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Questions and answers on monoclonal antibodies for veterinary use

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Background

Monoclonal antibodies (mAbs) are immunoglobulins (Ig) with a defined specificity derived from a single clone of cells. Their biological activities are characterised by a specific binding characteristic to an antigen and may be dependent on immune effector function such as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity.

Monoclonal antibodies may be generated by recombinant DNA (rDNA) technology, hybridoma technology, B lymphocyte immortalisation or other technologies (e.g. genetically engineered animals).

The range of clinical indications with potential for treatment with mAbs is very wide. Currently, in human medicine, a number are authorised for use as anti-cancer medicines and in medicines against diseases affecting the immune system, such as rheumatoid arthritis.

To date, the CVMP and its Scientific Advice Working Party (SAWP-V) have addressed a limited number of scientific advice requests concerning mAb products. This activity indicates that a number of mAbs for use as veterinary medicinal products are in development. Indeed, in February 2017, the CVMP recommended the granting of a marketing authorisation for Cytopoint (lokivetmab), the first monoclonal antibody in a veterinary medicine in the EU, intended for the treatment of dogs with atopic dermatitis.

Following a review of the scientific information relating to mAbs, a number of areas (in the form of questions) were identified that would benefit from consideration by relevant experts and the elaboration of specific guidance in the form of questions and answers (Q&A).

These questions, together with an answer, are presented below.

1. What guidance is currently available for the characterisation / setting of specifications for mAbs, and how appropriate and sufficient is it for mAbs intended for veterinary use?

1.1 What are general principles in characterisation / specification setting for veterinary monoclonal antibodies?

Appropriate analytical testing with relevant specifications is necessary for the consistent manufacture of veterinary medicinal products containing mAbs. The specifications set should take into account relevant quality attributes identified in characterisation studies. When setting specifications for mAbs for veterinary use, the Veterinary International Conference on Harmonisation (VICH) Guideline (GL) 40: Specifications: Test procedures and acceptance criteria for new biotechnology/biological veterinary medicinal products (EMEA/CVMP/VICH/811/04) [1] should be taken into consideration. To address the specifics of mAbs for veterinary use, in the absence of any veterinary specific guidance, applicants should consider the CHMP Guideline on production and quality control of monoclonal antibodies and related substances (EMA/CHMP/BWP/532517/2008) [2], specifically the sections relating to identity, purity and impurities, and potency, and the provisions of the European Pharmacopeia (Ph. Eur.) monograph 2031 on human mAbs for quantity and standard tests [3].

While the currently available human guidance mentioned above provides a useful reference, these documents are written from the perspective of human medicine based risk assessment and development. It is recognised that it may present a challenge to veterinary mAbs manufacturers to achieve the extent of characterisation/ quality control testing typically required for human mAbs. Further, it is recognised that the characterisation/quality control testing requirements should be proportionate to the assessed degree of risk to target animals and users of these products. Ultimately, setting relevant specifications for quality control testing should ensure

consistency of manufacture of a veterinary medicinal product that is safe and efficacious. On this point, it is considered that appropriate quality standards can be proposed by manufacturers of mAbs for veterinary indications taking into account data generated in pivotal safety and clinical efficacy studies. That is, the risk assessments and therefore quality expectations need to be viewed in a different context for veterinary mAbs, where the evaluation of safety and efficacy directly in the target species can be used to define and support product quality (characterisation, specifications and process- and product-related impurities) from the earliest stages of development. Where data generated in the context of pivotal safety and efficacy studies are used to define quality control standards (limits) for a product, those limits cannot be subsequently relaxed without confirming that the revised limits do not impact on either target animal safety or efficacy.

Further, the requirements in terms of quality control testing could vary depending on target species and posology. For example, process- and product-related related impurities could present different risks for a product when only given once in an animal's life time in comparison to mAbs to be administered for chronic diseases.

Guidance needs to allow different approaches to testing in development versus routine production and a case by case decision on testing and specifications. Depending on the level of development performed and the number of batches at time of submission of the marketing authorisation application there may be a need to carry developmental test methods into routine production for at least acceptable process validation or for a sufficient number of batches to have meaningful data to set appropriate specifications. More extensive developmental and process qualification testing can be used to show production consistency and to justify a reduced testing set on commercial product post-authorisation. Note that where a reduced testing set is accepted for routine testing of commercial product post-authorisation, more extensive developmental and process qualification testing (in particular, for process and product-related impurities) may be required to confirm production consistency in the event of process/site changes post authorisation.

Characterisation and testing requirements should be scientifically justified.

1.2 What specific considerations are needed for characterisation of veterinary mAbs?

Characterisation is performed in the development phase and, when necessary, following significant process changes. Characterisation is necessary to allow relevant specifications to be established, based on physicochemical properties, biological activity, immunochemical properties, purity and impurities.

At the time of submission of the marketing authorisation application, the product should have been compared with an appropriate reference standard, if available, and also the manufacturer should have established appropriately characterised in-house reference materials which will serve for biological and physicochemical testing of production lots.

a. How should physicochemical properties be tested?

Physicochemical properties include determination of the composition, physical properties, primary structure and, in some cases, higher-order structure of the desired product. Higher order structure (tertiary or even quaternary structure) is a parameter that could provide additional assurance in terms of consistency of production, but would not be required where there is adequate assurance based on primary structure and other tests (e.g. biological assay). For example, where the mechanism of action of a mAb is based simply on binding of antigen, information on primary structure together with an appropriate biological assay may be sufficient. However, where mechanism of action is more complex (e.g., Fc receptor binding with subsequent induction of

antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytoxicity (CDC)) and relevant biological assays are not available, investigations about higher order structure may be necessary.

VICH GL 40 indicates that "In some cases, information regarding higher-order structure of the desired product (the fidelity of which is generally inferred by its biological activity) may be obtained by appropriate physicochemical methodologies".

For physicochemical characterisation, in the VICH GL 40 (Appendix 6.1), the following tests are indicated as being suitable analytical techniques that could be performed:

- *6.1.1 Structural characterisation and confirmation: Amino acid sequence, amino acid composition, terminal amino acid sequence, peptide map, sulfhydryl group(s) and disulfide bridges, carbohydrate structure*
- *6.1.2 Physicochemical properties: Molecular weight or size, isoform pattern (determined by isoelectric focusing or other appropriate techniques), extinction coefficient (or molar absorptivity), electrophoretic patterns, liquid chromatographic patterns, spectroscopic profiles.*

  The parameters included above are examples of technical approaches which might be considered for structural characterization and confirmation, and evaluation of physicochemical properties of the desired product, drug substance and/or medicinal product. The specific technical approach employed will vary from product to product and alternative approaches, other than those mentioned may be appropriate.

b. How can biological activity of veterinary mAbs be demonstrated?

In vitro test methods for potency of the product (correlated to efficacy in the target species) are preferable to *in vivo* test methods (in accordance with Directive 2010/63/EC, *in vitro* test methods should be used, where possible, if they are a feasible and acceptable alternative to *in vivo* tests/studies). Such methods could include cell culture/biochemical/immuno assays. The applicant should select the most appropriate assay.

Where appropriately validated, a single potency assay is considered sufficient. A reference material is preferable, if available. However, an in house reference material may be established.

c. How should immunological properties of veterinary mAbs be evaluated?

Specificity for the target antigen can be used to characterise the mAb.

With the exception of tests for biological activity/specificity, characterisation of immunological properties for veterinary mAbs is not required from a quality perspective given that properties of clinical relevance can be evaluated in safety and efficacy studies in the target species.

The evaluation of mAb:target complex formation may be relevant, but it can also be in general addressed during preclinical/clinical tests and need not be studied from a quality point of view.

Depending on the proposed mechanism of action of the mAb, a one-time *in vitro* assay to address ADCC and CDC may be appropriate.

Identifying the epitope (biochemically) can help define the mechanism of action and this may be done at the developmental stage. Such development work is appropriate in the sense that it leads to a better understanding of the drug substance, but it would not be considered an essential part of the characterisation.

d. How should purity for the veterinary mAbs be tested?

Monoclonal antibodies commonly display several sources of heterogeneity (isomerisation, fragmentation, etc.) which may lead to a complex purity/impurity profile. As part of the determination of the purity of the mAb, the manufacturer should consider the structural heterogeneity and demonstrate its consistency during the manufacturing procedure.

The study of the purity is an essential requirement for a veterinary mAb and it should be assessed by a combination of methods. The methods generally include physicochemical properties such as molecular weight or size, isoform pattern, extinction coefficient, electrophoretic profiles, chromatographic data and spectroscopic profiles.

Suitable analytical techniques that can be used include size-exclusion chromatography (SEC) and/or isoelectric focusing (IEF), depending on whether there are differences in the binding capacities of charge forms.

The relative purity can be expressed as the specific activity (units of biological activity per mg of product).

e. How should veterinary mAbs be tested for impurities?

Impurities should be defined and limits determined. The biological activity of impurities may not need to be defined if justified with satisfactory safety/efficacy data.

Process-related impurities specific for mAbs include host-cell derived proteins (HCP), host and vector derived DNA and Protein A, if used. Product related impurities for mAbs include precursors and degradation products which could arise during the manufacture or storage.

Impurities can indicate issues/changes in the manufacturing process. Interpreting impurity profiles is difficult as there are no defined acceptable limits for veterinary mAbs. In order to determine appropriate quality control of impurities acceptance criteria should be based on data obtained from lots used in preclinical and clinical studies, and found to be safe, and manufacturing consistency lots.

Relevant analytical approaches for the detection of “impurities” are detailed in VICH GL 40 (Appendix 6.2 for impurities). As in the case of physicochemical characterisation, the specific technical approach employed will vary from product to product and alternative approaches, other than those mentioned may be appropriate. However, consideration should be given to establishing product specific specifications for routine testing (for example % monomer, % High Molecular Mass Species (HMMS) and % Low Molecular Mass Species (LMMS)) and process related impurities acceptance criteria (e.g. residual DNA, HCP and Protein A, if appropriate).

2. What quality control is needed for potential contaminants (chemical and biochemical materials, microorganisms, extraneous agents) of veterinary mAbs?

While VICH GL 40 cross-refers to ICH guidelines and specifications about source cells are also not included in VICH GL 40, the following veterinary guidance should be taken into account in development of veterinary mAbs (in line with veterinary immunological medicinal products):

- Sterility of the final product should be in line with Ph. Eur. 2.6.1: Sterility [4].
- Extraneous agents: CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010-Rev.1) [5] and CVMP reflection paper on methods found suitable within the EU for demonstrating freedom from extraneous agents of the seeds used for the production of immunological veterinary medicinal products (EMA/CVMP/IWP/251741/2015) [6].

- Ph. Eur. 5.1.7: Viral safety [7].
- Ph. Eur. 5.1.10 Guidelines for using the Test for Bacterial Endotoxins [8].
- VICH GL 34 Biologicals: testing for the detection of mycoplasma contamination (EMA/CVMP/VICH/463/2002) [9] and Ph. Eur. 2.6.7: Mycoplasma [10].
- Ph. Eur. 5.2.4: Cell cultures for the production of Veterinary Vaccines. The MCS and WCS should be investigated for the same range of viral contaminants [11].

Monoclonal antibodies can be obtained from immortalized B lymphocytes that are cloned and expanded as continuous cell lines or form rDNA-engineered cell lines. For mAbs obtained from rDNA cell lines the Ph. Eur. monograph 784 'Recombinant DNA technology, products of' [12] is applicable and also Guideline on live recombinant vector vaccines for veterinary use (EMEA/CVMP/004/04) could be considered [13].

3. What is required for stability testing for veterinary mAbs?

VICH GL 17 Stability testing of biotechnological/biological veterinary medicinal products (EMA/CVMP/VICH/501/99) is relevant for stability testing and the guidance given is adequate for veterinary mAbs [14]. The following tests can be performed in mAbs for veterinary use on active substance and final product:

- Active substance tests: Appearance, bioburden and bacterial endotoxins, product related substances and process-related impurities (host-cell derived proteins, host and vector derived DNA), identity, structural integrity, protein content, biological activity,
- Final product tests: Appearance, solubility, pH, osmolality, extractable volume, total protein, molecular size distribution, molecular identity and structural integrity, purity, stabilizer, water, sterility, bacterial endotoxins, biological activity.

These tests can also be considered to check the stability of the active substance and the finished product. Again, not all the tests need to be performed; it is a case by case decision and depends on the developmental characterisation studies performed.

4. Why are reproductive safety studies needed for the target animal safety evaluation of mAbs?

In accordance with existing veterinary guidance, reproductive safety studies are required for systemically absorbed pharmaceuticals or immunologicals when data suggest that the starting material from which the product is derived may pose a risk when used in breeding animals (VICH GL 43 Target animal safety: pharmaceuticals (CVMP/VICH/393388/2006) and VICH GL 44 Target animal safety for veterinary live and inactivated vaccines (EMA/CVMP/VICH/359665/20050) respectively [15, 16]. The goal of reproductive safety studies is to identify any adverse effects of the veterinary medicinal product on male or female reproduction, foetal development or on offspring viability. A number of mAbs developed for use in humans are known to have (potential) effects on fertility and foetal development.

5. Should an applicant wish to develop a mAb for use in breeding / pregnant animals, what safety data would be considered adequate to characterize the risk (or confirm the absence of a risk)?

The scope of this questions & answers document is restricted to monoclonal antibodies (mAb's) which are not intended for a reproductive indication by targeting reproductive function *per se*.

In general, the applicant must address the need to conduct specific reproductive safety studies in the target animal. Unless a scientific justification for the absence of risk can be presented, such

safety studies should be provided if the intended target population is to include breeding/pregnant animals. If such justification cannot be provided and if relevant data to allow an assessment of potential risks is not available, use in breeding animals cannot be recommended and an exclusion statement must be included on the product information. However, on basis of the general characteristics of a mAb it is expected that the absence of specific reproductive safety data can be justified in many situations, as outlined below.

Whereas the ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals (EMA/CHMP/ICH/731268/1998) on human medicinal products [17] describes a tiered approach for reproductive and developmental toxicity testing in laboratory animals for the safety evaluation of, for example, monoclonal antibodies, some of the existing guidelines for veterinary medicinal products (VICH GL 43 and VICH GL 44) provide a useful starting point for the key areas that need to be considered when addressing reproductive and developmental safety in the target species.

The requirement for specific reproductive safety and developmental safety studies would depend on specific toxicological concerns such as those associated with the characteristics of the monoclonal antibody, target class, mode of action, antibody specificity, and embryo-foetal exposure including degree of transplacental passage. Thus, the need for conducting specific reproductive safety studies in the target animal should be addressed using a risk-based assessment taking the following aspects into consideration:

1. Available scientific information can provide guidance regarding what can/should be evaluated in the development programs. The evaluation should include the following:

    a. Addressing the pharmacological and toxicological profile of the mAb. This includes the understanding of possible transplacental passage, taking into consideration e.g. the target nature, mode of action, class effects, species specific characteristics of the placental passage and mAb isotype (IgG1 can pass placenta) as well as an assessment of the anticipated foetal drug exposure levels.

    b. When the weight of evidence indicates that there can be an adverse effect on reproduction or pregnancy in the target species, an appropriate warning statement advising against use in breeding/ pregnant animals is needed.

    c. If the mAb crosses the placenta and relevant exposure levels in the foetus are expected for a mAb with unknown embryo-foetal safety risks, reproductive toxicity studies in the target species may be necessary when breeding animals will be treated during the reproductive period. This would in particular include mAbs intended for treatments that are expected to continue over extended periods of time.

2. In case reproductive safety studies are scientifically warranted, a study of developmental safety should preferably be performed in the target animal, since testing of reproductive safety in preclinical laboratory studies (e.g. in rodents) is typically not considered useful for extrapolation to the target animal species, in particular because the placentation differs amongst species. While IgG are directly transported through the placenta in humans and rodents (haemochorial placentas), and up to 10% in dogs and cats (endotheliochorial placentas), the intra-uterine passage of antibodies is prevented for horses and pigs (epitheliochorial placentae) and for ruminants (synepitheliochorial placenta). In addition, the pharmacological and pharmacokinetic profiles of mAbs are seldom comparable between laboratory animals and the target species for which the mAb is intended.

a. The test of developmental safety to detect any adverse effects on the pregnant female and development of the embryo-foetus can be performed separately or in conjunction with well-designed target animal safety (TAS) studies by including appropriate treatment periods and relevant endpoints according to the principles outlined in VICH GL 43 and VICH GL 44.

b. Such TAS studies, covering the entire reproductive process, would include measurements of e.g. the length of gestation post-initiation of treatment, number of viable offspring, foetal growth and structural changes of the foetus, the health and development of the offspring during the first 30 days of life. However, multi-generation reproduction toxicity studies according to the Commission Directive (2009/9/EC, section 3.4) should not be necessary.

c. Adequate measures to mitigate any risk identified according to the study outcome should be reflected in the SPC.

3. For products that are directed at a foreign target such as bacteria and viruses, in general no reproductive toxicity would be expected.

6. In the context of safety of mAbs for the target animal, what data need to be generated to characterise the potential for indirect effects?

6.1 For functional rather than histopathological/lesional abnormalities, would it be considered sufficient, in general, to rely on clinical findings in a well conducted target animal safety study or should more specific investigations be conducted?

TAS tests should include an evaluation of potential risks to the target species under the proposed conditions of use. For mAbs, the safety evaluation should also include an investigation of immunogenic potential and effects on immune function or other indirect effects. For example, eliciting effects due to direct/indirect interactions with the antigen or related antigens at the target site(s) or non-target site(s) (when considering a mAb directed at a specific target, it is possible that the target is expressed in tissues other than the tissue of interest with the potential for unwanted treatment-related effects in tissues other than the tissue of interest). While certain parameters can be evaluated in the context of conventional TAS studies (haematology, tissue histopathology, bone marrow evaluation, lymphocyte populations), there is no clear guidance on the approach to, or the required extent of, evaluation of indirect effects.

While the existing guidance e.g. VICH GL 43, VICH GL 44 and ICH S6 can provide a meaningful starting point for safety evaluation of mAbs intended for veterinary use, a more tailored approach is generally needed to support the TAS. Factors such as the nature of the mAb (IgG class, level of species-specific sequences), nature of the target molecule, mode of action, levels and location of expression of the target molecule, therapeutic indication and the duration of treatment, largely determine the safety aspects that need to be taken into consideration in overall safety evaluation. The applicant should also consider the usefulness of the planned studies for addressing user safety and consumer safety (where applicable) in order to avoid overlapping studies where possible.

In general, the extent of data needed to support safe use of a mAb should be informed by conducting a comprehensive risk assessment taking into account current scientific knowledge, human or animal experience with mAbs of the same class, the biology of the target and physiological pathways involved in the given disease. The following tools and information sources

could be used for such a risk assessment and to identify any additional data required to support the safety of a mAb:

- scientific literature
- information on previous therapeutic use and experience in other animal species or in humans
- databases and *in silico* tools for e.g. homology searches and epitope modelling.

The mode of action and the nature of the mAb largely determine the safety of the individual mAb. Toxicity related to the clinical use of mAbs is predominantly caused by exaggerated pharmacological effect(s), and potential safety concerns can be largely predicted by the pharmacology. Therefore, a thorough understanding of pharmacodynamic effects is a key to the safety evaluation. MAbs are highly specific in target binding and, therefore, they are often species-specific and pharmacologically active only in species having the correct target antigen. Due to the species-specific nature of mAbs, extrapolation of safety from laboratory animals or other species, including humans, may not always be appropriate. It is acknowledged that mAbs used in humans have generally proven to be safe. However, caution should be used when extrapolating safety from/to other species.

The most relevant safety information should be collected from target animals in a well-designed TAS study in healthy or, in some cases, in diseased animals as well as from field studies in diseased animals. Consideration should be given to the selection of an animal model for safety testing. Generally, healthy animals are used for safety testing. Healthy animals are useful for testing of mAbs that are intended to neutralize for example cytokines that are expressed at pathologically high levels. In such a situation, the anticipated pharmacological effect (i.e. return of cytokine levels to normal) is expected to restore normal physiological cytokine levels, and the possible adverse effects due to exaggerated pharmacological activity can be detected in healthy animals. Also, when immunological function is relevant for safety testing healthy animals can be useful. However, in some cases e.g. when the target molecule is expressed or present in clinically relevant levels only in disease condition, the most relevant safety information can be gained from diseased animals.

Adverse effects on the immune and other physiological systems

The factors that influence the extent and possibility of physiological systems to be affected include:

- **Mode of action (MoA)**

  The potential safety concerns related to mAbs targeting an antigen with well-understood downstream effects restricted to a specific pathway or a single organ or organ system can be more reliably predicted by the MoA and consequently, it may be possible to justify a focused safety data set. However, mAbs targeting antigens that have pleiotropic functions affecting multiple pathways and/or organ systems can be associated with higher risks that may also be more difficult to predict. Thus, they generally require a more extensive safety evaluation focusing on those vital systems that might be affected. In particular, mAbs targeting antigens that serve as central key regulators of one or more physiological systems need to be carefully evaluated and the extent of unintended effects needs to be understood.

  MAbs with a MoA leading to stimulation of downstream pathways may need more extensive safety evaluation than mAbs with a simple neutralizing MoA.

- **Effector functions of the Fc region**

  Depending on the IgG class and possible engineering of an antibody molecule the effector functions of the Fc region of the antibody may play a role in pharmacological activity and/or

safety. The antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are essential for the MoA and efficacy of, for example, cancer mAbs. In contrast, for some mAbs the effector functions are not part of the MoA and would be considered (unwanted) secondary activities that need to be evaluated from a TAS perspective.

Evaluation of unwanted effector functions is particularly important for mAbs targeting a cell-bound antigen which is expressed in tissues involved in vital physiological functions such as e.g. cardiovascular or central nervous system.

- **Self or non-self-antigens**

  The nature of the target antigen in terms of recognition in the target animal as self or non-self also influences the anticipated risks related to the clinical use. In principle, mAbs targeting a foreign antigen such as virus or other pathogen-specific antigen can be regarded as relatively safe in comparison to mAbs targeting antigens that are expressed at high non-physiological levels in certain tissue or organ or antigens that are expressed predominantly in a diseased state such as tumour-associated antigens.

6.2 How to evaluate adverse effects on the immune and other physiological systems?

For mAbs with immunomodulatory MoA there might be a need to evaluate the effects on the immune system. MAbs with immune suppressing activity may impair the functionality of the immune system which may render the treated animals more susceptible to certain opportunistic infections, or more prone to malignancies due to impaired immune surveillance. In addition to the histopathological and haematology data derived from the TAS study the potential risks can be addressed by including specialized investigations in the TAS study and/or additional studies to address specific issues. The potential for a mAb to cause any changes in the relative proportions of different types of immune cells should be addressed by immune cell phenotyping. The potential for functional impairment in mounting of an immune response, for example by investigation of the T cell dependent antigen response (TDAR), should be evaluated, when relevant. Depending on the perceived risk there might be a need for pathogen challenge tests or for evaluation of potential effect on the target animal's ability to induce adequate immune response to vaccination.

MAbs with immunomodulatory MoA may also typically induce development of autoimmune-type adverse effects. These aspects can normally be addressed in a conventional TAS study and in a field study.

The evaluation of effects on other physiological systems is normally part of the target animal studies and in most cases that is sufficient. However, in cases where there is a specific concern related to adverse effects on certain vital functions a more thorough and focused evaluation such as ECG, can be included in the TAS study or addressed in a separate safety study in target animals.

For mAbs intended for chronic use, evaluation of the potential for increased risk for malignancies could be included in a TAS study with a duration of 6 months or more. An adequate number of animals should be included to allow relevant risk evaluation, typically 8 animals per treatment group at a minimum. However, more reliable data could be gained from a long-term field study in target species. Usually there is only limited information available before authorization. Therefore, where carcinogenicity remains a concern, the risk should be addressed by adequate follow-up measures. For mAbs used only for short-term treatment, and where the risk can be considered negligible based on pharmacology, evaluation of the risk of carcinogenicity is not usually needed.

Immunogenicity i.e. development of antibody-drug antibodies (ADAs) is a relevant concern of both safety and efficacy. Development of binding ADAs may affect pharmacokinetic behavior of the

mAb by either increasing or decreasing clearance and may thus influence the mAb exposure. ADAs may also neutralize the mAb which may lead to loss of efficacy. Consequently, evaluation of an immunogenic potential should be an integral part of pharmacokinetic, pharmacodynamic, safety and efficacy studies.

6.3 How to evaluate effects due to interaction of the mAb with the target antigen at non-target site(s)?

The potential off-target effect due to interaction of the mAb with the target antigen on non-target tissues is an important safety aspect that needs to be considered. Risk assessment based on the weight of evidence from available scientific information on e.g. expression of the target molecule in non-target tissues in healthy and diseased animals and on human experience should be used to identify the need for further data.

Expression patterns (i.e. extent of tissues and organs expressing the target molecule and expression level in a given tissue) of certain target molecules can be significantly different in various species and limited information may be available. In particular, the information on potential differences in expression pattern of the target molecule in healthy and diseased animals may be scarce. Therefore, direct extrapolation from healthy to diseased animals or from one species to another may not be appropriate. The weight of evidence should be carefully balanced with respect to the perceived risks and the need for further safety data. If further safety data is warranted based on the risk assessment such information can be generated in tissue cross-reactivity studies with a panel of tissues from the target animal species. In general, however, the safety data derived from the TAS study and/or field study may be sufficient to address the potential risk and the dose level at which the unintended secondary effects at non-target sites are kept at an acceptable level.

6.4 How to evaluate effects due to interaction of the mAb with related antigens at the target/non-target site(s)?

The potential off-target effects related to interaction of the mAb with related antigens should be evaluated on a case by case basis. The pharmacological characterisation of the mAb should include evaluation of specificity of target binding and the potential for binding to related antigens. These data are normally generated in *in vitro* and/or cell based assays. The extent of such analyses should be designed based on the available information on related molecules.

In silico tools to compare amino acid sequences and target epitopes between the intended target molecule and related molecules may be used for initial identification of potential cross-reactivity. These analyses can be complemented with *in vitro* binding assays and/or functional cell based assays to demonstrate the possibility for unintended effects. These additional data can provide important information to guide in designing a tailored and more focused approach for safety evaluation in the TAS study in order to identify clinically relevant effects on related antigens. In case suitable pharmacodynamics markers are available the potential for clinically relevant effects can be evaluated in an appropriately designed TAS study or in a field study.

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