes |
| Zessly | Infliximab | Remicade | 1 | Chimpanzee (not used) | | | no data | no data |
| Zinbryta | Daclizumab | | 1 | cynomolgus | | | no data | no data |
| Zinplava | Bezlotoxumab | | 2 | mouse | hamster | | na/non-human target | yes |
| Zirabev | Bevacizumab | Avastin | 1 | cynomolgus | | | no data | no data |

**Annex 4:** Pharmacodynamic drug-drug interaction studies

| Medicine name | Active substance | Bio similar | PD drug-drug interaction studies | EPAR: justification for absence or presence of PD-DDI Studies |
|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | no | no | no justification |
| Aimovig | Erenumab | no | no | No drug-drug interactions were expected based on the mechanism of AMG 334 |
| Ajovy | Fremanezumab | no | no data | |
| Amgevita | Adalimumab | yes | no | In line with relevant guidelines including the CHMP guidance on similar biological medicinal products containing monoclonal antibodies (EMA/CHMP/BMWP/403543/2010). |
| Arzerra | Ofatumumab | no | no | unnecessary for biologic with high specificity and affinity for CD20 |
| Bavencio | Avelumab | no | no | The same justification as PK DDI |
| Benlysta | Belimumab | no | no | high specificity and affinity |
| Besponsa | Inotuzumab ozogamicin | no | no | Intended as single agent. Concomitant medicines (i.e. steroids, anti–infectives etc.) that may be used at the time of treatment were administered during the clinical trials and thus specific non clinical studies are not considered warranted. |
| Blincyto | Blinatumomab | no | yes | The influence of dexamethasone and indomethacin pre-treatment on blinatumomab-dependent cell lysis and cytokine production was examined in vitro using human PBMCs and NALM-6 cells (study 103-PCD-0071). |
| Blitzima | Rituximab | yes | no | biosimilar |
| Cablivi | Caplacizumab | no | no | this is an antibody/Nanobody that specifically interacts |
| Cimzia | Certolizumab pegol | no | no | no potential pharmacodynamic drug interactions at the p55 and p75 TNF receptor level are considered likely, as certolizumab pegol should not be used with other anti-TNFα agents. |
| Cinqaero | Reslizumab | no | yes | had an additive effect with oral prednisolone for suppression of BAL fluid eosinophilia in allergic B6D2F1/J mice (D26940). |
| Cosentyx | Secukinumab | no | no | Fully human antibody and there is no direct evidence for the role of IL 17A in the expression of CYP450 enzymes. |
| Crysvita | Burosumab | no | no data | |
| Cyltezo | Adalimumab | yes | no | biosimilar |
| Cyramza | Ramucirumab | no | no | no justification |
| Darzalex | Daratumumab | no | no, but | comprehensive testing of combination therapies in primary and secondary PD!!! |
| Dupixent | Dupilumab | no | no data | |
| Emgality | Galcanezumab | no | no | Due to the high target specificity |
| Empliciti | Elotuzumab | no | no | due to the absence of adequate animal models |
| Entyvio | Vedolizumab | no | no | does not modulate production of cytokines, which can affect drug metabolism. |
| Fasenra | Benralizumab | no | no | The justification for this approach has been provided (not listed in EPAR) |

| Medicine name | Active substance | Bio similar | PD drug-drug interaction studies | EPAR: justification for absence or presence of PD-DDI Studies |
|---|---|---|---|---|
| Flixabi | Infliximab | yes | no | in line with relevant guidelines including the CHMP guidance on similar biological medicinal products containing monoclonal antibodies (EMA/CHMP/BMWP/403543/2010) |
| Gazyvaro | Obinutuzumab | no | yes | Using isolated human PBMCs pre-incubated with 5 nM or 5 µM chlorambucil, no impact on the ADCC function of obinutuzumab was observed (Report 1050168). number of studies addressing the anti-tumour activity of obinutuzumab in combination with various regimens in SCID mice tumour xenograft models Including cyclophosphamide, cyclophosphamide/vincristine, doxorubicin, bendamustine, fludarabine, rituximab. In vitro prednisolone (1-100 µg/mL) decreased ADCC and NK activity. |
| Halimatoz | Adalimumab | yes | no | biosimilar |
| Hefiya | Adalimumab | yes | no | biosimilar |
| Hemlibra | Emicizumab | no | yes | FVIII and by-passing agents *in vitro*, *in vivo*, interference with various diagnostic assays |
| Herzuma | Trastuzumab | yes | no | biosimilar |
| Hulio | Adalimumab | yes | no | re not deemed necessary by the CHMP. |
| Hyrimoz | Adalimumab | yes | no | biosimilar |
| Idacio | Adalimumab | yes | no data | |
| Ilaris | Canakinumab | no | no | no justification |
| Ilumetri | Tildrakizumab | no | no data | |
| Imfinzi | Durvalumab | no | no | no justification |
| Imraldi | Adalimumab | yes | no | biosimilar, are not deemed necessary |
| Inflectra | Infliximab | yes | no | No requirements for PD drug interactions studies have been detailed in relevant guidelines including the CHMP guidance on similar biological medicinal products containing monoclonal antibodies (EMA/CHMP/BMWP/403543/2010). |
| Kadcyla | Trastuzumab emtansine | no | yes | The influence of Trastuzumab on MMTV-HER2 Fo5 Tumour response to T-DM1 conjugates |
| Kanjinti | Trastuzumab | yes | no data | |
| Kevzara | Sarilumab | no | yes | approved in combination with methotrexate (MTX). disease modifying anti rheumatic drugs act differently from sarilumab, IL-6Rα, thus PD interactions are not expected |
| Keytruda | Pembrolizumab | no | no | known potential in relation to cytotoxic, immunosuppressive or immunomodulatory therapies. In the SmPC section 4.5 it is reported to avoid the use of systemic corticosteroids or other immunosuppressants before start. "Potential PD interaction with systemic immunosuppressants" is listed among the missing information in the RMP. |
| Kromeya | Adalimumab | yes | no data | |
| Kyntheum | Brodalumab | no | no | no drug-drug interactions were expected based on the putative mechanism of brodalumab |
| Lartruvo | Olaratumab | no | no | no justification |
| Lemtrada | Alemtuzumab | no | no | no justification |

| Medicine name | Active substance | Bio similar | PD drug-drug interaction studies | EPAR: justification for absence or presence of PD-DDI Studies |
|---|---|---|---|---|
| Libtayo | Cemiplimab | no | no | no justification |
| Mvasi | Bevacizumab | yes | no | biosimilar |
| Mylotarg | Gemtuzumab ozogamicin | no | no | only PK-justification: were presented and indicated a low potential to inhibit activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 at clinically relevant concentrations. Combination therapy studies are presented in primary PD |
| Nivolumab BMS | Nivolumab | no | yes | in combination with anti-CTLA (ipilimumab) and anti-LAG-3 (BMS-986016) monoclonal antibodies. |
| Nucala | Mepolizumab | no | no | the CHMP considered acceptable given the nature mepolizumab (high affinity and high specificity) |
| Ocrevus | Ocrelizumab | no | no | no justification |
| Ogivri | Trastuzumab | yes | no | biosimilar |
| Ontruzant | Trastuzumab | yes | no | biosimilar |
| Opdivo | Nivolumab | no | yes | in combination with anti-CTLA (ipilimumab) and anti-LAG-3 (BMS-986016) monoclonal antibodies. |
| Perjeta | Pertuzumab | no | yes | in combination with other anti-cancer agents (cisplatin, gemcitabine, capecitabine, erlotinib, paclitaxel and bevacizumab) in several xenograft models for non-small cell lung carcinoma, colon carcinoma, mammary tumour, and ovarian carcinoma. |
| Portrazza | Necitumumab | no | part of primary PD | in combination with gemcitabine and cisplatin, paclitaxel and cisplatin, and/or pemetrexed and cisplatin, the most common platinum doublets used in first-line treatment of patients with metastatic NSCLC |
| Poteligeo | Mogamulizumab | no | no | no drug-drug interactions were expected based on the putative mechanism of mogamulizumab |
| Praluent | Alirocumab | no | yes | in combination with atorvastatin in APOE*Leiden.CETP mice and Cynomolgus monkeys |
| Praxbind | Idarucizumab | no | part of primary PD | with other anti-coagulants or anti-platelet agents or coagulation factor concentrates |
| Prolia | Denosumab | no | part of primary PD | OVX cynomolgus monkeys with 6 months pretreatment of alendronate before 6 months treatment of denosumab |
| Qarziba | Dinutuximab beta | no | no | no justification |
| Removab | Catumaxomab | no | yes | Influence of steroids (dexamethasone, hydrocortisone) on cytokine release |
| Remsima | Infliximab | yes | no | biosimilar |
| Repatha | Evolocumab | no | no | high specificity, considered via TCR |
| Ritemvia | Rituximab | yes | no | biosimilar |
| Rixathon | Rituximab | yes | no | biosimilar |
| Riximyo | Rituximab | yes | no data | |
| RoActemra | Tocilizumab | no | no | no justification |
| Scintimun | Besilesomab | no | no | no justification |

| Medicine name | Active substance | Bio similar | PD drug-drug interaction studies | EPAR: justification for absence or presence of PD-DDI Studies |
|---|---|---|---|---|
| Simponi | Golimumab | no | no | Due to the high binding specificity of golimumab for its target, TNFα, it is unlikely to have pharmacodynamic interactions with co-administered drugs. |
| Skyrizi | Risankizumab | no | no data | |
| Solymbic | Adalimumab | yes | no | biosimilar |
| Stelara | Ustekinumab | no | no | because of the high binding specificity of CNTO 1275 (STELARA, ustekinumab) for its target, IL-12/23p40, it is unlikely to have pharmacodynamic interactions with co- administered drugs |
| Sylvant | Siltuximab | no | yes | in combination with a variety of anti-cancer therapeutics bortezomib, dexamethasone or melphalan have been evaluated. |
| Takhzyro | Lanadelumab | no | no | antibody that specifically targets pKal. |
| Taltz | Ixekizumab | no | no | no justification |
| Tecentriq | Atezolizumab | no | no | known potential in relation to immunomodulatory therapies. In the SmPC section 4.5 it is reported to avoid the use of systemic corticosteroids or other immunosuppressants before start. |
| Trazimera | Trastuzumab | yes | no data | |
| Tremfya | Guselkumab | no | no | due to the high specificity |
| Trogarzo | Ibalizumab | no | yes | the potential to have a synergistic effect with other anti-retroviral agents when tested in combination in a low passage clinical isolate, namely enfuvirtide, abacavir, and atazanavir. effect of the combination ibalizumab and maraviroc on activity was measured |
| Truxima | Rituximab | yes | no | biosimilar |
| Ultomiris | Ravulizumab | no | no | no justification |
| Unituxin | Dinutuximab | no | no | no justification |
| Xgeva | Denosumab | no | no | no justification |
| Yervoy | Ipilimumab | no | yes | in combination with immunomodulatory antibodies to human CD137 (BMS-663513, 2 studies) or to PD-1 receptor (MDX- 1106, 1 study) was studied in cynomolgus monkeys. Effect of dexamethasone on the anti-tumour activity of anti-mouse CTLA-4 monoclonal antibody (mAb) was made (study MDX-010-001-R). |
| Zessly | Infliximab | yes | no | biosimilar |
| Zinbryta | Daclizumab | no | no | no justification |
| Zinplava | Bezlotoxumab | no | no data | |
| Zirabev | Bevacizumab | yes | no | biosimilar |

**Annex 5:** Pharmacokinetic Studies

| Medicine name | Active substance | Bio-similar | Intended clinical admi-nistration route | Pharmaco-kinetic Study | Pharmaco-kinetics Species | Absorption | Distribution | Metabolism | Excretion | PK drug interactions | Immunogenicity (ADA) Detection |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | no | IV | dedicated | cynomolgus, rats | basic PK-data conjugate | yes conjugate | yes *in vitro* conjugate | yes conjugate | yes | PK |
| Aimovig | Erenumab | no | SC | dedicated | cynomolgus | basic PK-data | no data | no data | no data | no data | PK |
| Ajovy | Fremanezumab | no | SC | dedicated | cynomolgus, mice, rats, rabbits | yes | yes | no | no | not expected | PK |
| Amgevita | Adalimumab | yes | SC | part of repeat dose toxicity | cynomolgus | yes comparative | no | no | no | no | no data |
| Arzerra | Ofatumumab | no | IV | part of repeat dose toxicity | cynomolgus | basic PK-data (but stated there were no Absorption study) | no | no | no | no | part of repeat dose |
| Bavencio | Avelumab | no | IV | dedicated | cynomolgus, mouse | basic PK-data | no data | no | no | no | part of repeat dose and primary PD |
| Benlysta | Belimumab | no | IV | dedicated | cynomolgus, mouse | basic PK-data | no data | no | no | not expected | PK + part of repeat dose |
| Besponsa | Inotuzumab ozogamicin | no | IV | dedicated + repeat dose toxicity | cynomolgus, mice, rats, rabbits | basic PK-data conjugate, no values | yes conjugate | yes conjugate | yes conjugate | yes | part of repeat dose |
| Blincyto | Blinatumomab | no | IV | dedicated | cynomolgus, chimpanzee, mice, rats | basic PK-data | no data | no data | yes | no data | PK |
| Blitzima | Rituximab | yes | IV | part of repeat dose toxicity | cynomolgus | basic PK-data | no | no | no | no data | part of repeat dose |
| Cablivi | Caplacizumab | no | SC + IV | part of single dose toxicity | cynomolgus, mouse, guinea pig | yes | yes | no | no | no | part of repeat dose |
| Cimzia | Certolizumab pegol | no | SC | dedicated | cynomolgus, rats | yes conjugate | yes conjugate | yes conjugate | yes conjugate | no data | part of repeat dose |
| Cinqaero | Reslizumab | no | SC | dedicated | cynomolgus, mice, rats, rabbits | yes | no | no | no | yes | PK |

| Medicine name | Active substance | Bio-similar | Intended clinical admi-nistration route | Pharmaco-kinetic Study | Pharmaco-kinetics Species | Absorption | Distribution | Metabolism | Excretion | PK drug interactions | immunogenicity in animals measured |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cosentyx | Secukinumab | no | SC | dedicated | cynomolgus | yes comparative | yes | no | yes | not expected | PK |
| Crysvita | Burosumab | no | SC | dedicated + repeat dose toxicity | cynomolgus, rabbit | basic PK-data no values | no data | no data | no data | no data | PK + part of repeat dose, ePPND, JAS |
| Cyltezo | Adalimumab | yes | SC | dedicated | cynomolgus | yes comparative | no | no | no | no data | PK |
| Cyramza | Ramucirumab | no | IV | part of repeat dose toxicity | cynomolgus, mouse | basic PK-data | no data | no data | no data | no data | part of repeat dose |
| Darzalex | Daratumumab | no | IV | part of repeat dose toxicity | cynomolgus, chimpanzee | basic PK-data | no data | no data | no data | no data | part of repeat dose |
| Dupixent | Dupilumab | no | SC | part of single dose toxicity | cynomolgus, rats | basic PK-data | no | no | no | not expected | part of repeat dose |
| Emgality | Galcanezumab | no | SC | dedicated | cynomolgus, rats | basic PK-data | yes | no | no | not expected | no data |
| Empliciti | Elotuzumab | no | IV | dedicated | rhesus, mouse | basic PK-data | no data | no | no | not expected | no |
| Entyvio | Vedolizumab | no | IV | part of repeat dose toxicity | cynomolgus, rabbit | basic PK-data (but stated there were no Absorption study) | no data | no | no | no | PK |
| Fasenra | Benralizumab | no | SC | dedicated | cynomolgus, rabbit | basic PK-data | no data | no data | no | no data | part of acute toxicity study |
| Flixabi | Infliximab | yes | IV | dedicated | rat, mouse model | basic PK-data | no data | no data | no data | no data | PK |
| Gazyvaro | Obinutuzumab | no | IV | dedicated + repeat dose toxicity + ePPND | cynomolgus, mouse | basic PK-data | no | no | no | no | no data |
| Halimatoz | Adalimumab | yes | SC | dedicated | cynomolgus, rabbit | yes comparative | no data | no data | no data | no data | part of repeat dose |
| Hefiya | Adalimumab | yes | SC | dedicated + repeat dose toxicity | cynomolgus, rabbit | yes comparative | no data | no data | no data | no data | part of repeat dose |
| Hemlibra | Emicizumab | no | SC | dedicated | cynomolgus, mouse | basic PK-data | no | no | no | no | PK + part of repeat dose |

| Medicine name | Active substance | Bio-similar | Intended clinical admi-nistration route | Pharmaco-kinetic Study | Pharmaco-kinetics Species | Absorption | Distribution | Metabolism | Excretion | PK drug interactions | immunogenicity in animals measured |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Herzuma | Trastuzumab | yes | SC + IV | part of repeat dose toxicity | cynomolgus | yes comparative | no data | no data | no data | no data | no data |
| Hulio | Adalimumab | yes | SC | dedicated + repeat dose toxicity | cynomolgus, mouse | yes comparative | no | no | no | no | part of repeat dose |
| Hyrimoz | Adalimumab | yes | SC | dedicated + repeat dose toxicity | cynomolgus, rabbit | yes comparative | no data | no data | no data | no data | part of repeat dose |
| Idacio | Adalimumab | yes | SC | part of repeat dose toxicity | cynomolgus | basic PK-data no values | no data | no data | no data | no data | no data |
| Ilaris | Canakinumab | no | SC | dedicated + repeat dose toxicity | rhesus, marmoset, mouse, | basic PK-data | no data | no data | no data | no data | no data |
| Ilumetri | Tildrakizumab | no | SC + IV | dedicated | cynomolgus, mouse | yes | yes | no | no | not expected | PK |
| Imfinzi | Durvalumab | no | IV | part of repeat dose toxicity | cynomolgus | basic PK-data | no data | no | no | no data | part of repeat dose |
| Imraldi | Adalimumab | yes | SC | part of repeat dose toxicity | cynomolgus | yes comparative TK | no | no | no | no | part of repeat dose |
| Inflectra | Infliximab | yes | IV | dedicated | rat | yes comparative | no | no | no | no | part of repeat dose |
| Kadcyla | Trastuzumab emtansine | no | IV | dedicated | cynomolgus, rats | yes conjugate | yes conjugate | yes conjugate | yes conjugate | yes | part of acute and repeat dose |
| Kanjinti | Trastuzumab | yes | IV | part of repeat dose toxicity | cynomolgus | yes comparative | no data | no data | no data | no data | no data |
| Kevzara | Sarilumab | no | SC | dedicated | cynomolgus, rats | basic PK-data | no | no | no | no | part of repeat dose |
| Keytruda | Pembrolizumab | no | IV | dedicated | cynomolgus | basic PK-data | no data | no | no | No | PK |
| Kromeya | Adalimumab | yes | SC | part of repeat dose toxicity | cynomolgus | yes comparative | no data | no data | no data | no data | part of repeat dose |
| Kyntheum | Brodalumab | no | SC | dedicated + repeat dose toxicity | cynomolgus, rabbit | yes | yes | no | no | not expected | part of repeat dose |
| Lartruvo | Olaratumab | no | IV | dedicated | cynomolgus, mouse | yes | no | no | no | no data | part of repeat dose |
| Lemtrada | Alemtuzumab | no | IV | dedicated | cynomolgus, mouse | basic PK-data | no data | no | no | no | part of repeat dose |

| Medicine name | Active substance | Bio-similar | Intended clinical admi-nistration route | Pharmaco-kinetic Study | Pharmaco-kinetics Species | Absorption | Distribution | Metabolism | Excretion | PK drug interactions | immunogenicity in animals measured |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Libtayo | Cemiplimab | no | IV | dedicated + repeat dose toxicity | cynomolgus | basic PK-data | no data | no data | no data | no data | part of repeat dose |
| Mvasi | Bevacizumab | yes | IV | dedicated | cynomolgus, rats | basic PK-data | no | no | no | no data | part of repeat dose |
| Mylotarg | Gemtuzumab ozogamicin | no | IV | dedicated + repeat dose toxicity | cynomolgus, rat, dog | basic PK-data conjugate | yes conjugate | yes conjugate | yes conjugate | yes | PK |
| Nivolumab BMS | Nivolumab | no | IV | dedicated | cynomolgus | basic PK-data | no data | no | no | no data | PK |
| Nucala | Mepolizumab | no | SC + IV ? | part of single and repeat dose toxicity | cynomolgus | yes | no | no | no | no data | part of repeat dose |
| Ocrevus | Ocrelizumab | no | IV | dedicated | cynomolgus, mice, rats | basic PK-data | Yes tissue distribution study | no | no data | no data | PK |
| Ogivri | Trastuzumab | yes | IV | dedicated | cynomolgus | basic PK-data | no data | no | no | no | PK |
| Ontruzant | Trastuzumab | yes | SC + IV | no data | no data | no data | no | no | no | no | no data |
| Opdivo | Nivolumab | no | IV | dedicated | cynomolgus | basic PK-data | no | no | no | no data | PK |
| Perjeta | Pertuzumab | no | IV | dedicated + DART | cynomolgus, rats | yes | no data | no | no | yes | PK |
| Portrazza | Necitumumab | no | IV | dedicated + repeat dose toxicity | cynomolgus, mouse | yes | no | no | no | no | PK + part of repeat dose |
| Poteligeo | Mogamulizumab | no | IV | dedicated + repeat dose toxicity | cynomolgus | yes | yes | no | no | not expected | part of acute, repeat dose + PPND |
| Praluent | Alirocumab | no | SC | dedicated | cynomolgus | yes | no | no | no data | no data | PK |
| Praxbind | Idarucizumab | no | IV | dedicated + repeat dose toxicity | rhesus, rat | yes | no data | no data | yes | not expected | no data |
| Prolia | Denosumab | no | SC | dedicated | cynomolgus, mice, rats | yes | yes | yes | yes | not expected | PK |

| Medicine name | Active substance | Bio-similar | Intended clinical admi-nistration route | Pharmaco-kinetic Study | Pharmaco-kinetics Species | Absorption | Distribution | Metabolism | Excretion | PK drug interactions | immunogenicity in animals measured |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Qarziba (beta Apeiron) | Dinutuximab beta | no | IV | dedicated + repeat dose toxicity | guinea pig | yes | yes *in vivo* biodistribution data | no | no | no data | part of repeat dose |
| Removab | Catumaxomab | no | Intraperito neal Infusion | dedicated | mouse surrogate | yes | yes | no | no | no | part of acute toxicity study |
| Remsima | Infliximab | yes | IV | dedicated | rat | yes | no | no | no | no | part of repeat dose |
| Repatha | Evolocumab | no | SC | dedicated | cynomolgus, hamster | yes | yes | no | no | not expected | part of repeat dose + PPND |
| Ritemvia | Rituximab | yes | IV | dedicated + repeat dose toxicity | cynomolgus | yes comparative | no | no | no | no | part of repeat dose |
| Rixathon | Rituximab | yes | IV | dedicated | cynomolgus | yes comparative | no data | no data | no data | no data | part of acute and repeat dose |
| Riximyo | Rituximab | yes | IV | dedicated | cynomolgus | yes comparative | no data | no data | no data | no data | part of acute and repeat dose |
| RoActemra | Tocilizumab | no | IV | dedicated | cynomolgus, rats | basic PK-data | yes | yes | yes | cannot be excluded, no data | part of repeat dose |
| Scintimun | Besilesomab | no | IV | dedicated | cynomolgus, rats | yes | yes | yes | yes | no | part of repeat dose |
| Simponi | Golimumab | no | SC | part of single and repeat dose toxicity | cynomolgus | yes | yes | no | no | no | part of repeat dose |
| Skyrizi | Risankizumab | no | SC | part of single, repeat dose toxicity and PPND | cynomolgus | yes | yes | no | no | not expected | PK |
| Solymbic | Adalimumab | yes | SC | part of repeat dose toxicity | cynomolgus | yes | no | no | no | no | no data |
| Stelara | Ustekinumab | no | SC | part of single and repeat dose toxicity | cynomolgus | yes | yes | no | no | no data | part of repeat dose |
| Sylvant | Siltuximab | no | IV | dedicated + repeat dose toxicity | cynomolgus | basic PK-data (but stated there were no Absorption study) | yes foetus | no | no | cannot be excluded, no data | PK + part of repeat dose |

| Medicine name | Active substance | Bio-similar | Intended clinical admi-nistration route | Pharmaco-kinetic Study | Pharmaco-kinetics Species | Absorption | Distribution | Metabolism | Excretion | PK drug interactions | immunogenicity in animals measured |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Takhzyro | Lanadelumab | no | SC | dedicated + repeat dose toxicity + ePPND | cynomolgus, rats | yes | yes | no | no | no data | PK + part of repeat dose |
| Taltz | Ixekizumab | no | SC | dedicated + repeat dose toxicity | cynomolgus | yes | no | no | no | no | no data |
| Tecentriq | Atezolizumab | no | IV | dedicated + repeat dose toxicity | cynomolgus | basic PK-data | no | no | no | no | part of repeat dose |
| Trazimera | Trastuzumab | yes | IV | dedicated | mouse surrogate | yes comparative | no data | no data | no data | no data | no data |
| Tremfya | Guselkumab | no | SC | dedicated + repeat dose toxicity | cynomolgus, guinea pig | yes | no | no | no | not expected | part of acute, repeat dose + ePPND |
| Trogarzo | Ibalizumab | no | IV | dedicated + repeat dose toxicity + PD + ePPND | cynomolgus, rhesus | yes | no data | no | no | not expected | part of repeat dose + ePPND |
| Truxima | Rituximab | yes | IV | part of repeat dose toxicity | cynomolgus | yes comparative | no | no | no | no | part of repeat dose |
| Ultomiris | Ravulizumab | no | IV | dedicated | cynomolgus, rabbit | yes | no | no | no | no data | PK + Local Tolerance |
| Unituxin | Dinutuximab | no | IV | literatur review | mice, rats, dog | basic PK-data | yes | no | no | no | no data |
| Xgeva | Denosumab | no | SC | dedicated | cynomolgus, mice, rats | yes | yes | yes | yes | no | part of acute and repeat dose |
| Yervoy | Ipilimumab | no | IV | part of repeat dose toxicity | cynomolgus | yes | no | no | no | not expected | part of repeat dose |
| Zessly | Infliximab | yes | IV | dedicated | rat | yes comparative | no | no | no | no | part of repeat dose |
| Zinbryta | Daclizumab | no | SC | dedicated + repeat dose toxicity + DART | cynomolgus | yes | yes | no | no | no | PK + part of repeat dose |
| Zinplava | Bezlotoxumab | no | IV | dedicated | hamster | basic PK-data | yes *in vivo* tissue distr. studies | no | no data | no | part of repeat dose |
| Zirabev | Bevacizumab | yes | IV | part of repeat dose toxicity | cynomolgus, rats | basic PK-data | no | no | no | no | PK + part of repeat dose |

**Annex 6:** Species used in Pharmacokinetic studies

*Bold font: non-active species*

| Medicine name | Active substance | Bio-similar | Pharmaco-kinetic Study | Number of active species | All active species | All Species used in Pharmacokinetic studies | Not active Rodent used | Not active Non-Rodent used |
|---|---|---|---|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | no | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Aimovig | Erenumab | no | dedicated | 1 | cynomolgus | cynomolgus | | |
| Ajovy | Fremanezumab | no | dedicated | 3 | cynomolgus, rat, rabbit | cynomolgus, rat, rabbits, **mouse** | mouse | |
| Amgevita | Adalimumab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Arzerra | Ofatumumab | no | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Bavencio | Avelumab | no | dedicated | 2 | cynomolgus, rat | cynomolgus, **mouse** | mouse | |
| Benlysta | Belimumab | no | dedicated | 2 | cynomolgus, mouse | cynomolgus, mouse | | |
| Besponsa | Inotuzumab ozogamicin | no | dedicated + repeat dose toxicity | 0 | no | **cynomolgus, mouse, rat, rabbits** | mouse, rat | cynomolgus, rabbit |
| Blincyto | Blinatumomab | no | dedicated | 1 | chimpanzee | chimpanzee, **cynomolgus, mouse, rat** | mouse, rat | cynomolgus |
| Blitzima | Rituximab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Cablivi | Caplacizumab | no | part of single dose toxicity | 2 | cynomolgus, guinea pig | cynomolgus, guinea pig, **mouse** | mouse | |
| Cimzia | Certolizumab pegol | no | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Cinqaero | Reslizumab | no | dedicated | 2 | cynomolgus, rabbit | cynomolgus, rabbits, **mouse, rat** | mouse, rat | |
| Cosentyx | Secukinumab | no | dedicated | 3 | cynomolgus, rhesus, marmoset | cynomolgus | | |
| Crysvita | Burosumab | no | dedicated + repeat dose toxicity | 2 | cynomolgus, rabbit | cynomolgus, rabbit | | |
| Cyltezo | Adalimumab | yes | dedicated | 1 | cynomolgus | cynomolgus | | |

| Medicine name | Active substance | Bio-similar | Pharmaco-kinetic Study | Number of active species | All active species | All Species used in Pharmacokinetic studies | Not active Rodent used | Not active Non-Rodent used |
|---|---|---|---|---|---|---|---|---|
| Cyramza | Ramucirumab | no | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |
| Darzalex | Daratumumab | no | part of repeat dose toxicity | 1 | chimpanzee | chimpanzee, **cynomolgus** | | cynomolgus |
| Dupixent | Dupilumab | no | part of single dose toxicity | 0 | no | **cynomolgus, rat** | rat | cynomolgus |
| Emgality | Galcanezumab | no | dedicated | 3 | cynomolgus, rabbit, rat | cynomolgus, rat | | |
| Empliciti | Elotuzumab | no | dedicated | 0 | no | **rhesus, mouse** | mouse | rhesus |
| Entyvio | Vedolizumab | no | dedicated | 3 | cynomolgus, rhesus, rabbit | cynomolgus, rabbit | | |
| Fasenra | Benralizumab | no | dedicated | 1 | cynomolgus | cynomolgus, **rabbit** | | rabbit |
| Flixabi | Infliximab | yes | dedicated | 0 | no | **rat, mouse model** | rat, mouse model | |
| Gazyvaro | Obinutuzumab | no | dedicated + repeat dose toxicity + ePPND | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |
| Halimatoz | Adalimumab | yes | dedicated | 1 | cynomolgus | cynomolgus, **rabbit** | | rabbit |
| Hefiya | Adalimumab | yes | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus, **rabbit** | | rabbit |
| Hemlibra | Emicizumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |
| Herzuma | Trastuzumab | yes | part of repeat dose toxicity | 0 | no | **cynomolgus** | | |
| Hulio | Adalimumab | yes | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |
| Hyrimoz | Adalimumab | yes | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus, **rabbit** | | rabbit |
| Idacio | Adalimumab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Ilaris | Canakinumab | no | dedicated + repeat dose toxicity | 1 | marmoset | rhesus, marmoset, **mouse** | mouse | |
| Ilumetri | Tildrakizumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |

| Medicine name | Active substance | Bio-similar | Pharmaco-kinetic Study | Number of active species | All active species | All Species used in Pharmacokinetic studies | Not active Rodent used | Not active Non-Rodent used |
|---|---|---|---|---|---|---|---|---|
| Imfinzi | Durvalumab | no | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Imraldi | Adalimumab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Inflectra | Infliximab | yes | dedicated | 0 | no | **rat** | rat | |
| Kadcyla | Trastuzumab emtansine | no | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Kanjinti | Trastuzumab | yes | part of repeat dose toxicity | 0 | no | **cynomolgus** | | |
| Kevzara | Sarilumab | no | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Keytruda | Pembrolizumab | no | dedicated | 1 | cynomolgus | cynomolgus | | |
| Kromeya | Adalimumab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Kyntheum | Brodalumab | no | dedicated + repeat dose toxicity | 1 | cynomolgus, rabbit (week) | cynomolgus, **rabbit** | | rabbit |
| Lartruvo | Olaratumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |
| Lemtrada | Alemtuzumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse** | mouse | |
| Libtayo | Cemiplimab | no | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Mvasi | Bevacizumab | yes | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Mylotarg | Gemtuzumab ozogamicin | no | dedicated + repeat dose toxicity | 0 | no | **cynomolgus, rat, dog** | rat | cynomolgus, dog |
| Nivolumab BMS | Nivolumab | no | dedicated | 1 | cynomolgus | cynomolgus | | |
| Nucala | Mepolizumab | no | part of single and repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Ocrevus | Ocrelizumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse, rat** | mouse, rat | |
| Ogivri | Trastuzumab | yes | dedicated | 0 | no | **cynomolgus** | | |
| Ontruzant | Trastuzumab | yes | no data | 0 | no | no data | no data | |
| Opdivo | Nivolumab | no | dedicated | 1 | cynomolgus | cynomolgus | | |

| Medicine name | Active substance | Bio-similar | Pharmaco-kinetic Study | Number of active species | All active species | All Species used in Pharmacokinetic studies | Not active Rodent used | Not active Non-Rodent used |
|---|---|---|---|---|---|---|---|---|
| Perjeta | Pertuzumab | no | dedicated + DART | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Portrazza | Necitumumab | no | dedicated + repeat dose toxicity | 1 | cynomolgus, rabbit (weak) | cynomolgus, **mouse** | mouse | |
| Poteligeo | Mogamulizumab | no | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Praluent | Alirocumab | no | dedicated | 4 | cynomolgus, rat, mouse (weak), hamster (weak) | cynomolgus | | |
| Praxbind | Idarucizumab | no | dedicated + repeat dose toxicity | 2 | rhesus rat | rhesus, rat | | |
| Prolia | Denosumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse, rat** | mouse, rat | |
| Qarziba (beta Apeiron) | Dinutuximab beta | no | dedicated + repeat dose toxicity | 2 | cynomolgus, guinea pig | guinea pig | | |
| Removab | Catumaxomab | no | dedicated | 0 | no | **mouse surrogate** | mouse surrogate | |
| Remsima | Infliximab | yes | dedicated | 0 | no | rat | rat | |
| Repatha | Evolocumab | no | dedicated | 2 | cynomolgus, hamster | cynomolgus, hamster | | |
| Ritemvia | Rituximab | yes | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Rixathon | Rituximab | yes | dedicated | 1 | cynomolgus | cynomolgus | | |
| Riximyo | Rituximab | yes | dedicated | 1 | cynomolgus | cynomolgus | | |
| RoActemra | Tocilizumab | no | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Scintimun | Besilesomab | no | dedicated | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Simponi | Golimumab | no | part of single and repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Skyrizi | Risankizumab | no | part of single, repeat dose toxicity and PPND | 1 | cynomolgus | cynomolgus | | |
| Solymbic | Adalimumab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Stelara | Ustekinumab | no | part of single and repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |

| Medicine name | Active substance | Bio-similar | Pharmaco-kinetic Study | Number of active species | All active species | All Species used in Pharmacokinetic studies | Not active Rodent used | Not active Non-Rodent used |
|---|---|---|---|---|---|---|---|---|
| Sylvant | Siltuximab | no | dedicated + repeat dose toxicity | 7 | cynomolgus, chimpanzee, baboon, pigtailed macaque, cotton-top tamarin, marmoset, rhesus | cynomolgus | | |
| Takhzyro | Lanadelumab | no | dedicated + repeat dose toxicity + ePPND | 2 | cynomolgus, rat, mouse (waek) | cynomolgus, rat | | |
| Taltz | Ixekizumab | no | dedicated + repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Tecentriq | Atezolizumab | no | dedicated + repeat dose toxicity | 2 | cynomolgus, mouse | cynomolgus | | |
| Trazimera | Trastuzumab | yes | dedicated | 0 | | **mouse surrogate** | mouse surrogate | |
| Tremfya | Guselkumab | no | dedicated + repeat dose toxicity | 2 | cynomolgus, guinea pig | cynomolgus, guinea pig | | |
| Trogarzo | Ibalizumab | no | dedicated + repeat dose toxicity + PD + ePPND | 2 | cynomolgus, rhesus | cynomolgus, rhesus | | |
| Truxima | Rituximab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Ultomiris | Ravulizumab | no | dedicated | 0 | no | **cynomolgus, rabbit** | | cynomolgus, rabbit |
| Unituxin | Dinutuximab | no | literatur review | 0 | no | **mouse, rat, dog** | mouse, rat | dog |
| Xgeva | Denosumab | no | dedicated | 1 | cynomolgus | cynomolgus, **mouse, rat** | mouse, rat | |
| Yervoy | Ipilimumab | no | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus | | |
| Zessly | Infliximab | yes | dedicated | 1 | Chimpanzee (but not used) | **rat** | rat | |
| Zinbryta | Daclizumab | no | dedicated + repeat dose toxicity + DART | 1 | cynomolgus | cynomolgus | | |
| Zinplava | Bezlotoxumab | no | dedicated | 2 | mouse, hamster | hamster | | |
| Zirabev | Bevacizumab | yes | part of repeat dose toxicity | 1 | cynomolgus | cynomolgus, **rat** | rat | |

**Annex 7:** Immunogenicity and Pharmacokinetic Drug-Drug Interaction Studies

| Medicine Name | Active substance | Bio-similar | Intended clinical administra-tion route | Immunogenicity in animals measured | PK Drug Interactions | EPAR: justification for absence or presence of PK DDI-Studies |
|---|---|---|---|---|---|---|
| Adcetris | Brentuximab vedotin | no | IV | PK | yes | MMAE did not induce any CYP isozymes in vitro. MMAE had a weak inhibitory effect on CYP3A4, but it is unlikely to have any clinical relevance. MMAE was not a substrate of BCRP or MRP2, but it was a substrate of P-gp. MMAE inhibits P-gp, however, not at clinically relevant concentrations. Therefore, interactions via P-gp inhibition are not expected. In addition, MMAE was shown not to be a substrate for OATP1B1, OATP1B3, OCT2, OAT1, OAT3 uptake transporters. However, inhibition of these uptake transporters by MMAE has not been studied. Placental transfer of brentuximab vedotin, MMAE and TAb was shown in the rat. |
| Aimovig | Erenumab | no | SC | PK | no data | |
| Ajovy | Fremanezumab | no | SC | PK | no | Do not metabolize via CYP P450 enzymes. The mechanism of action of a drug may have an effect on CYP450 enzymes or on transporters through cytokine dependent modulation but for fremanezumab this is unlikely and no such evidence was found.<br>Assessed in the clinical PK section. |
| Amgevita | Adalimumab | yes | SC | no data | no | |
| Arzerra | Ofatumumab | no | IV | part of repeat dose | no | The potential interaction with methotrexate assessed in the clinical PK section. |
| Bavencio | Avelumab | no | IV | part of repeat dose and primary PD | no | Avelumab is primarily metabolised through catabolic pathways, therefore, it is not expected that avelumab will have pharmacokinetic drug-drug interactions with other medicinal products. |
| Benlysta | Belimumab | no | IV | PK + part of repeat dose | not expected | Does not undergo metabolism by the cytochrome P450 enzymes. Concomitant medications were explored in the clinical PK section. |
| Besponsa | Inotuzumab ozogamicin | no | IV | part of repeat dose | no data | |
| Blincyto | Blinatumomab | no | IV | PK | no data | |
| Blitzima | Rituximab | yes | IV | part of repeat dose | no data | |
| Cablivi | Caplacizumab | no | SC + IV | part of repeat dose | no data | |
| Cimzia (PEG) | Certolizumab pegol | no | SC | part of repeat dose | no data | |

| Medicine Name | Active substance | Bio-similar | Intended clinical administra-tion route | Immunogenicity in animals measured | PK Drug Interactions | EPAR: justification for absence or presence of PK DDI-Studies |
|---|---|---|---|---|---|---|
| Cinqaero | Reslizumab | no | SC | PK | yes | In vitro study in human hepatocytes to investigate potential direct cytotoxicity of IL-5 and reslizumab, and to investigate the effect on expression of selected CYP450 enzymes (DM-2013-017) |
| Cosentyx | Secukinumab | no | SC | PK | not expected | Typical metabolizing enzymes, such as CYP450's and UGT's, etc. are not involved in the proteolytic degradation of immunoglobulins. This was agreed by the CHMP. |
| Crysvita | Burosumab | no | SC | PK + part of repeat dose, ePPND, JAS | no data | |
| Cyltezo | Adalimumab | yes | SC | PK | no data | |
| Cyramza | Ramucirumab | no | IV | part of repeat dose | no data | |
| Darzalex | Daratumumab | no | IV | part of repeat dose | no data | |
| Dupixent | Dupilumab | no | SC | part of repeat dose | no | does not involve cytochrome P450 (CYP450)-mediated metabolism or interaction with cell membrane transporters, therefore pharmacokinetic interactions with small molecule drugs are limited. However, published literature suggests IL-4 plays a role in the regulation of CYP. Assessed in the clinical PK section. |
| Emgality | Galcanezumab | no | SC | no data | not expected | Pharmacokinetic interactions with other drugs that rely on renal or hepatic mechanisms for their clearance are not expected, and no drug-drug interaction studies were conducted |
| Empliciti | Elotuzumab | no | IV | no | not expected | The expected *in vivo* degradation of mAbs is to small peptides and amino acids via biochemical pathways that are independent of drug metabolizing enzymes, such as CYP enzymes, so no drug-drug interactions are anticipated. |
| Entyvio | Vedolizumab | no | IV | PK | no | does not modulate production of cytokines, which can affect drug metabolism. The lack of specific PK drug interaction data was accepted by the CHMP given the biological nature of the product. |
| Fasenra | Benralizumab | no | SC | part of acute toxicity study | no data | |
| Flixabi | Infliximab | yes | IV | PK | no data | |
| Gazyvaro | Obinutuzumab | no | IV | no data | no | is not expected to have direct effect on the activity or expression of cytochrome P450 enzymes or drug transporters. |
| Halimatoz | Adalimumab | yes | SC | part of repeat dose | no data | |
| Hefiya | Adalimumab | yes | SC | part of repeat dose | no data | |
| Hemlibra | Emicizumab | no | SC | PK + part of repeat dose | no | no justification |

| Medicine Name | Active substance | Bio-similar | Intended clinical administra-tion route | Immunogenicity in animals measured | PK Drug Interactions | EPAR: justification for absence or presence of PK DDI-Studies |
|---|---|---|---|---|---|---|
| Herzuma | Trastuzumab | yes | SC + IV | no data | no data | |
| Hulio | Adalimumab | yes | SC | part of repeat dose | no | |
| Hyrimoz | Adalimumab | yes | SC | part of repeat dose | no data | |
| Idacio | Adalimumab | yes | SC | no data | no data | |
| Ilaris | Canakinumab | no | SC | no data | no data | |
| Ilumetri | Tildrakizumab | no | SC + IV | PK | yes | ...not metabolized via CYP450 enzymes. However, a potential impact of tildrakizumab on the PK of other drugs was evaluated in vitro in cultures of human hepatocytes. The study showed that expression of IL-23 receptor is negligible in human hepatocytes and IL-23 does not affect expression of CYP1A2 and CYP 3A4. |
| Imfinzi | Durvalumab | no | IV | part of repeat dose | no data | |
| Imraldi | Adalimumab | yes | SC | part of repeat dose | no | |
| Inflectra | Infliximab | yes | IV | part of repeat dose | no | |
| Kadcyla | Trastuzumab emtansine | no | IV | part of acute and repeat dose | yes | In vitro metabolism studies suggested that DM1 does not induce or inhibit P-450–mediated metabolism at the highest concentration tested (600 ng/mL). DM1 appeared to be a substrate but not an inhibitor of P-gp when tested at 0.5 µM in MDCKII- MDR1 cells. The clinical data indicates concomitant treatment with OATP 1B1 and 1B3 inhibitors results in pharmacokinetic drug interactions. DDI database analyse. |
| Kanjinti | Trastuzumab | yes | IV | no data | no data | |
| Kevzara | Sarilumab | no | SC | part of repeat dose | no | not metabolized via CYP450 enzymes. However, according to literature, the expression of hepatic CYP450 enzymes is suppressed by cytokines such as IL-6. Thus, CYP450 expression may be reversed when IL-6 signalling is inhibited by sarilumab. This issue is adequately addressed in the SmPC. |
| Keytruda | Pembrolizumab | no | IV | PK | no | is designed to modulate the functional activity of T lymphocytes, there is potential for PD drug-drug interactions in relation to cytotoxic, immunosuppressive or immunomodulatory therapies. In the SmPC section 4.5 it is reported to avoid the use of systemic corticosteroids or other immunosuppressants before start of pembrolizumab treatment. "Potential PD interaction with systemic immunosuppressants" is missing information in the RMP. |
| Kromeya | Adalimumab | yes | SC | part of repeat dose | no data | |
| Kyntheum | Brodalumab | no | SC | part of repeat dose | no | biotechnology-derived substances do not metabolize via CYP P450 enzymes. |

| Medicine Name | Active substance | Bio-similar | Intended clinical administra-tion route | Immunogenicity in animals measured | PK Drug Interactions | EPAR: justification for absence or presence of PK DDI-Studies |
|---|---|---|---|---|---|---|
| Lartruvo | Olaratumab | no | IV | part of repeat dose | no data | |
| Lemtrada | Alemtuzumab | no | IV | part of repeat dose | no | is an unlikely candidate for cytochrome P450 mediated drug-drug interactions and therefore, no drug-drug interaction studies were performed. This was accepted by the CHMP |
| Libtayo | Cemiplimab | no | IV | part of repeat dose | no data | |
| Mvasi | Bevacizumab | yes | IV | part of repeat dose | no data | |
| Mylotarg | Gemtuzumab ozogamicin | no | IV | PK | yes | Gemtuzumab ozogamicin did not cause induction of CYP3A4 in the transfected HepG2 cells at up to 0.0424 µM of gemtuzumab ozogamicin. N-Ac-γ-calicheamicin DMH did not cause induction of CYP1A2, CYP2B6, or CYP3A4 mRNA expression and/or enzyme activity. N-Ac-γ-calicheamicin DMH inhibited UGT1A1 activity. N-Ac-γ-calicheamicin DMH showed little or no inhibition of the bidirectional transport of digoxin (P-gp substrate) or pitavastatin (BCRP substrate) |
| Nivolumab BMS | Nivolumab | no | IV | PK | no data | |
| Nucala | Mepolizumab | no | SC + IV nicht ganz klar | part of repeat dose | no data | |
| Ocrevus | Ocrelizumab | no | IV | PK | no data | |
| Ogivri | Trastuzumab | yes | IV | PK | no | |
| Ontruzant | Trastuzumab | yes | SC + IV | no data | no | |
| Opdivo | Nivolumab | no | IV | PK | no data | |
| Perjeta | Pertuzumab | no | IV | PK | yes | The potential for pharmacokinetic drug interactions between pertuzumab and bevacizumab (humanized monoclonal antibody against VEGF) were investigated in a single-dose pharmacokinetic study in SD rats. No substantial differences, and therefore no PK interactions, were apparent between the PK parameters of the rats given a single agent and those of the rats given combination treatment. |
| Portrazza | Necitumumab | no | IV | PK + part of repeat dose | no | no justification |
| Poteligeo | Mogamulizumab | no | IV | part of acute, repeat dose + PPND | not expected | no drug-drug interactions were expected in the case of a mAb drug. |
| Praluent | Alirocumab | no | SC | PK | no data | |
| Praxbind | Idarucizumab | no | IV | no data | no data | |

| Medicine Name | Active substance | Bio-similar | Intended clinical administra-tion route | Immunogenicity in animals measured | PK Drug Interactions | EPAR: justification for absence or presence of PK DDI-Studies |
|---|---|---|---|---|---|---|
| Prolia | Denosumab | no | SC | PK | no data | |
| Qarziba (beta Apeiron) | Dinutuximab beta | no | IV | part of repeat dose | no data | |
| Removab | Catumaxomab | no | Intraperitoneal Infusion | part of acute toxicity study | no | no justification |
| Remsima | Infliximab | yes | IV | part of repeat dose | no | |
| Repatha | Evolocumab | no | SC | part of repeat dose + PPND | TCR study | |
| Ritemvia | Rituximab | yes | IV | part of repeat dose | no | |
| Rixathon | Rituximab | yes | IV | part of acute and repeat dose | no data | |
| Riximyo | Rituximab | yes | IV | part of acute and repeat dose | no data | |
| RoActemra | Tocilizumab | no | IV | part of repeat dose | cannot be excluded, no data | |
| Scintimun | Besilesomab | no | IV | part of repeat dose | no | no justification |
| Simponi | Golimumab | no | SC | part of repeat dose | no data | |
| Skyrizi | Risankizumab | no | SC | PK | no | On the basis of that any potential small molecule co-dosed with risankizumab is unlikely to share the same elimination mechanisms as this antibody. Nonetheless inhibition of IL-23 by risankizumab in patients may suppress directly or indirectly the pathophysiological expression of downstream cytokines such as IL-17, IL-6, IL-10, IFNγ and TNF-α. Some of these cytokines are known to impact CYP isoforms. A clinical study carried out by the applicant demonstrated that 150mg risankizumab administered SC every four weeks had no effect on the AUC of CYP probe drugs or their metabolites. |
| Solymbic | Adalimumab | yes | SC | no data | no | |
| Stelara | Ustekinumab | no | SC | part of repeat dose | no data | |
| Sylvant | Siltuximab | no | IV | PK + part of repeat dose | cannot be excluded, no data | |

| Medicine Name | Active substance | Bio-similar | Intended clinical administra-tion route | Immunogenicity in animals measured | PK Drug Interactions | EPAR: justification for absence or presence of PK DDI-Studies |
|---|---|---|---|---|---|---|
| Takhzyro | Lanadelumab | no | SC | PK + part of repeat dose | no data | |
| Taltz | Ixekizumab | no | SC | no data | no | no justification |
| Tecentriq | Atezolizumab | no | IV | part of repeat dose | no | no justification |
| Trazimera | Trastuzumab | yes | IV | no data | no data | |
| Tremfya | Guselkumab | no | SC | part of acute, repeat dose + ePPND | not expected | In vitro, IL-23 did not alter the expression or activity of multiple CYP enzymes (i.e., 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4) in cryopreserved human hepatocytes, suggesting that potential interactions between guselkumab and CYP substrates are unlikely. Therefore, no nonclinical *in vivo* drug-drug interaction studies to evaluate the effect of guselkumab on other drugs were conducted, which was considered acceptable by CHMP. |
| Trogarzo | Ibalizumab | no | IV | part of repeat dose + ePPND | not expected | Degraded not via CYPs and Phase II enzymes, nor is a substrate for drug transporters. Therefore, no pharmacokinetic drug-drug interactions are expected. |
| Truxima | Rituximab | yes | IV | part of repeat dose | no | |
| Ultomiris | Ravulizumab | no | IV | PK + Local Tolerance | no data | |
| Unituxin | Dinutuximab | no | IV | no data | no | no justification |
| Xgeva | Denosumab | no | SC | part of acute and repeat dose | no | no justification |
| Yervoy | Ipilimumab | no | IV | part of repeat dose | not expected | independent of cytochrome P450 enzymes. Consequently, ipilimumab is not expected to have interactions with molecules that are metabolized by these enzymes. However, the potential toxicity of ipilimumab administered alone or in combination with BMS-663513 (primary PD) was evaluated. |
| Zessly | Infliximab | yes | IV | part of repeat dose | no | |
| Zinbryta | Daclizumab | no | SC | PK + part of repeat dose | no data | |
| Zinplava | Bezlotoxumab | no | IV | part of repeat dose | no | Since bezlotoxumab is eliminated by catabolism, no metabolic drug-drug interactions are expected. |
| Zirabev | Bevacizumab | yes | IV | PK + part of repeat dose | no | |

**Annex 8:** Single and Repeat-dose Toxicity Program

| Medicine Name | Single-dose Toxicity Studies | Repeat-dose Toxicity (supportive) | Repeat-dose Toxicity Studies (RTDS, pivotal) | Duration pivotal RDTS (longest) | Duration RDTS in Weeks (calculated) | Recovery RDTS in Weeks (calculated) |
|---|---|---|---|---|---|---|
| Adcetris | part of repeat dose toxicity, 4 rats, 3 cynomolgus | 1 rat, 4 cynomolgus | 1 rat, 3 cynomolgus, GLP | 24 weeks+9 weeks recovery | 24 | 9 |
| Aimovig | no data | no | cynomolgus | 1 months | 4 | no data |
| Ajovy | yes, 1 in rat | rats, cynomolgus, 1 and 3 month | cynomolgus | 26 weeks | 26 | no data |
| Amgevita | no | no | comparative | 1 months | 4 | no data |
| Arzerra | part of repeat dose toxicity | 2 non GLP, cynomolgus, | 4 cynomolgus GLP | 7 mon.+ 6 mon. recovery | 30 | 26 |
| Bavencio | part of single dose PK | 2xmouse, rat, non GLP | 2 cynomolgus GLP | 13 weeks + 8 weeks recovery | 13 | 8 |
| Benlysta | part of repeat dose toxicity | 1 non GLP, cynomolgus | 2 cynomolgus GLP | 6 mo + 8 mo recovery | 26 | 34 |
| Besponsa | yes, 1 rat non-GLP, 1 rat GLP, 1 Cynomolgus GLP | 2 rat GLP | 2 cynomolgus GLP | 26 weeks | 26 | Tox. studies of shorter duration |
| Blincyto | part of single dose PK | 3 mouse surrogate model, GLP | 1 chimpanzee, mouse | 13 weeks+4 weeks recovery | 13 | 4 |
| Blitzima | no | no | 1 cynomolgus comparative GLP | 8 weeks | 8 | no data |
| Cablivi | yes, 1 Guinea pig, 2 cynomolgus GLP | no | 1 Guinea pig, 4 cynomolgus GLP | 26 weeks | 26 | no data |
| Cimzia | yes, 1 cynomolgus | at liest one rat | 2 cynomolgus GLP | 26 weeks | 26 | 13 |
| Cinqaero | yes, 1 mouse, 1 rat, 1 cynomolgus, GLP | 3 mouse GLP | 3 cynomolgus GLP | 6 months | 26 | 6 |
| Cosentyx | yes, 1 cynomolgus | no | 4 cynomolgus | 26 weeks with a 13 weeks recovery | 26 | 13 |
| Crysvita | yes, rabbit | yes, rabbit | 1 juvenile, 2 adult cynomolgus | 40 weeks | 40 | duration unknown |
| Cyltezo | no | no | comparative | 5 weeks | 5 | no data |
| Cyramza | part of repeat dose toxicity and primary PD | no | 2 cynomolgus GLP | 39 weeks | 39 | Tox. studies of shorter duration |

| Medicine Name | Single-dose Toxicity Studies | Repeat-dose Toxicity (supportive) | Repeat-dose Toxicity Studies (RTDS, pivotal) | Duration pivotal RDTS (longest) | Duration RDTS in Weeks (calculated) | Recovery RDTS in Weeks (calculated) |
|---|---|---|---|---|---|---|
| Darzalex | part of single dose PK and repeat dose toxicity | no | 1 chimpanzee, 1 cynomolgus | 6 weeks | 6 | 2 |
| Dupixent | part of repeat dose toxicity | no | cynomolgus (surrogate Abs) | 26 weeks | 26 | no data |
| Emgality | no data | no | 3 rats, cynomolgus | 6 months | 26 | duration unknow |
| Empliciti | yes, rhesus (off-target toxicity) | no | no | no | no | no |
| Entyvio | yes, 1 Cynomolgus non-GLP, 1 Cynomolgus GLP | no | 1 rabbit, 5 cynomolgus | 6 months | 26 | 12 |
| Fasenra | yes, cynomolgus non-GLP | no | 3 cynomolgus | 39 weeks | 39 | 12 |
| Flixabi | no | no | no | no | no | no |
| Gazyvaro | part of repeat dose toxicity | no | 3 cynomolgus GLP | 26 weeks+37 weeks recovery | 26 | 37 |
| Halimatoz | no | no | comparative | 4 weeks | 4 | no data |
| Hefiya | no | no | comparative | 4 weeks | 4 | no data |
| Hemlibra | part of repeat dose toxicity | 1 non GLP, cynomolgus | 3 cynomolgus GLP | 26 weeks+13 weeks recovery | 26 | 13 |
| Herzuma | no | no | comparative | 4 weeks | 4 | no data |
| Hulio | no | no | comparative | 4 weeks | 4 | no data |
| Hyrimoz | no | no | comparative | 4 weeks | 4 | no data |
| Idacio | no | no | comparative | 4 weeks | 4 | no data |
| Ilaris | part of local tolerance study | 1 murine surogate | 5 marmoset | 26 weeks+6 weeks recovery | 26 | 6 |
| Ilumetri | part of Pharmacokinetics | no | 2 cynomolgus | 9 months | 39 | duration unknow |
| Imfinzi | no | 2 cynomolgus non-GLP, 2 cynomolgus GLP | 2 cynomolgus GLP | 13 weeks+8 weeks recovery | 13 | 8 |
| Imraldi | no | no | comparative | 4 weeks | 4 | no data |
| Inflectra | no | 1 with comparator only, dose finding, rat | 2 rat comparative GLP | 2 weeks | 2 | no data |
| Kadcyla | yes, 5 rat, 1 cynomolgus | 1 rat non-GLP | 2 cynomolgus non-GLP and 2 cynomolgus GLP | 6 weeks+3 weeks recovery | 6 | 3 |
| Kanjinti | no | 14 day rat | 1 cynomolgus comparative GLP | 4 weeks | 4 | no data |

| Medicine Name | Single-dose Toxicity Studies | Repeat-dose Toxicity (supportive) | Repeat-dose Toxicity Studies (RTDS, pivotal) | Duration pivotal RDTS (longest) | Duration RDTS in Weeks (calculated) | Recovery RDTS in Weeks (calculated) |
|---|---|---|---|---|---|---|
| Kevzara | no | no | 5 cynomolgus | 26 weeks | 26 | duration unknow |
| Keytruda | no | no | 2 cynomolgus | 6 months + 4 months recovery | 26 | 16 |
| Kromeya | no | no | 1 cynomolgus comparative GLP | 4 weeks | 4 | no data |
| Kyntheum | no | no | 3 cynomolgus | 6 months+6 months recovery | 26 | 26 |
| Lartruvo | part of repeat dose toxicity | no | 3 cynomolgus GLP | 39 weeks+8 weeks recovery | 39 | 8 |
| Lemtrada | yes, 3 cynomolgus | 1 non-GLP dose ranging cynomolgus | 1 cynomolgus GLP | 1 months | 4 | single dose tox. studies |
| Libtayo | no | no | 3 cynomolgus | 26 weeks | 26 | 12 |
| Mvasi | no | no | 1 cynomolgus comparative GLP | 4 weeks | 4 | no data |
| Mylotarg | yes, 1 mouse, 2 rat, 1 cynomolgus, 1 chimpanzee | no | 1 rat, 3 cynomolgus, 1 rabbit | 12 weeks | 12 | Tox. studies of shorter duration |
| Nivolumab BMS | yes, cynomolgus | no | 2 cynomolgus GLP | 3 months | 13 | 4 |
| Nucala | yes, cynomolgus | no | 2 cynomolgus | 6 months | 26 | duration unknow |
| Ocrevus | no | no | 3 cynomolgus | at least 149 days | 21 | Tox. studies of shorter duration |
| Ogivri | no | no | 1 cynomolgus comparative GLP | 4 weeks | 4 | no data |
| Ontruzant | no | no | 1 cynomolgus comparative GLP | 4 weeks | 4 | no data |
| Opdivo | yes, cynomolgus | no | 2 cynomolgus GLP | 3 months | 13 | 4 |
| Perjeta | no | no | 2 cynomolgus GLP | 26 weeks | 26 | 8 |
| Portrazza | no | no | 2 cynomolgus GLP | 26 weeks+8 weeks recovery | 26 | 8 |
| Poteligeo | yes, cynomolgus non-GLP | no | 3 cynomolgus GLP | 26 weeks | 26 | Tox. studies of shorter duration |
| Praluent | no data | no | 2 rat and cynomolgus | 26 weeks | 26 | duration unknow |

| Medicine Name | Single-dose Toxicity Studies | Repeat-dose Toxicity (supportive) | Repeat-dose Toxicity Studies (RTDS, pivotal) | Duration pivotal RDTS (longest) | Duration RDTS in Weeks (calculated) | Recovery RDTS in Weeks (calculated) |
|---|---|---|---|---|---|---|
| Praxbind | yes, non-GLP, rat | 1 rat GLP,<br>1 Rhesus escalating dose non GLP | 3 Rhesus GLP | 2 weeks+28 days recovery; rat 4 weeks + 4 weeks recovery | 2 | 4 |
| Prolia | part of safety pharmacology | no | 2 cynomolgus | 6 months+6 months recovery | 26 | 26 |
| Qarziba (beta Apeiron) | part of single dose PK and repeat dose toxicity | 1 GLP juvenile guinea pig | 1 cynomolgus GLP | 15 days, (36-15) days recovery | 7 | 4 |
| Removab | yes, cynomolgus, mouse (surrogate), rat | no | no | no | no | no |
| Remsima | no | 1 with comparator only, dose finding, rat | 2 comparative GLP rat | 2 weeks | 2 | no data |
| Repatha | part of repeat dose toxicity | no | cynomolgus, hamster | 6 months | 26 | no data |
| Ritemvia | no | no | 1 cynomolgus comparative GLP | 8 weeks | 8 | no data |
| Rixathon | no data | no | comparative | 4 weeks+6 months recovery | 4 | 26 |
| Riximyo | part of Pharmacokinetics | no | comparative | 4 weeks+6 months recovery | 4 | 26 |
| RoActemra | yes, cynomolgus | mouse surrogate | 2 cynomolgus | 6 months | 26 | no data |
| Scintimun | yes, mouse and rat | no | rat, cynomolgus | 30 days | 4 | no data |
| Simponi | part of single dose PK and repeat dose toxicity | mouse surrogate | 3 cynomolgus | 6 months + 3 month recovery | 26 | 13 |
| Skyrizi | yes, cynomolgus | no | 2 cynomolgus | 26 weeks | 26 | 8 |
| Solymbic | no | no | comparative | 1 months | 4 | no data |
| Stelara | part of single dose PK | yes | 2 cynomolgus GLP | 26-weeks with a 12-weeks recovery | 26 | 12 |
| Sylvant | no | 1 cynomolgus | 2 cynomolgus GLP | 6 months | 26 | 13 |
| Takhzyro | yes, rat, cynomolgus | 1 rat | 2 cynomolgus | 6 months | 26 | Tox. studies of shorter duration |
| Taltz | no | no | 2 cynomolgus | 39 weeks+16 weeks recovery | 39 | 16 |

| Medicine Name | Single-dose Toxicity Studies | Repeat-dose Toxicity (supportive) | Repeat-dose Toxicity Studies (RTDS, pivotal) | Duration pivotal RDTS (longest) | Duration RDTS in Weeks (calculated) | Recovery RDTS in Weeks (calculated) |
|---|---|---|---|---|---|---|
| Tecentriq | part of single dose PK | 1 non-GLP mouse | 2 cynomolgus GLP | 26 weeks | 26 | 13 |
| Trazimera | no | no | mouse comparative | 2 weeks | 4 | no data |
| Tremfya | yes, cynomolgus non GLP | 1 guinea pig non GLP | 1 cynomolgus GLP | 24 weeks | 24 | 12 |
| Trogarzo | yes, cynomolgus | no | cynomolgus, rhesus | 9 months | 39 | Tox. studies of shorter duration |
| Truxima | no | no | 1 cynomolgus comparative GLP | 8 weeks | 8 | no data |
| Ultomiris | no | 1 murine surogate dose range finding | 1 murine surogate | 26 weeks+4 weeks recovery | 26 | 4 |
| Unituxin | literatur review | no | dog, rat | 4 weeks+6 weeks recovery | 4 | 6 |
| Xgeva | part of repeat dose toxicity | no | 2 cynomolgus | 12 months+13 week srecovery | 52 | 13 |
| Yervoy | part of repeat dose toxicity | no | 7 cynomolgus | 6 months | 26 | no data |
| Zessly | part of repeat dose toxicity | no | rat comparative | 2 weeks | 2 | no data |
| Zinbryta | yes, cynomolgus | 2 cynomolgus | 2 cynomolgus | 39 weeks | 39 | no data |
| Zinplava | yes, mouse | no | 2 mouse | 3 weeks | 3 | no data |
| Zirabev | no | rat GLP | 1 cynomolgus comparative GLP | 1 months | 4 | no data |

**Annex 9:** Species used in Toxicology (Single and Repeat-Dose) Studies

*Bold font: non-active species*

| Medicine Name | Bio-similar | Number of Active Species | Active Species | All Species used in Toxicology Studies | Non Active Rodents used in Toxicology Studies | Non Active Non-Rodent used in Toxicology Studies |
|---|---|---|---|---|---|---|
| Adcetris | no | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Aimovig | no | 1 | cynomolgus | cynomolgus | | |
| Ajovy | no | 3 | cynomolgus, rat, rabbit | cynomolgus, rat | | |
| Amgevita | yes | 1 | cynomolgus | cynomolgus | | |
| Arzerra | no | 1 | cynomolgus | cynomolgus | | |
| Bavencio | no | 2 | cynomolgus, rat | cynomolgus, **mouse,** rat | mouse CD-1 | |
| Benlysta | no | 2 | cynomolgus, mouse | cynomolgus | | |
| Besponsa | no | 0 | no | **cynomolgus, rat** | rat | cynomolgus |
| Blincyto | no | 1 | chimpanzee | chimpanzee, **mouse model** | mouse model | |
| Blitzima | yes | 1 | cynomolgus | cynomolgus | | |
| Cablivi | no | 2 | cynomolgus, guinea pig | cynomolgus, guinea pig | | |
| Cimzia | no | 1 | cynomolgus | cynomolgus | | |
| Cinqaero | no | 2 | cynomolgus, rabbit | cynomolgus, **mouse, rat** | mouse CD-1, rat | |
| Cosentyx | no | 3 | cynomolgus, rhesus, marmoset | cynomolgus | | |
| Crysvita | no | 2 | cynomolgus, rabbit | cynomolgus, rabbit | | |
| Cyltezo | yes | 1 | cynomolgus | cynomolgus | | |
| Cyramza | no | 1 | cynomolgus | cynomolgus | | |
| Darzalex | no | 1 | chimpanzee | chimpanzee, **cynomolgus** | | cynomolgus |
| Dupixent | no | 0 | no | **cynomolgus (surrogate AB)** | | cynomolgus |
| Emgality | no | 3 | cynomolgus, rabbit, rat | cynomolgus, rat | | |
| Empliciti | no | 0 | no | **Rhesus (single dose off-target tox)** | | rhesus |
| Entyvio | no | 3 | cynomolgus, rhesus, rabbit | cynomolgus, rabbit | | |
| Fasenra | no | 1 | cynomolgus | cynomolgus | | |
| Flixabi | yes | 0 | no | no | | |
| Gazyvaro | no | 1 | cynomolgus | cynomolgus | | |
| Halimatoz | yes | 1 | cynomolgus | cynomolgus | | |
| Hefiya | yes | 1 | cynomolgus | cynomolgus | | |
| Hemlibra | no | 1 | cynomolgus | cynomolgus | | |

| Medicine Name | Bio-similar | Number of Active Species | Active Species | All Species used in Toxicology Studies | Non Active Rodents used in Toxicology Studies | Non Active Non-Rodent used in Toxicology Studies |
|---|---|---|---|---|---|---|
| Herzuma | yes | 0 | no | **cynomolgus** | | |
| Hulio | yes | 1 | cynomolgus | cynomolgus | | |
| Hyrimoz | yes | 1 | cynomolgus | cynomolgus | | |
| Idacio | yes | 1 | cynomolgus | cynomolgus | | |
| Ilaris | no | 1 | marmoset | marmoset, **mouse model** | mouse model | |
| Ilumetri | no | 1 | cynomolgus | cynomolgus | | |
| Imfinzi | no | 1 | cynomolgus | cynomolgus | | |
| Imraldi | yes | 1 | cynomolgus | cynomolgus | | |
| Inflectra | yes | 0 | no | **rat (off-target tox)** | | |
| Kadcyla | no | 1 | cynomolgus | cynomolgus, **rat** | rat | |
| Kanjinti | yes | 0 | no | **cynomolgus, rat** | rat | |
| Kevzara | no | 1 | cynomolgus | cynomolgus | | |
| Keytruda | no | 1 | cynomolgus | cynomolgus | | |
| Kromeya | yes | 1 | cynomolgus | cynomolgus | | |
| Kyntheum | no | 1 | cynomolgus, rabbit (weak) | cynomolgus | | |
| Lartruvo | no | 1 | cynomolgus | cynomolgus | | |
| Lemtrada | no | 1 | cynomolgus | cynomolgus | | |
| Libtayo | no | 1 | cynomolgus | cynomolgus | | |
| Mvasi | yes | 1 | cynomolgus | cynomolgus | | |
| Mylotarg | no | 0 | no | **cynomolgus, chimpanzee, mouse, rat, rabbit** | mouse CD-1, rat | cynomolgus, chimpanzee, rabbit |
| Nivolumab BMS | no | 1 | cynomolgus | cynomolgus | | |
| Nucala | no | 1 | cynomolgus | cynomolgus | | |
| Ocrevus | no | 1 | cynomolgus | cynomolgus | | |
| Ogivri | yes | 0 | no | **cynomolgus** | | |
| Ontruzant | yes | 0 | no | **cynomolgus** | | |
| Opdivo | no | 1 | cynomolgus | cynomolgus | | |
| Perjeta | no | 1 | cynomolgus | cynomolgus | | |
| Portrazza | no | 1 | cynomolgus, rabbit (weak) | cynomolgus | | |
| Poteligeo | no | 1 | cynomolgus | cynomolgus | | |
| Praluent | no | 4 | cynomolgus, rat, mouse (weak), hamster (weak) | cynomolgus, rat | | |

| Medicine Name | Bio-similar | Number of Active Species | Active Species | All Species used in Toxicology Studies | Non Active Rodents used in Toxicology Studies | Non Active Non-Rodent used in Toxicology Studies |
|---|---|---|---|---|---|---|
| Praxbind | no | 2 | rhesus rat | rhesus | | |
| Prolia | no | 1 | cynomolgus | cynomolgus | | |
| Qarziba (beta Apeiron) | no | 2 | cynomolgus, guinea pig | cynomolgus, guinea pig | | |
| Removab | no | 0 | no | **cynomolgus, rat, mouse (surrogate)** | | cynomolgus, rat, mouse (surrogate) |
| Remsima | yes | 0 | no | **rat** | rat | |
| Repatha | no | 2 | cynomolgus, hamster | cynomolgus, hamster | | |
| Ritemvia | yes | 1 | cynomolgus | cynomolgus | | |
| Rixathon | yes | 1 | cynomolgus | cynomolgus | | |
| Riximyo | yes | 1 | cynomolgus | cynomolgus | | |
| RoActemra | no | 1 | cynomolgus | cynomolgus | | |
| Scintimun | no | 1 | cynomolgus | cynomolgus, **mouse, rat** | mouse, rat | |
| Simponi | no | 1 | cynomolgus | cynomolgus, **mouse model** | mouse model | |
| Skyrizi | no | 1 | cynomolgus | cynomolgus | | |
| Solymbic | yes | 1 | cynomolgus | cynomolgus | | |
| Stelara | no | 1 | cynomolgus | cynomolgus | | |
| Sylvant | no | 7 | cynomolgus, chimpanzee, baboon, pigtailed macaque, cotton-top tamarin, marmoset, rhesus | cynomolgus | | |
| Takhzyro | no | 2 | cynomolgus, rat, mouse (weak) | cynomolgus, rat | | |
| Taltz | no | 1 | cynomolgus | cynomolgus | | |
| Tecentriq | no | 2 | cynomolgus, mouse | cynomolgus | | |
| Trazimera | yes | 0 | no | **mouse CD-1** | | |
| Tremfya | no | 2 | cynomolgus, guinea pig | cynomolgus, guinea pig | | |
| Trogarzo | no | 2 | cynomolgus, rhesus | cynomolgus, rhesus | | |
| Truxima | yes | 1 | cynomolgus | cynomolgus | | |
| Ultomiris | no | 0 | no | **mouse model** | mouse model | |
| Unituxin | no | 0 | no | **rat, dog** | rat | dog |
| Xgeva | no | 1 | cynomolgus | cynomolgus | | |
| Yervoy | no | 1 | cynomolgus | cynomolgus | | |
| Zessly | yes | 1 | chimpanzee (not used) | **rat off-target toxicity** | rat | |

| Medicine Name | Bio-similar | Number of Active Species | Active Species | All Species used in Toxicology Studies | Non Active Rodents used in Toxicology Studies | Non Active Non-Rodent used in Toxicology Studies |
|---|---|---|---|---|---|---|
| Zinbryta | no | 1 | cynomolgus | cynomolgus | | |
| Zinplava | no | 2 | mouse, hamster | mouse | | |
| Zirabev | yes | 1 | cynomolgus | cynomolgus | | |

**Annex 10:** Genotoxicity studies

| Medicine name | Biosimilar | Active substance | Genotoxicity | Linker | Conjugate |
|---|---|---|---|---|---|
| Adcetris | no | Brentuximab vedotin | yes | cleavable linker | monomethyl auristatin E (MMAE) disrupts mitotic spindle |
| Aimovig | no | Erenumab | no | | |
| Ajovy | no | Fremanezumab | no | | |
| Amgevita | yes | Adalimumab | no | | |
| Arzerra | no | Ofatumumab | no | | |
| Bavencio | no | Avelumab | no | | |
| Benlysta | no | Belimumab | no | | |
| Besponsa | no | Inotuzumab ozogamicin | yes 3 *in vitro* 1 *in vivo* | acid cleavable linker | N acetyl gamma calicheamicin dimethylhydrazide= double-stranded DNA breaks |
| Blincyto | no | Blinatumomab | no | | |
| Blitzima | yes | Rituximab | no | | |
| Cablivi | no | Caplacizumab | no | | |
| Cimzia | no | Certolizumab pegol | yes | maleimide linker | polyethylene glycol (PEG) in order to extend plasma half-life |
| Cinqaero | no | Reslizumab | yes 2 *in vitro* | | |
| Cosentyx | no | Secukinumab | no | | |
| Crysvita | no | Burosumab | no | | |
| Cyltezo | yes | Adalimumab | no data | | |
| Cyramza | no | Ramucirumab | no | | |
| Darzalex | no | Daratumumab | no | | |
| Dupixent | no | Dupilumab | no | | |
| Emgality | no | Galcanezumab | no | | |
| Empliciti | no | Elotuzumab | no | | |
| Entyvio | no | Vedolizumab | no | | |
| Fasenra | no | Benralizumab | no | | |
| Flixabi | yes | Infliximab | no | | |
| Gazyvaro | no | Obinutuzumab | no | | |
| Halimatoz | yes | Adalimumab | no | | |
| Hefiya | yes | Adalimumab | no | | |
| Hemlibra | no | Emicizumab | no | | |
| Herzuma | yes | Trastuzumab | no | | |
| Hulio | yes | Adalimumab | no | | |
| Hyrimoz | yes | Adalimumab | no | | |
| Idacio | yes | Adalimumab | no data | | |
| Ilaris | no | Canakinumab | no | | |

| Medicine name | Biosimilar | Active substance | Genotoxicity | Linker | Conjugate |
|---|---|---|---|---|---|
| Ilumetri | no | Tildrakizumab | no | | |
| Imfinzi | no | Durvalumab | no | | |
| Imraldi | yes | Adalimumab | no | | |
| Inflectra | yes | Infliximab | no | | |
| Kadcyla | no | Trastuzumab emtansine | yes, *in vitro* and *in vivo* rat, cynomolgus | thioether bond | microtubule-inhibitory maytansinoid, DM1 |
| Kanjinti | yes | Trastuzumab | no | | |
| Kevzara | no | Sarilumab | no | | |
| Keytruda | no | Pembrolizumab | no | | |
| Kromeya | yes | Adalimumab | no data | | |
| Kyntheum | no | Brodalumab | no | | |
| Lartruvo | no | Olaratumab | no | | |
| Lemtrada | no | Alemtuzumab | no | | |
| Libtayo | no | Cemiplimab | no | | |
| Mvasi | yes | Bevacizumab | no | | |
| Mylotarg | no | Gemtuzumab ozogamicin | yes, *in vitro* and *in vivo* mouse | AcBut (4-(4-acetylphenoxy) butanoic acid) linker | N acetyl gamma calicheamicin=double-stranded DNA breaks |
| Nivolumab BMS | no | Nivolumab | no | | |
| Nucala | no | Mepolizumab | no | | |
| Ocrevus | no | Ocrelizumab | no | | |
| Ogivri | yes | Trastuzumab | no | | |
| Ontruzant | yes | Trastuzumab | no | | |
| Opdivo | no | Nivolumab | no | | |
| Perjeta | no | Pertuzumab | no | | |
| Portrazza | no | Necitumumab | no | | |
| Poteligeo | no | Mogamulizumab | no | | |
| Praluent | no | Alirocumab | no | | |
| Praxbind | no | Idarucizumab | no | | |
| Prolia | no | Denosumab | no | | |
| Qarziba (beta Apeiron) | no | Dinutuximab beta | no | | |
| Removab | no | Catumaxomab | no | | |
| Remsima | yes | Infliximab | no | | |
| Repatha | no | Evolocumab | no | | |
| Ritemvia | yes | Rituximab | no | | |
| Rixathon | yes | Rituximab | no | | |

| Medicine name | Biosimilar | Active substance | Genotoxicity | Linker | Conjugate |
|---|---|---|---|---|---|
| Riximyo | yes | Rituximab | no | | |
| RoActemra | no | Tocilizumab | no | | |
| Scintimun | no | Besilesomab Technetium (99mTc)-labeled | yes | Radioactive labelled mAb | |
| Simponi | no | Golimumab | no | | |
| Skyrizi | no | Risankizumab | no | | |
| Solymbic | yes | Adalimumab | no | | |
| Stelara | no | Ustekinumab | no | | |
| Sylvant | no | Siltuximab | no | | |
| Takhzyro | no | Lanadelumab | no | | |
| Taltz | no | Ixekizumab | no | | |
| Tecentriq | no | Atezolizumab | no | | |
| Trazimera | yes | Trastuzumab | no | | |
| Tremfya | no | Guselkumab | no | | |
| Trogarzo | no | Ibalizumab | no | | |
| Truxima | yes | Rituximab | no | | |
| Ultomiris | no | Ravulizumab | no | | |
| Unituxin | no | Dinutuximab | no | | |
| Xgeva | no | Denosumab | no | | |
| Yervoy | no | Ipilimumab | no | | |
| Zessly | yes | Infliximab | no | | |
| Zinbryta | no | Daclizumab | no | | |
| Zinplava | no | Bezlotoxumab | no | | |
| Zirabev | yes | Bevacizumab | no | | |

**Annex 11** Carcinogenicity studies

*WoE Approach - Weight of the Evidence Approach*

| Medicine Name | Bio similar | Carcino-genicity Study performed | WoE Approach Factor 1: Risk Assessment | WoE Approach Factor 2: Intended Indication | WoE Approach Factor 3: Literature review or other Source | WoE Approach Factor 4: Treatment Duration | WoE Approach Factor 5: Feasibility | Measures |
|---|---|---|---|---|---|---|---|---|
| Adcetris | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Aimovig | no | no | low risk | Analgesics | other non-clinical studies +literature review | long term treatment | not feasible | |
| Ajovy | no | no | low risk | Analgesics | literature review | long term treatment | | |
| Amgevita | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Arzerra | no | no | low risk | CLL | other non-clinical studies (repeat dose)+literature review | long term treatment | not feasible | |
| Bavencio | no | no | not performed | Advanced Cancer | | | | |
| Benlysta | no | no | low risk | | repeat dose toxicity studies | long term treatment | not feasible | |
| Besponsa | no | no | not applicable ICH S9 | Advanced Cancer | | | | RMP Important potential risks |
| Blincyto | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Blitzima | yes | no | not applicable biosimilar | Advanced Cancer | | | | RMP Important potential risks |
| Cablivi | no | no | low risk | | repeat dose toxicity studies | short term treatment | | |
| Cimzia | no | no | high risk | Immunosuppressants | literature review | long term treatment | not feasible | SmPC/RMP Important potential risks |
| Cinqaero | no | yes | not certain evidence | Other systemic drugs for obstructive airway diseases | | long term treatment | | SmPC/RMP Important potential risks |

| Medicine Name | Bio similar | Carcino-genicity Study performed | WoE Approach Factor 1: Risk Assessment | WoE Approach Factor 2: Intended Indication | WoE Approach Factor 3: Literature review or other Source | WoE Approach Factor 4: Treatment Duration | WoE Approach Factor 5: Feasibility | Measures |
|---|---|---|---|---|---|---|---|---|
| Cosentyx | no | no | low risk | Immunosuppressants | literature review | long term treatment | not feasible | RMP Important potential risks |
| Crysvita | no | no | low risk | | repeat dose toxicity studies + literature review | long term treatment | | |
| Cyltezo | yes | no data | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Cyramza | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Darzalex | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Dupixent | no | no | low risk | | other non-clinical studies +literature review | long term treatment | | RMP Important potential risks |
| Emgality | no | no | unpredictable | Analgesics | literature review | short term treatment | | |
| Empliciti | no | no | unpredictable | Advanced Cancer | | | not feasible | SmPC |
| Entyvio | no | no | no data | Immunosuppressants | in vitro human tumour tissue | long term treatment | not feasible | SmPC/RMP |
| Fasenra | no | no | unpredictable | Drugs for obstructive airway diseases | literature review | long term treatment | not feasible | RMP Important potential risks |
| Flixabi | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Gazyvaro | no | no | pointless | Advanced Cancer | | | | RMP Important potential risks |
| Halimatoz | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Hefiya | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Hemlibra | no | no | not performed | Antihemorrhagics | not applicable ICH S6 | long term treatment | | |
| Herzuma | yes | no | not applicable biosimilar | Advanced Cancer | | | | |

| Medicine Name | Bio similar | Carcino-genicity Study performed | WoE Approach Factor 1: Risk Assessment | WoE Approach Factor 2: Intended Indication | WoE Approach Factor 3: Literature review or other Source | WoE Approach Factor 4: Treatment Duration | WoE Approach Factor 5: Feasibility | Measures |
|---|---|---|---|---|---|---|---|---|
| Hulio | yes | no | not applicable biosimilar | Immunosuppressants, TNFα inhibitors | | | | RMP Important potential risks |
| Hyrimoz | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Idacio | yes | no data | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Ilaris | no | no | low risk | Interleukin inhibitors | literature review | long term treatment | | SmPC/RMP Important potential risks |
| Ilumetri | no | no | low risk | immunmodulators | other non-clinical studies +literature review | long term treatment | | |
| Imfinzi | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Imraldi | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Inflectra | yes | no | not applicable biosimilar | Immunosuppressants, TNFα inhibitors | | | | RMP Important potential risks |
| Kadcyla | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Kanjinti | yes | no | not applicable biosimilar | Advanced Cancer | | | | |
| Kevzara | no | no | low risk | immunmodulators | other non-clinical studies (repeat dose)+literature review | long term treatment | | RMP Important potential risks |
| Keytruda | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Kromeya | yes | no data | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Kyntheum | no | no | not certain evidence | immunmodulators | repeat dose toxicity studies + literature review | long term treatment | | RMP Important potential risks |
| Lartruvo | no | no | not applicable ICH S9 | Advanced Cancer | | | | RMP Missing Information |

| Medicine Name | Bio similar | Carcino-genicity Study performed | WoE Approach Factor 1: Risk Assessment | WoE Approach Factor 2: Intended Indication | WoE Approach Factor 3: Literature review or other Source | WoE Approach Factor 4: Treatment Duration | WoE Approach Factor 5: Feasibility | Measures |
|---|---|---|---|---|---|---|---|---|
| Lemtrada | no | no | known risk | Immunosuppressants | literature review | long term treatment | not feasible | RMP Important potential risks |
| Libtayo | no | no | known risk | Advanced Cancer | | | | |
| Mvasi | yes | no | not applicable biosimilar | Advanced Cancer | | | | |
| Mylotarg | no | no | low risk | Advanced Cancer | | | | RMP Important potential risks |
| Nivolumab BMS | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Nucala | no | no | not performed | Drugs for obstructive airway diseases | CHMP advice | long term treatment | | |
| Ocrevus | no | no | not performed | Immunosuppressants | ?? not applicable ICH S6 | long term treatment | | SmPC/RMP Important potential risks |
| Ogivri | yes | no | not applicable biosimilar | Advanced Cancer | | | | |
| Ontruzant | yes | no | not applicable biosimilar | Advanced Cancer | | | | |
| Opdivo | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Perjeta | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Portrazza | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Poteligeo | no | no | low risk | Advanced Cancer | | | | |
| Praluent | no | no | low risk | | repeat dose toxicity studies | long term treatment | | |
| Praxbind | no | no | no risk | anti dabigatran mAB | | short term treatment | | |
| Prolia | no | no | not certain evidence | | primary pharmacodynamic study | long term treatment | | |

| Medicine Name | Bio similar | Carcino-genicity Study performed | WoE Approach Factor 1: Risk Assessment | WoE Approach Factor 2: Intended Indication | WoE Approach Factor 3: Literature review or other Source | WoE Approach Factor 4: Treatment Duration | WoE Approach Factor 5: Feasibility | Measures |
|---|---|---|---|---|---|---|---|---|
| Qarziba (beta Apeiron) | no | no | low risk | Advanced Cancer | | short term treatment | | |
| Removab | no | no | not applicable ICH S9 | Advanced Cancer | | | | |
| Remsima | yes | no | not applicable biosimilar | Immunosuppressants, TNFα inhibitors | | | | RMP Important potential risks |
| Repatha | no | part of repeat dose | low risk | | repeat dose toxicity studies | long term treatment | | |
| Ritemvia | yes | no | not applicable biosimilar | Advanced Cancer | | | | RMP Important potential risks |
| Rixathon | yes | no | not applicable biosimilar | Advanced Cancer | | | | RMP Important potential risks |
| Riximyo | yes | no | not applicable biosimilar | Advanced Cancer | | | | RMP Important potential risks |
| RoActemra | no | no data | known risk | Immunosuppressants | | long term treatment | | RMP Important potential risks |
| Scintimun | no | no | low risk | diagnostic containing an antibody for a single use | | one-time use | | SmPC/RMP Important potential risks |
| Simponi | no | no | high risk | Immunosuppressants | | | not feasible | RMP Important potential risks |
| Skyrizi | no | no | low risk | Immunosuppressants | other non-clinical studies +literature review | | not feasible | RMP Important potential risks |
| Solymbic | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Stelara | no | no | not certain evidence | immunmodulators | | long term treatment | not feasible | SmPC/RMP |
| Sylvant | no | no | low risk | | other non-clinical studies | long term treatment | | RMP Important potential risks |
| Takhzyro | no | no | low risk | | other non-clinical studies (repeat dose)+literature review | long term treatment | | |

| Medicine Name | Bio similar | Carcino-genicity Study performed | WoE Approach Factor 1: Risk Assessment | WoE Approach Factor 2: Intended Indication | WoE Approach Factor 3: Literature review or other Source | WoE Approach Factor 4: Treatment Duration | WoE Approach Factor 5: Feasibility | Measures |
|---|---|---|---|---|---|---|---|---|
| Taltz | no | no | low risk | Immunosuppressants | other non-clinical studies (repeat dose)+literature review | long term treatment | | RMP Important potential risks |
| Tecentriq | no | no | low risk | Advanced Cancer | | | | |
| Trazimera | yes | no | not applicable biosimilar | Advanced Cancer | | | | |
| Tremfya | no | no | low risk | Immunosuppressants | literature review | long term treatment | not feasible | RMP Important potential risks |
| Trogarzo | no | no | low risk | Antivirals for systemic use | | long term treatment | not feasible | SmPC/RMP/Post Marketing Surv |
| Truxima | yes | no | not applicable biosimilar | Advanced Cancer | | | | RMP Important potential risks |
| Ultomiris | no | no | low risk | | other non-clinical studies | long term treatment | | RMP Important potential risks |
| Unituxin | no | no | low risk | Advanced Cancer | | short term treatment | | |
| Xgeva | no | no | not certain evidence | | primary pharmacodynamic study | long term treatment | | RMP |
| Yervoy | no | no | not applicable ICH S9 | Advanced Cancer | | | not feasible | |
| Zessly | yes | no | not applicable biosimilar | Immunosuppressants | | | | RMP Important potential risks |
| Zinbryta | no | no | low risk | Immunosuppressants | literature review | long term treatment | | RMP Important potential risks |
| Zinplava | no | no | no risk | anti clostriduim difficile mAB | | | | |
| Zirabev | yes | no | not applicable biosimilar | Advanced Cancer | | | | |

**Annex 12:** Fertility and early embryonic Development Studies (FEED)

| Medicine Name | Biosimilar | FEED Studies | justification for no | FEED Species | FEED conducted in relevant species |
|---|---|---|---|---|---|
| Adcetris | no | no? | | testicular toxicity in rats!! | |
| Aimovig | no | no data | | no data | |
| Ajovy | no | yes | no indications | rats and rabbits | yes rat |
| Amgevita | yes | no | | no | |
| Arzerra | no | no | low risk / patients age / SmPC | no | |
| Bavencio | no | part of repeat dose | risk is already identified | | |
| Benlysta | no | part of repeat dose | no indications / SmPC | 6 month cynomolgus | |
| Besponsa | no | part of repeat dose | risk is already identified | | |
| Blincyto | no | part of repeat dose | no indications / SmPC | mouse surrogate | |
| Blitzima | yes | part of repeat dose | | 8 weeks cynomolgus | |
| Cablivi | no | part of repeat dose | no indications | cynomolgus | |
| Cimzia | no | yes | no indications | rat homologous agent | no |
| Cinqaero | no | yes | no indications | 2 GLP CD-1 mouse | no |
| Cosentyx | no | yes | no indications | mouse surrogate | no |
| Crysvita | no | part of repeat dose | Mineralization of the testes, monitoring of serum phosphate | cynomolgus | |
| Cyltezo | yes | no | | no | |
| Cyramza | no | no | risk is already identified, adressed in SmPC/ RMP-potential risk | | |
| Darzalex | no | no data | not feasible /patients age, adressed in SmPC | the majority of patients is beyond reproductive age | |
| Dupixent | no | yes | no indications | mouse surrogate | no active |
| Emgality | no | yes | no indications, testis weight was affected only | rat | yes rat |

| Medicine Name | Biosimilar | FEED Studies | justification for no | FEED Species | FEED conducted in relevant species |
|---|---|---|---|---|---|
| Empliciti | no | no | lack of an adequate animal model/ unknown risk/ SmPC | | |
| Entyvio | no | part of repeat dose | no indications /SmPC | cynomolgus | |
| Fasenra | no | part of repeat dose | no indications /SmPC | cynomolgus | |
| Flixabi | yes | no | | no | |
| Gazyvaro | no | part of repeat dose | no indications, but intended clinical setting with fertility risk bearing clorambucil | cynomolgus | |
| Halimatoz | yes | no | | no | |
| Hefiya | yes | no | | no | |
| Hemlibra | no | part of repeat dose | no indications | cynomolgus | |
| Herzuma | yes | no | | no | |
| Hulio | yes | no | | no | |
| Hyrimoz | yes | no | | no | |
| Idacio | yes | no data | | | |
| Ilaris | no | yes + part of repeat dose | no indications | full panel mouse surrogate, marmosets | yes marmoset |
| Ilumetri | no | part of repeat dose | no indications /not expected | cynomolgus | |
| Imfinzi | no | part of repeat dose (male only) | no jutification given | cynomolgus | |
| Imraldi | yes | no | | no | |
| Inflectra | yes | no | | no | |
| Kadcyla | no | no | risk is already identified /SmpC | | |
| Kanjinti | yes | no | | no | |
| Kevzara | no | yes | no indications | mouse surrogate | no |
| Keytruda | no | no | advanced cancer | few data available, SmPC section 5.3 | |
| Kromeya | yes | no | | no | |

| Medicine Name | Biosimilar | FEED Studies | justification for no | FEED Species | FEED conducted in relevant species |
|---|---|---|---|---|---|
| Kyntheum | no | part of repeat dose | no indications | cynomolgus, rabbit | |
| Lartruvo | no | no | risk is already identified, advanced cancer | | |
| Lemtrada | no | yes | affected | transgenic mouse | no |
| Libtayo | no | part of repeat dose | no indications | cynomolgus | |
| Mvasi | yes | no | | no | |
| Mylotarg | no | yes | affected | rat | no active |
| Nivolumab BMS | no | no | advanced cancer | advanced cancer | |
| Nucala | no | yes | no indications | mouse CD-1 rat hololog ous ab | no |
| Ocrevus | no | yes | no indications | cynomolgus | yes cynomolgus |
| Ogivri | yes | no | | no | |
| Ontruzant | yes | no | | no | |
| Opdivo | no | no data | | no data | |
| Perjeta | no | part of repeat dose | advanced cancer /no indications | | |
| Portrazza | no | no | advanced cancer /unknown risk | | |
| Poteligeo | no | part of repeat dose | no indications | | |
| Praluent | no | no data | | no data | |
| Praxbind | no | tissue cross reactivity study | not expected | low risk | |
| Prolia | no | yes + part of repeat dose | no indications | cynomolgus | yes cynomolgus |
| Qarziba (beta Apeiron) | no | part of repeat dose | Risk for foetus is already identified + co medication with a potential teratogen. no indications for fertility, mammary gland absence only | cynomolgus, guinea pig (for fertility) | |
| Removab | no | no data | advanced cancer | no | |
| Remsima | yes | no | | no | |
| Repatha | no | yes in Hamster + part of repeat dose | no indications | hamster cynomolgus | yes hamster cynomolgus |

| Medicine Name | Biosimilar | FEED Studies | justification for no | FEED Species | FEED conducted in relevant species |
|---|---|---|---|---|---|
| Ritemvia | yes | part of repeat dose | | | |
| Rixathon | yes | no | | no | |
| Riximyo | yes | no | | no | |
| RoActemra | no | part of repeat dose | no indications | cynomolgus | |
| Scintimun | no | part of repeat dose | no indications | cynomolgus? | |
| Simponi | no | yes | affected | mouse surrogate | no |
| Skyrizi | no | part of repeat dose | no indications /SmPC | cynomolgus | |
| Solymbic | yes | no | | no | |
| Stelara | no | yes | female affected, male not affected | mouse surrogate | no |
| Sylvant | no | yes | no indications | mouse surrogate | no |
| Takhzyro | no | yes | no indications | cynomolgus | yes cynomolgus |
| Taltz | no | yes | no indications | cynomolgus GLP | yes cynomolgus |
| Tecentriq | no | part of repeat dose | female affected, male not affected | cynomolgus | |
| Trazimera | yes | no | | no | |
| Tremfya | no | yes | no indications | guinea pigs | yes guinea pigs |
| Trogarzo | no | no | patients age | the majority of patients is beyond reproductive age | |
| Truxima | yes | part of repeat dose | | | |
| Ultomiris | no | yes | no indications | mouse surrogate | no active |
| Unituxin | no | no | advanced cancer | advanced cancer | |
| Xgeva | no | part of repeat dose | no indications | cynomolgus | |
| Yervoy | no | part of repeat dose (male only) + tissue cross reactivity | testis weights affected | cynomolgus | |
| Zessly | yes | no | | no | |
| Zinbryta | no | yes | no indications | cynomolgus | yes cynomolgus |
| Zinplava | no | part of repeat dose +tissue cross reactivity studies | not expected/ non-human target /SmPC | | |
| Zirabev | yes | no | | no | |

**Annex 13:** Embryo-Foetal Development (EFD) and Pre-and Postnatal Development (PPND) Studies

*Bold font: non-active species*

| Medicine Name | Bio similar | Embryo-Foetal Development (EFD) Studies | Species used for EFD | EFD conducted in Relevant Species | Pre-and Postnatal Development (PPND) Studies | Species used for PPND | PPND conducted in Relevant Species |
|---|---|---|---|---|---|---|---|
| Adcetris | no | yes | **rat** | no | no, the risk is already identified + advanced cancer | | |
| Aimovig | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Ajovy | no | yes | rabbit | yes | yes, rat | rat | yes |
| Amgevita | yes | no | | | no | | |
| Arzerra | no | yes, cynomolgus | cynomolgus | yes | no, the patients is beyond reproductive age + no species +no binding | | |
| Bavencio | no | no, the risk is already identified | | | no, the risk is already identified | | |
| Benlysta | no | ePPND | cynomolgus | yes | ePPND cynomolgus | cynomolgus | yes |
| Besponsa | no | yes, 2 rat 1 rabbit, is beleaved to have potential impair | **rat, rabbit** | no active species | no data | | |
| Blincyto | no | yes | **mouse surrogate** | no | no data | | |
| Blitzima | yes | no | | | no | | |
| Cablivi | no | yes | guinea pig | yes | no, no jusification | | |
| Cimzia | no | yes | **rat** | no | yes rat | **rat** | no |
| Cinqaero | no | yes, 2 (1 GLP, 1 non GLP) mouse CD-1 and rabbit | Rabbit, **Mouse** | yes and no | yes, GLP, mouse, cynomolgus | cynomolgus, **mouse** | yes +no |
| Cosentyx | no | yes GLP | **mouse surrogate** | no | yes mouse surrogate | **mouse surrogate** | no |
| Crysvita | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Cyltezo | yes | no | | | no | | |
| Cyramza | no | no, the risk is already identified | | | no, the risk is already identified | | |
| Darzalex | no | no, the majority of patients is beyond reproductive age | | | no, the majority of patients is beyond reproductive age | | |

| Medicine Name | Bio similar | Embryo-Foetal Development (EFD) Studies | Species used for EFD | EFD conducted in Relevant Species | Pre-and Postnatal Development (PPND) Studies | Species used for PPND | PPND conducted in Relevant Species |
|---|---|---|---|---|---|---|---|
| Dupixent | no | ePPND | **cynomolgus** | no active species | ePPND | **cynomolgus** | no active species |
| Emgality | no | yes (rat rabbit) | rat, rabbit | yes | yes | rat | yes |
| Empliciti | no | no animal studies are irrelevant because of absent of reactive species | | | no, risk unknown, combination with lenalidomide, which is contraindicated during pregnancy | | |
| Entyvio | no | yes, rabbit | rabbit | yes | yes, cynomolgus | cynomolgus | yes |
| Fasenra | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Flixabi | yes | no | | | no | | |
| Gazyvaro | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Halimatoz | yes | no | | | no | | |
| Hefiya | yes | no | | | no | | |
| Hemlibra | no | no, no impair expected, the majority of patients are male | | | no, low risk,+ target population are male | | |
| Herzuma | yes | no | | | no | | |
| Hulio | yes | no | | | no | | |
| Hyrimoz | yes | no | | | no | | |
| Idacio | yes | no data | | | no data | | |
| Ilaris | no | yes, marmoset +surrogate | marmoset, **mouse surrogate** | yes and no | yes, surrogate | **mouse surrogate** | no |
| Ilumetri | no | yes, cynomolgus | cynomolgus | yes | yes, cynomolgus | cynomolgus | yes |
| Imfinzi | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Imraldi | yes | no | | | no | | |
| Inflectra | yes | no | | | no | | |
| Kadcyla | no | no, the risk is already identified | | | no, the risk is already identified | | |
| Kanjinti | yes | no | | | no | | |

| Medicine Name | Bio similar | Embryo-Foetal Development (EFD) Studies | Species used for EFD | EFD conducted in Relevant Species | Pre-and Postnatal Development (PPND) Studies | Species used for PPND | PPND conducted in Relevant Species |
|---|---|---|---|---|---|---|---|
| Kevzara | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Keytruda | no | no, the risk is already identified (literatur assessment) | | | no, the risk is already identified | | |
| Kromeya | yes | no | | | no | | |
| Kyntheum | no | ePPND cinomolgus, dose range finding rabbit | cynomolgus, rabbit | yes | ePPND cinomolgus, dose range finding rabbit | cynomolgus, rabbit | yes |
| Lartruvo | no | yes | **mouse surrogate** | no | no, no jusification | | |
| Lemtrada | no | 1 GLP mouse model | **mouse surrogate** | no | 2 GLP mouse model | **mouse surrogate** | no |
| Libtayo | no | no, because is beleaved to have potential impair | | | no, the risk is already identified | | |
| Mvasi | yes | no | | | no | | |
| Mylotarg | no | yes | **rat** | no | no data | | |
| Nivolumab BMS | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Nucala | no | yes cynomolgus | cynomolgus | yes | yes, cynomolgus | cynomolgus | yes |
| Ocrevus | no | yes cynomolgus | cynomolgus | yes | yes, cynomolgus | cynomolgus | yes |
| Ogivri | yes | no | | | no | | |
| Ontruzant | yes | no | | | no | | |
| Opdivo | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Perjeta | no | yes | cynomolgus | yes | no, S9 and S6 (advanced cancer) | | |
| Portrazza | no | no, the risk is already identified | | | no, the risk is already identified + advanced cancer | | |
| Poteligeo | no | yes | cynomolgus | yes | no, S9 (advanced cancer) | | |
| Praluent | no | yes, rats + ePPND cinomolgus | ePPND cynomolgus, rat | yes | ePPND cynomolgus | cynomolgus | yes |
| Praxbind | no | no, tissue cross reactivity studes only | | | no, no jusification (non-human target?) | | |
| Prolia | no | yes | cynomolgus | yes | no data | | |

| Medicine Name | Bio similar | Embryo-Foetal Development (EFD) Studies | Species used for EFD | EFD conducted in Relevant Species | Pre-and Postnatal Development (PPND) Studies | Species used for PPND | PPND conducted in Relevant Species |
|---|---|---|---|---|---|---|---|
| Qarziba (beta Apeiron) | no | no, the risk is already identified, co medication with a potential teratogen, the patients are under reproductive age (10 years) | | | no, the risk is already identified, combination with a potential teratogen, the patients are under reproductive age (10 years) | | |
| Removab | no | no, no justification (advanced cancer?) | | | no, no jusification | | |
| Remsima | yes | no | | | no | | |
| Repatha | no | ePPND cynomolgus | ePPND cynomolgus | yes | ePPND cynomolgus | cynomolgus | yes |
| Ritemvia | yes | no | | | no | | |
| Rixathon | yes | no | | | no | | |
| Riximyo | yes | no | | | no | | |
| RoActemra | no | yes, cynomolgus+ mouse model | cynomolgus, **mouse surrogate** | yes and no | yes cynomolgus | cynomolgus | yes |
| Scintimun | no | no, no justification (diagnostic for single use?) | | | no, no jusification (target population potreproductive women?) | | |
| Simponi | no | yes, cynomolgus+ 1 mouse + 1 mouse ePPND | cynomolgus, ePPND **mouse** | yes and no | yes, cynomolgus +1 mouse + 1 mouse ePPND | cynomolgus, **mouse** | yes +no |
| Skyrizi | no | ePPND | cynomolgus | yes | ePPND | cynomolgus | yes |
| Solymbic | yes | no | | | no | | |
| Stelara | no | yes cynomolgus | cynomolgus | yes | yes, cynomolgus | cynomolgus | yes |
| Sylvant | no | yes, cynomolgus | cynomolgus | yes | yes, cynomolgus | cynomolgus | yes |
| Takhzyro | no | ePPND cynomolgus | cynomolgus | yes | ePPND cynomolgus | cynomolgus | yes |
| Taltz | no | yes, GLP, cynomolgus | cynomolgus | yes | yes, GLP, cynomolgus | cynomolgus | yes |
| Tecentriq | no | no, the risk is already identified | | | no, the risk is already identified | | |
| Trazimera | yes | no | | | no | | |

| Medicine Name | Bio similar | Embryo-Foetal Development (EFD) Studies | Species used for EFD | EFD conducted in Relevant Species | Pre-and Postnatal Development (PPND) Studies | Species used for PPND | PPND conducted in Relevant Species |
|---|---|---|---|---|---|---|---|
| Tremfya | no | ePPND cynomolgus | ePPND cynomolgus | yes | ePPND cynomolgus | cynomolgus | yes |
| Trogarzo | no | no, no justification | | | yes, cynomolgus | cynomolgus | yes |
| Truxima | yes | no | | | no | | |
| Ultomiris | no | yes | **mouse surrogate** | no active species | 1 murine surrogate | **mouse surrogate** | no active species |
| Unituxin | no | no, indication: neuroblastoma | | | no data | | |
| Xgeva | no | yes, cynomolgus | cynomolgus | yes | no, no concerns | | |
| Yervoy | no | ePPND ongoing | not specified | | no, ePPND ongoing | | |
| Zessly | yes | no | | | no | | |
| Zinbryta | no | yes, 2 pilot and pivotal cynomolgus | cynomolgus | yes | yes, cynomolgus | cynomolgus | yes |
| Zinplava | no | no, an antibody against foreign target | | | no, an antibody against foreign target | | |
| Zirabev | yes | no | | | no | | |

**Annex 14**: Juvenile Animal Studies

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Adcetris | no | Brentuximab vedotin | no | JAS not submitted, which is acceptable as brentuximab vedotin is intended for the treatment of patients with relapsed or refractory HL and sALCL | waiver requested | PIP was not yet completed |
| Aimovig | no | Erenumab | no data | no data are available.. in paediatric.. patients | waiver requested | PIP was not yet completed |
| Ajovy | no | Fremanezumab | no data | no information | waiver requested | PIP was not yet completed |
| Amgevita | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Arzerra | no | Ofatumumab | no data | Ofatumumab is covered by a class waiver on the 'treatment of chronic lymphocytic leukaemia', a condition which is not applicable to children. Arzerra is not recommended for use in children below 18 years due to insufficient data on safety and/or efficacy. | covered by a class waiver | PIP should be completed |
| Bavencio | no | Avelumab | no data | Treatment of Merkel cell carcinoma. The waiver applies to:<br>• The paediatric population from birth to less than 2 years of age;<br>• on the grounds that the disease or condition for which the specific medicinal product is intended does not occur in the specified paediatric subset(s).<br>The waiver applies to:<br>• The paediatric population from 2 years to less than 18 years of age;<br>• on the grounds that the specific medicinal product does not represent a significant therapeutic benefit as clinical studies(s) are not feasible. | covered by a product specific waiver | PIP was completed |
| Benlysta | no | Belimumab | no data | Systemic lupus erythematosus<br>The waiver applies to:<br>− Children from birth to less than 5 years<br>− on the grounds that the disease or condition for which the specific medicinal product is intended does not occur in the specified paediatric subset(s). | covered by a product specific waiver | PIP was completed |
| Besponsa | no | Inotuzumab ozogamicin | no data | no information | no information | PIP was not yet completed |
| Blincyto | no | Blinatumomab | yes mouse surrogate | ALL represents about 15% of adult and 80% of paediatric leukaemia affecting all ages with two incidence peaks. One is in late adulthood and one in children aged 2 to 5 years. Non-clinical studies were performed *in vivo* xenograft models of adult and paediatric acute lymphocytic leukemia (ALL). | not applicable | PIP was not yet completed |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Blitzima | yes | Rituximab | no data | Paediatric requirements Not applicable | | |
| Cablivi | no | Caplacizumab | no | No juvenile toxicity studies were performed with caplacizumab considering that the intended clinical use is for treatment of adult patients with thrombotic thrombocytopenia. P/0189/2016 | waiver requested | PIP was not yet completed |
| Cimzia | no | Certolizumab pegol | no data | no information, no PIP avail. | no information | not available |
| Cinqaero | no | Reslizumab | yes mouse cynomolgus | A PIP has been agreed in order to obtain data in the paediatric population between 6 and 18 years of age. The Applicant has not requested an indication in the paediatric population. Limited data are available in patients > 12 years old and no data in patients <12 years old. | paediatric subset covered by waiver | PIP was not yet completed |
| Cosentyx | no | Secukinumab | no | Since the submission aims at treatment of adult patients juvenile animal studies are not needed. This was agreed by the CHMP. | waiver requested | PIP was not yet completed |
| Crysvita | no | Burosumab | yes mouse surrogate + cynomolgus | mouse surrogate and cynomolgus | not applicable | PIP was not yet completed |
| Cyltezo | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Cyramza | no | Ramucirumab | no data | granted class waiver CW/1/2011 | covered by a class waiver | PIP was completed |
| Darzalex | no | Daratumumab | no data | granted class waiver CW/1/2011 | covered by a class waiver | PIP was completed |
| Dupixent | no | Dupilumab | no data | A waiver was granted for the paediatric population from birth to less than 6 months on the grounds that the specific medicinal product is likely to be unsafe.<br>A deferral for one or more measures was granted for the paediatric population from 6 months to 18 years of age for the treatment of atopic dermatitis. | covered by a product specific waiver | PIP was completed |
| Emgality | no | Galcanezumab | yes rat | juvenile toxicity study in rats was conducted to assess potential effects on growth and development to support paediatric development. | not applicable | PIP was not yet completed |
| Empliciti | no | Elotuzumab | no data | granted class waiver CW/1/2011 | covered by a class waiver | PIP was completed |
| Entyvio | no | Vedolizumab | no | Toxicology studies in juvenile animals have not been conducted. The Applicant argued that α4β7 integrin does not play a role in mammalian growth and development (literature references have been provided) | waiver requested | PIP was not yet completed |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Fasenra | no | Benralizumab | no | The lack of studies with dosing of juvenile animals is also accepted, considering that the medicine is intended for adults, the lack of non-clinical studies in the agreed paediatric investigation plan, and the results from the enhanced pre- and post-natal development study. | waiver requested | PIP was not yet completed |
| Flixabi | yes | Infliximab | no | Paediatric requirements Not applicable | | |
| Gazyvaro | no | Obinutuzumab | no | Obinutuzumab is not indicated for treatment of the paediatric population and Obinutuzumab has been granted a waiver for paediatric development. The lack of studies in juvenile animals is considered acceptable. | covered by a product specific waiver | PIP should be completed |
| Halimatoz | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Hefiya | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Hemlibra | no | Emicizumab | no | Juvenile studies were not performed. However, the available 13-week SC toxicity study in monkeys of 3 years of age with once weekly dosing supports treatment of adolescent humans at 12 years of age and older [Baldrick 2010]. Toxicology studies in juvenile animals have not been conducted and are not considered meaningful for emicizumab. | waiver requested | PIP was not yet completed |
| Herzuma | yes | Trastuzumab | no | Paediatric requirements Not applicable | | |
| Hulio | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Hyrimoz | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Idacio | yes | Adalimumab | no data | Paediatric requirements Not applicable | | |
| Ilaris | no | Canakinumab | yes mouse surrogate | A juvenile animal study was conducted with the surrogate mAb 01BSUR. Mice were treated once weekly with 01BSUR from day 7 to day 70 post partum. | not applicable | PIP was not yet completed |
| Ilumetri | no | Tildrakizumab | no | In accordance with the agreed PIP, juvenile animal toxicity studies were not conducted to support a future use of tildrakizumab in paediatric patients. | waiver requested | PIP was not yet completed |
| Imfinzi | no | Durvalumab | no data | granted class waiver CW/1/2011 | covered by a class waiver | PIP was completed |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Imraldi | yes | Adalimumab | no | Separate study in paediatric patients is not usually required in the biosimilarity setting. The applicant has selected the population to cover the age group from 18 to 75 years, which is acceptable. | | |
| Inflectra | yes | Infliximab | no | Paediatric requirements Not applicable | | |
| Kadcyla | no | Trastuzumab emtansine | no data | granted class waiver CW/1/2011 | covered by a class waiver | PIP was completed |
| Kanjinti | yes | Trastuzumab | no | Paediatric requirements Not applicable | | |
| Kevzara | no | Sarilumab | yes mouse surrogate | To support the use of sarilumab in paediatric patients, toxicity was evaluated with the surrogate mAb in juvenile mice, although such study was not considered necessary in the agreed PIP. | not applicable | PIP was not yet completed |
| Keytruda | no | Pembrolizumab | no data | product specific waiver requested | waiver requested | PIP was not yet completed |
| Kromeya | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Kyntheum | no | Brodalumab | part of repeat dose | The PDCO opinion (P/0235/2014) granted a waiver with respect to the population from birth to less than 4 years old and a deferral for studies in population from 4 to 18 years of age for completion of studies by January 2026. At the time of submission of the application, the PIP P/0235/2014 was not yet completed as some measures were deferred. | paediatric subset covered by waiver | PIP was not yet completed |
| Lartruvo | no | Olaratumab | no data | Missing Information: Use in paediatric patients | waiver requested | PIP was not yet completed |
| Lemtrada | no | Alemtuzumab | no data | With respect to the paediatric population, the CHMP considered that a waiver was granted for the paediatric subsets in the range from birth to less than 10 years and a deferral was granted for conducting and submitting results of a clinical trial in MS patients 10 to less than 18 years. The CHMP noted that the information is adequately covered in the Product Information. | paediatric subset covered by waiver | PIP was not yet completed |
| Libtayo | no | Cemiplimab | no | the risk is already identified,hence it is acceptable that no studies have been performed to test the potential of cemiplimab for carcinogenicity, genotoxicity, pre and postnatal development and juvenile studies | waiver requested | PIP was not yet completed |
| Mvasi | yes | Bevacizumab | no | Paediatric requirements Not applicable | | |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Mylotarg | no | Gemtuzumab ozogamicin | no data | The results of the population modelling showed that the PK behaviour of gemtuzumab ozogamicin (hP67.6 antibody and unconjugated calicheamicin) is similar between adult and paediatric AML patients following the 9 mg/m2 dosing regimen (SmPC section 5.2).<br>In a large survey evaluating the age effect on AML biology and response to therapy among paediatric patients, differences were seen in infants, but a distinct biology in TYA patients could not be identified | waiver requested | PIP was not yet completed |
| Nivolumab BMS | no | Nivolumab | part of ePPND cynomolgus, repeat dose cynomolgus | applied for class waiver CW/1/2011 | covered by a class waiver | PIP was not yet completed |
| Nucala | no | Mepolizumab | no data | There are limited data available in the paediatric population (59 subjects with eosinophilic esophagitis, 19 subjects with severe asthma). Paediatric pharmacokinetics was predictable from adults, after taking into account bodyweight. Mepolizumab pharmacokinetics in adolescent subjects with severe eosinophilic asthma included in the phase 3 studies was consistent with adults. | waiver requested | PIP was not yet completed |
| Ocrevus | no | Ocrelizumab | ongoing cynomolgus | A juvenile toxicity study in cynomolgus monkeys has been started. In the PIP agreed upon with EMA no nonclinical studies are mentioned. However the study is a requirement from authorities in different regions, and when the study is completed it should be submitted to the EMA as well. | not applicable | PIP was not yet completed |
| Ogivri | yes | Trastuzumab | no | Paediatric requirements Not applicable | | |
| Ontruzant | yes | Trastuzumab | no | Paediatric requirements Not applicable | | |
| Opdivo | no | Nivolumab | yes cynomolgus + ePPND | The potential developmental effects of nivolumab were examined in the ePPND study that included assessments in infant monkeys up to 6 month old. In addition, pivotal toxicity studies up to 3 months in duration included cynomolgus monkeys as young as 2 years of age, which is approximately equivalent to a 6-year-old human. | not applicable | PIP was not yet completed |
| Perjeta | no | Pertuzumab | no data | granted class waiver P/345/2010 breast cancer | covered by a class waiver | PIP was completed |
| Portrazza | no | Necitumumab | no data | granted class waiver CW/1/2011 | covered by a class waiver | PIP was completed |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Poteligeo | no | Mogamulizumab | no | No juvenile Toxicity studies were conducted with mogamulizumab, as it is not developed for pediatric indication.The waiver applies to:<br>• all subsets of the paediatric population from birth to less than 18 years of age;<br>on the grounds that clinical studies with the specific medicinal product cannot be expected to be of significant therapeutic benefit to or fulfil a therapeutic need of the specified paediatric subset(s). | covered by a product specific waiver | PIP was completed |
| Praluent | no | Alirocumab | no | However, the F1-offspring had been exposed to alirocumab in utero and through colostral milk and there appeared to be a decrease in IgG in the TDAR in the F1-offspring. However, based on a weight of evidence approach, this single finding is considered not to be clinically relevant.?? | waiver requested | PIP was not yet completed |
| Praxbind | no | Idarucizumab | no data | lack of juvenile and paediatric population data | waiver requested | PIP was not yet completed |
| Prolia | no | Denosumab | no data | granted class waiver P/89/2008 Treatment of menopausal and other perimenopausal disorders. | covered by a class waiver | PIP was completed |
| Qarziba (beta Apeiron) | no | Dinutuximab beta | part of repeat dose | guinea pig, paediatric data available | not applicable | PIP was not yet completed |
| Removab | no | Catumaxomab | no data | Section 4.2 of the SPC was updated to note that catumaxomab is not recommended for use in children below the ages of 18 years due to the lack of data on safety and efficacy. | no information | no information on PIP |
| Remsima | yes | Infliximab | no | Paediatric requirements Not applicable | | |
| Repatha | no | Evolocumab | part of repeat dose and EFPD | No dedicated juvenile animal studies have been performed (and none are planned), but the completed studies provided adequate nonclinical safety support for evaluation of the intended paediatric population<br>(agreed with the PCDO):<br>(1) a 3-months toxicity study with Cynomolgus monkeys between age of 3 and 7 years and (2) an Embryo-foetal and Postnatal Development (EFPD) were infants could be followed. The first of these studies tested evolocumab in Cynomolgus monkeys of 2.5 years and older (in the 6-weeks study) | not applicable | PIP was not yet completed |
| Ritemvia | yes | Rituximab | no | Paediatric requirements Not applicable | | |
| Rixathon | yes | Rituximab | no | Paediatric requirements Not applicable | | |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Riximyo | yes | Rituximab | no | Paediatric requirements Not applicable | | |
| RoActemra | no | Tocilizumab | no data | Missing information for relevant populations ... paediatric patients | no information | no information on PIP |
| Scintimun | no | Besilesomab | no | No studies in juvenile animals. There were no data in the paediatric population apart from literature which is not relevant to the indication of osteomyelitis. Thus, technetium (99mTc) besilesomab is not recommended for use in children below 18 years of age due to insufficient data on safety and efficacy (see SPC section 4.2, 5.1 and 5.2). | paediatric population excluded | no information on PIP |
| Simponi | no | Golimumab | no | No studies in which the offspring (juvenile animals) are further dosed and/or further evaluated were submitted. | no information | no information on PIP |
| Skyrizi | no | Risankizumab | part of ePPND | In the ePPND study infants were observed for six months post-partum, no dedicated juvenile toxicology studies were carried out and this is acceptable. The ePPND study conducted in cynomolgus monkeys revealed no risankizumab-related effects in infant monkeys up to six months of age. Based on the pharmacology of risankizumab, the toxicity data, including lack of toxicity of the immune system, and as the major organ systems are developed in cynomolgus monkey by 6 months of age and humans by 2 years, this data is considered to sufficiently address the safety profile for all age groups. | not applicable | PIP was not yet completed |
| Solymbic | yes | Adalimumab | no | Paediatric requirements Not applicable | | |
| Stelara | no | Ustekinumab | part of safety pharmacologycynomolgus | juvenile F1 monkeys exposed to ustekinumab indirectly following treatment of their respective dams. | not applicable | PIP was not yet completed |
| Sylvant | no | Siltuximab | no data | grant a waiver for all subsets of the paediatric population and condition Castleman's disease | covered by a product specific waiver | PIP was completed |
| Takhzyro | no | Lanadelumab | part of repeat dose | The 27 lanadelumab doses administered in the 6-month study in juvenile to adolescent cynomolgus monkeys (2.7-3.3 years) receiving lanadelumab doses 5, 25 or 50 mg/kg/week were well-tolerated. T | not applicable | PIP was not yet completed |
| Taltz | no | Ixekizumab | no data | There is also no information on the use of ixekizumab in the paediatric population. The applicant has been requested to include this as missing information in the RMP. | paediatric population excluded | PIP was not yet completed |
| Tecentriq | no | Atezolizumab | part of primary PD study | Non-Clinical Biomarker Study in Paediatric Tumour Tissue | not applicable | PIP was not yet completed |

| Medicine Name | Bio similar | Active Substance | Juvenile Animal Studies performed | JAS / Peadiatric data: Comments and Justification | Waiver | PIP |
|---|---|---|---|---|---|---|
| Trazimera | yes | Trastuzumab | no | Paediatric requirements Not applicable | | |
| Tremfya | no | Guselkumab | part of ePPND | Further studies addressing toxicity of guselkumab in juvenile animals, in addition to the ePPND study, were not conducted, which was considered acceptable by CHMP. | not applicable | PIP was not yet completed |
| Trogarzo | no | Ibalizumab | no data | The dose of ibalizumab was estimated for adolescent (18-12) and children (6-11) based on adult pop-PK model adjusted by CD4 count and bodyweight. However the model is not considered qualified for its purposes, and without paediatric data the estimated dose regimen in paediatric population cannot be verified. Paediatric indication is not requested in this application, thus no questions are raised. | paediatric population excluded | PIP was not yet completed |
| Truxima | yes | Rituximab | no | Paediatric requirements Not applicable | | |
| Ultomiris | no | Ravulizumab | no data | no waiver, no non-clinical study obligations | no non-clinical study obligations | PIP was completed |
| Unituxin | no | Dinutuximab | yes tissue cross reactivity in juvenile human tissue panel + cynomolgus planned | The Applicant has suggested that a juvenile toxicity study in monkeys of 5 months duration will be performed in order to evaluate the effects of dinutuximab on the central and peripheral nervous system. | not applicable | PIP was not yet completed |
| Xgeva | no | Denosumab | no | No specific studies were conducted and this is justified with reference to the indication not including patients less than 9 years old and the age of monkeys used in the toxicology programme. The indications of treatment of postmenopausal osteoporosis and treatment of multiple myeloma fall within the scope of the decision on class waivers (EMEA/245439/2008). | covered by a class waiver | PIP was not yet completed |
| Yervoy | no | Ipilimumab | no | No formal juvenile studies were submitted to support the proposed indication. Treatment of melanoma in all subsets of the paediatric population is granted with class waiver (P/63/2010) | covered by a class waiver | PIP was completed |
| Zessly | yes | Infliximab | no | Paediatric requirements Not applicable | | |
| Zinbryta | no | Daclizumab | no data | paediatric data are available throught previouse authorisation | not applicable | PIP was not yet completed |
| Zinplava | no | Bezlotoxumab | no data | two paediatric clinical trials planned, but no data on juvenile animals | not applicable | PIP was not yet completed |
| Zirabev | yes | Bevacizumab | no | Paediatric requirements Not applicable | | |

**Annex 15:** Local Tolerance, Immunotoxicity, Human Tissue Cross-Reactivity and Ecotoxicity studies

*Bold font: non-active species*

| Medicine Name | Biosimilar | Local Tolerance Studies | Dedicated Local Tolerance Study Species | Local Tolerance Study in Relevant Species | Immunotoxicity | Human Tissue directed TCR | Ecotoxicity |
|---|---|---|---|---|---|---|---|
| Adcetris | no | part of repeat dose | | | no data | Secondary PD | yes (conjugate) |
| Aimovig | no | part of repeat dose | | | no data | no data | no |
| Ajovy | no | yes +<br>part of repeat dose | rabbit | yes | no data | Other toxicity studies | no |
| Amgevita | yes | part of repeat dose | | | no data | no data | no |
| Arzerra | no | part of repeat dose | | | part of dose ranging toxicity study, cynomolgus | Secondary PD | no |
| Bavencio | no | part of repeat dose | | | no data | no data | no |
| Benlysta | no | yes | cynomolgus | yes | no data | Secondary PD | no |
| Besponsa | no | part of single- and repeat-dose | | | no data | Other toxicity studies | yes |
| Blincyto | no | yes | **rabbit** | no | no data | Other toxicity studies | no |
| Blitzima | yes | part of repeat dose | | | no data | Primary PD | no |
| Cablivi | no | yes | **rabbit** | no | no data | Secondary PD | no |
| Cimzia | no | yes | **rat** | no | dedicated +<br>repeat dose, cynomolgus, rat +<br>in vitro mouse and human macrophage viability tests with PEG | Safety Pharmacology | yes |
| Cinqaero | no | yes | rabbit, **rat** | yes +no | no studies | Primary PD | no |
| Cosentyx | no | part of repeat dose | | | Four studies were conducted to address the risk of infection | Other toxicity studies | no |
| Crysvita | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Cyltezo | yes | part of repeat dose | | | part of repeat dose | Other toxicity studies | no |
| Cyramza | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Darzalex | no | part of repeat dose | | | no data | Other toxicity studies | no |

| Medicine Name | Biosimilar | Local Tolerance Studies | Dedicated Local Tolerance Study Species | Local Tolerance Study in Relevant Species | Immunotoxicity | Human Tissue directed TCR | Ecotoxicity |
|---|---|---|---|---|---|---|---|
| Dupixent | no | part of repeat dose (injection site) | | | no data | no data | no |
| Emgality | no | part of repeat dose (injection site) | | | no data | no data | no |
| Empliciti | no | yes | **rabbit** | no active | no data | Other toxicity studies | no |
| Entyvio | no | yes | rabbit | yes | part of repeat dose toxicity study, cynomolgus | primary PD | no |
| Fasenra | no | yes | not specified | | part of repeat dose toxicity study, cynomolgus | Toxicology program | no |
| Flixabi | yes | no | | | no data | no data | no |
| Gazyvaro | no | part of repeat dose (injection site) | | | no data | secondary PD | no |
| Halimatoz | yes | yes | **rabbit** | no | no data | Toxicology program | no |
| Hefiya | yes | yes | **rabbit** | no | no data | Toxicology program | no |
| Hemlibra | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Herzuma | yes | part of repeat dose | | | no data | no data | no |
| Hulio | yes | part of repeat dose | | | part of repeat dose toxicity study | no data | no |
| Hyrimoz | yes | yes | **rabbit** | no | no data | Toxicology program | no |
| Idacio | yes | no data | | | no data | no data | no |
| Ilaris | no | part of repeat dose | | | part of repeat dose toxicity study mouse surrogate, marmoset, juvenile immunotoxicity study is planned. | Other toxicity studies | no |
| Ilumetri | no | yes | **rabbit** | no | Part of PPND study in the surviving infants | Toxicology program | no |
| Imfinzi | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Imraldi | yes | part of repeat dose | | | part of repeat dose toxicity study | no data | no |
| Inflectra | yes | part of repeat dose | | | no data | Primary PD | no |
| Kadcyla | no | part of single- and repeat-dose (injection site) | | | no data | Other toxicity studies | yes |

| Medicine Name | Biosimilar | Local Tolerance Studies | Dedicated Local Tolerance Study Species | Local Tolerance Study in Relevant Species | Immunotoxicity | Human Tissue directed TCR | Ecotoxicity |
|---|---|---|---|---|---|---|---|
| Kanjinti | yes | part of repeat dose | | | no data | Toxicology program | no |
| Kevzara | no | part of repeat dose | | | Part of ePPND study | no data | no |
| Keytruda | no | part of repeat dose | | | no data | Toxicology program | no |
| Kromeya | yes | no data | | | no data | no data | no |
| Kyntheum | no | part of repeat doseand ePPND | | | part of repeat dose toxicity study, cynomolgus | Primary PD | no |
| Lartruvo | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Lemtrada | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Libtayo | no | part of repeat dose | | | no data | no data | no |
| Mvasi | yes | part of repeat dose | | | no data | no data | no |
| Mylotarg | no | part of single- and repeat-dose | | | no data | Not human tissues? | yes |
| Nivolumab BMS | no | part of repeat dose | | | no data | Not human tissues? | no |
| Nucala | no | part of repeat dose | | | dedicated in mouse surrogate + part of FEED study | Secondary PD | no |
| Ocrevus | no | yes + part of repeat dose | not specified | | part of the repeat dose and reproductive toxicity studies. | Secondary PD clinical | no |
| Ogivri | yes | part of PK and repeat-dose | | | no data | no data | no |
| Ontruzant | yes | no | | | no data | no data | no |
| Opdivo | no | part of single- and repeat-dose | | | no data | Toxicology program | no |
| Perjeta | no | part of repeat dose | | | no data | Secondary PD | no |
| Portrazza | no | part of repeat dose | | | no data | Other toxicity studies | no |
| Poteligeo | no | part of single-, repeat dose and PPND | | | part of repeat dose toxicity study | no data | no |
| Praluent | no | no data | | | no data | PK | no |

| Medicine Name | Biosimilar | Local Tolerance Studies | Dedicated Local Tolerance Study Species | Local Tolerance Study in Relevant Species | Immunotoxicity | Human Tissue directed TCR | Ecotoxicity |
|---|---|---|---|---|---|---|---|
| Praxbind | no | yes + part of repeat dose | **rabbit** | no | Hemolysis of blood | Other toxicity studies | no |
| Prolia | no | part of repeat dose | | | literature data | no data | no |
| Qarziba (beta Apeiron) | no | part of repeat dose (injection site) | | | no data | Toxicology program + PK | no |
| Removab | no | yes | **rabbit** | no active | part of single dose toxicity study | Secondary PD | no |
| Remsima | yes | part of repeat dose | | | no data | Primary PD | no |
| Repatha | no | yes + part of repeat dose | not specified | | part of repeat dose toxicity studies | Other toxicity studies | no |
| Ritemvia | yes | part of repeat dose | | | no data | Primary PD | no |
| Rixathon | yes | part of repeat dose | | | no data | Secondary PD | no |
| Riximyo | yes | part of repeat dose | | | no data | Secondary PD | no |
| RoActemra | no | no data | | | no data | no data | no |
| Scintimun | no | yes | **rabbit** | no | no studies | Primary PD | yes |
| Simponi | no | yes | cynomolgus | yes | part of EFD (cord blood) and PPND (neonate) | Other toxicity studies | no |
| Skyrizi | no | part of repeat dose | | | part of repeat dose toxicity studies | Other toxicity studies | no |
| Solymbic | yes | part of repeat dose | | | no data | no data | no |
| Stelara | no | part of repeat dose | | | part of repeat dose toxicity studies | safety Pharmacology | no |
| Sylvant | no | no | | | part of repeat dose toxicity studies | Other toxicity studies | no |
| Takhzyro | no | part of repeat dose (injection site) | | | no data | Other toxicity studies | no |
| Taltz | no | part of repeat dose (injection site) | | | part of repeat dose toxicity studies | Other toxicity studies | no |
| Tecentriq | no | part of repeat dose | | | part of repeat dose toxicity studies | Secondary PD | no |
| Trazimera | yes | no | | | no data | no data | no |
| Tremfya | no | part of single-, repeat dose and ePPND | | | part of repeat dose toxicology and the ePPND studies, cynomolgus | Other toxicity studies | no |

| Medicine Name | Biosimilar | Local Tolerance Studies | Dedicated Local Tolerance Study Species | Local Tolerance Study in Relevant Species | Immunotoxicity | Human Tissue directed TCR | Ecotoxicity |
|---|---|---|---|---|---|---|---|
| Trogarzo | no | yes | cynomolgus | yes | no data | Toxicology program | no |
| Truxima | yes | part of repeat dose | | | no data | Primary PD | no |
| Ultomiris | no | yes | **rabbit cynomolgus** | no active | no data | Secondary PD | no |
| Unituxin | no | injection site reaction adressed in SmPC | | | no data | Other toxicity studies | no |
| Xgeva | no | part of repeat dose | | | part of repeat dose toxicity studies | Other toxicity studies | no |
| Yervoy | no | part of repeat dose | | | part of repeat dose toxicity and other studies | Toxicology program | no |
| Zessly | yes | part of repeat dose | | | no studies | no data | no |
| Zinbryta | no | yes + part of repeat dose | **rabbit** | no | part of the repeat dose and reproductive toxicity studies | no data | no |
| Zinplava | no | no data | | | no data | Toxicology program | no |
| Zirabev | yes | part of repeat dose | | | no data | no data | no |

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

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