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Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use.

Draft

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Executive summary

This guideline addresses issues relating to the unwanted immunogenicity of monoclonal antibodies intended for clinical use. These include the variability of immunogenicity of mAbs and its consequences, prediction and minimizing immunogenicity, the clinical consequences of immunogenicity, assay related problems, assessing neutralizing antibodies induced by monoclonal antibodies and consideration of a risk-based approach for the evaluation of immunogenicity of monoclonal antibodies.

1. Introduction (background)

Unwanted immunogenicity can be a significant problem in the treatment of patients with therapeutic biologicals. The importance of the unwanted immunogenicity problem has led to the preparation and adoption of the 'Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins' by the CHMP (adopted April 2008, referred to henceforth as 'the general guideline'), which in principle is applicable to monoclonal antibodies (mAbs). However, some specific aspects of immunogenicity are exclusively or primarily relevant for mAbs, novel mAb derivatives (eg Fab fragments, scfv, nanobodies, minibodies) or biosimilar mAbs and these are addressed in this guideline.

Monoclonal Antibodies (mAbs) comprise a large important class of therapeutic biologicals. The range of clinical indications with potential for treatment with mAbs is very wide. Many mAb products are known to be associated with unwanted immunogenicity and in some cases the immunogenicity causes impaired clinical responses or rarely serious adverse reactions which require clinical intervention. The wide range of mAbs in development, and approved for different clinical indications precludes specific guidelines that are pertinent to all situations. This guideline addresses the major quality and clinical aspects that are important to consider in order to adequately address the problems with detection of and risk related to the development of an immune response to the particular mAb in the particular clinical indication sought.

2. Scope

The general principles adopted and explained in this document mainly apply to the development of an unwanted immune response against a therapeutic or in vivo diagnostic mAb in recipients and how to systematically evaluate this. The guideline applies to mAbs, their derivatives, and products of which they are components, e.g., conjugates.

This guideline should be read in conjunction with other relevant guidelines, e.g.:

- Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins
- Guidelines on similar biological (biosimilar) medicinal products
- Guideline on Development, Production, Characterisation and Specifications for Monoclonal Antibodies and Related Products
- European Pharmacopeia monograph on monoclonal antibodies
- Guidelines on comparability of biotechnology-derived medicinal products after a change in the manufacturing process.

This guideline is primarily aimed at products at final development stage (e.g. marketing authorization application stage). However, many of the principles are relevant to earlier phases of development.

3. Legal basis

This guideline should to be read in conjunction with the introduction and general principles (4) and part III of the Annex I to Directive 2001/83 as amended.

4. Variability of immunogenicity of mAbs and its consequences

The factors discussed here are part of risk estimation in a risk-based approach of unwanted immunogenicity.

The immunogenicity of mAbs is complex and there are a number of often poorly understood factors which makes it difficult to predict with any certainty whether a therapeutically or diagnostically administered monoclonal antibody is likely to provoke an immunogenic response. For heterologous e.g. rodent sequence or human chimaeric mAbs, recognition of the antibody as being foreign is the primary basis for antibody mediated immunity. In such situations, antibodies can be produced against various epitopes present on different parts of the molecule e.g. anti-Fab, anti-Fc. Production of monoclonal antibodies identical to the endogenous human amino acid sequence can reduce the risk of immunogenicity but may not eliminate it because factors other than primary sequence contribute to immunologic potential. In such cases, especially with humanised or human sequence mAbs the immune response is predominantly anti-idiotypic (as the CDRs are unique in sequence for mAbs), which clearly can compromise clinical responses to the mAb. In some cases, antibodies can be induced against the constant region of human or humanised mAbs and this can affect the immunobiological function of the mAb. There is less experience with clinical use of emerging constructs and these may add to the perception of risk. Special consideration should be given to next generation products, for example, bivalent mAbs.

The formulation, container system (including container closure systems), or storage conditions can impact on the immunogenicity profile of the product. These factors may influence the immunogenic properties by interactions of ingredients with the therapeutic protein and the container closure system e.g. modification of protein conformation, extraction of impurities acting as immune adjuvants, provoking alterations such as aggregation, particulates or deamidation . Altered glycosylation patterns may decrease or enforce the immunogenic properties of the molecule, e.g. by shielding the protein backbone or triggering innate immune responses by Toll-like receptors. Non-typical glycosylation patterns, e.g. as may occur with entirely novel expression systems, may give rise to altered immunogenicity compared to what is usually observed with more commonly used expression systems. Other factors that contribute to immunogenicity include impurities arising from the production method, route, dose and frequency of administration.

Patient related factors may influence immunogenicity e.g., differences in major histocompatability and human leukocyte antigen alleles among recipients and the physiological status of each patient. The latter includes the individual history of previous microbial and viral infections. The rate of antibody formation is also influenced by the individual immune responsiveness. Immunogenicity for mAbs can be age related i.e. protein turnover is different in children compared to adults and this can result in differences in observed immunogenicity, e.g. for antibodies used in treatment of juvenile arthritis compared to rheumatoid arthritis at a comparable dose. Disease related factors also strongly influence immunogenicity as does concomitant treatment. Furthermore, previous exposure to similar or related monoclonal antibodies can also influence immunogenicity. Therapeutic antibodies used in a repeated dosing scheme or with intermittent dosing scheme changes have a higher likelihood to induce immunogenicity than single use mAbs.

Whether antibodies against a mAb have clinically significant effects depends on the binding site of the antibody, the affinity of the antibody for the mAb and the titre of the antibodies that develop. Antibodies against mAbs can transiently occur and then disappear during treatment or persist throughout treatment or for longer. For some monoclonal antibody therapies, the development of antibodies has no apparent adverse clinical consequences but for others it reduces efficacy or is associated with therapy related adverse events.

5. Approaches which may be helpful in predicting and reducing the unwanted immunogenicity of mAbs.

The design and selection of the mAb is the responsibility of the applicant. In-vitro approaches with the aim of predicting immunogenicity have been developed (see general guidelines). In-silico modelling may help to identify T-cell epitopes but does not predict whether immunogenicity will occur. Confirmation/identification of T-cell epitopes using in-vitro cell based assays has been refined and is often applied to therapeutic mAbs. The relatively large size of the mAb molecule makes it likely that each molecule will contain several such epitopes. Both T-helper and T-regulatory epitopes have apparently been identified on mAbs.

Various strategies for reducing the immunogenicity of mAb therapeutics are currently being considered. These involve protocols for induction of tolerance to the mAb or 'de-immunizing' the mAb by deletion of relevant T-cell epitopes. Deletion of T-helper epitopes may result in reduced immunogenicity, whereas the reverse would be the case for deletion of T-regulatory epitopes.

6. The clinical consequences of immunogenicity of mAbs

The clinical consequences described following antibody development against mAbs include loss or reduction of efficacy, local reactions, serum sickness/immune complex-mediated disease, and major allergic reactions (e.g. urticaria, bronchospasm, bronchoconstriction).The severity of the consequences of these different reactions can be affected by the underlying health status of the patient, e.g. a severe IgE-mediated allergic reaction is more likely to result in serious consequences for a patient with chronic asthma, and this potential reaction would be particularly serious if the patient was on home therapy.

It is important to note that not all induced antibodies are present in the serum i.e. they may be present in various organs. It is important during the clinical development to measure antibody levels, PK, PD markers, efficacy and safety simultaneously and over a period of repeated treatments. This allows assessment of the clinical significance of antibody development, and also whether the antibody effect changes over time, which could occur as a result of affinity maturation of the antibody response or /and epitope spreading. Unexpected clinical observations (e.g., loss of efficacy or considerable differences in PK) could be the result of undetected antibodies and should be further investigated.

Treatment with mAb can lead to the development of any class of immunoglobulin, although IgG is the most commonly induced class. In some cases, low affinity IgM antibodies can be induced. Antibodies can reduce the PK, PD and efficacy and can result in neutralisation of the mAb. The ability to measure induced antibody in the serum is limited by the clearances of complexes. Formation of immune complexes can lead to serum sickness which presents with features including haematuria, fever, arthralgia and in severe cases acute renal failure.

In some instances, IgE testing needs to be considered for patients if the mAb contains non-human carbohydrate structures. Another instance where development of IgE testing should be considered is where the incidence of allergic reactions is high on first administration during early clinical

development of the product. The availability of an appropriate IgE assay allows exclusion of those subjects with a positive result.

IgA antibody testing may only be needed on a case-by-case basis depending on the route of administration but is not usually required. IgA antibodies induced by biological products have been described and are usually accompanied by IgG antibodies. IgA antibodies are more likely to result from airway/gut administration of the mAb and such antibodies are present in secretions. Detection of mucosal immune responses using secreted fluids such as sputum as the sample is comparatively insensitive due to interfering matrix effects. The testing strategy needs to take this into account.

In many cases, the incidence of immune response is too low to be fully identified during Phase III clinical studies. Therefore systematic post-authorisation monitoring may be necessary and should be adequately organised to capture clinical signs that could be related to immunogenicity. The involvement of antibodies in this should be established by conducting appropriate assays.

Following marketing of mAbs, the features of major reactions such as serum sickness or severe allergic reactions are diagnosed clinically. In cases where adverse events follow administration of the implicated mAb, the reactions are attributed to an antibody response. The same rationale applies in cases where loss of efficacy is observed. In view of the potential seriousness of unwanted immunogenicity, it is important that confirmation and characterization of antibody induction is conducted.

Because detection of antibodies against mAbs is rarely monitored in clinical practice, it is unclear – other than in instances of obvious clinical evidence of one of the presentations listed above whether the development of antibodies to mAbs has additional unrecognised consequences.

Detection of antibodies in low dose cohorts does not necessarily justify termination of treatment. The need to terminate treatment because of antibody formation can only be assessed in combination with clinical findings and requires careful assessment and monitoring.

7. Problems experienced with screening and confirmatory assays used in assessing immunogenicity of mAbs

7.1. Assays for antibody detection

The general guideline outlines relevant information on assays and strategies that apply to mAb products. In principle, any immunoassay format can be used to measure antibodies against mAbs. However, assays used to detect antibodies against mAbs are often more problematic and difficult than those employed for other biologicals like G-CSF, EPO and interferons. Experience has shown that measuring antibodies against antibodies can be technically very challenging. Many standard assay formats involve the use of anti-immunoglobulin reagents such as antibodies against immunoglobulins, protein A or protein G, but these are inappropriate for use in detecting antibodies against mAbs as they very often bind to the product itself. Thus, for example simple ELISAs and radio-immunoprecipitation assays are not usually suitable for use with mAbs unless they are adapted to overcome this problem. Therefore, different assay approaches have to be adopted/developed. A common approach is to use the 'bridging' ELISA format which does not require anti-immunoglobulin reagents and so can be directly applied to studies with mAbs. However, this procedure may be less sensitive than other immunoassay methods and can require significant development effort to produce a suitable assay. It also will not efficiently detect the IgG_4 antibodies which can be produced in some cases. Another approach is to use a Surface Plasmon Resonance (SPR) procedure. This does not require anti-immunoglobulin reagents for detecting antibodies against mAbs. It is a real-time procedure and is therefore fast and also detects rapidly dissociating antibodies which can be missed by other methods.

However, as SPR simply detects protein binding to the coated chip it needs to be confirmed that the signal is caused by antibodies. It can be less sensitive than other methods for detecting high affinity antibodies and, in the absence of automated sampling systems may have a low throughput.

Samples (normally serum or plasma) may contain substances that interfere with the assays which produce false positive or negative results and/or incorrect assessment of antibody content. Well known examples of this are complement components, mannose binding protein, Fc receptors, complement receptor 1 and rheumatoid factors, but other substances can also cause problems. Assays often need to be 'tailored' to reduce artefacts and achieve acceptable background signal levels, sensitivity and specificity.

7.2. Presence of mAb product in samples

MAb products are usually administered in relatively high doses. They have relatively long half lives in circulation and even fragments persist in blood for several days. This can cause significant problems in detection of antibody responses due to the presence of mAb product in samples collected for antibody assessment. This normally results in an artefactually low estimate of antibody content of affected samples and can be so pronounced as to cause false negative results. Several approaches have been proposed to overcome this problem. One possibility is to delay sampling until levels of mAb product have declined sufficiently to no longer cause problems. This has been claimed to resolve the problem with some mAb products, but requires careful assessment as it has the potential to fail to detect immunogenicity, as induced antibodies have declined to undetectable levels by the time the samples are taken. Another approach is to use methodology which is least affected by the problem. Some ECL based immunoassays seem much less affected by residual product in samples than other methods, including conventional bridging ELISAs. A commonly described procedure for dealing with the problem is to include a preliminary antigen-antibody dissociation step in the assay design so that any complexes present are disrupted before antibody is detected. Various versions of assays which include acid incubations, sometimes coupled with affinity separation of product have been described for this. A final possibility is to dilute samples so that residual product present is insufficient to interfere with the assay. This approach needs care as it may result in a false negative assessment of immunogenicity if the assay is not sufficiently sensitive to detect antibodies in the diluted samples. In some cases it may be necessary to assay samples for the amount of residual mAb.

7.3. Confirmatory Assays

Confirmatory assays can suffer from the same problems as screening assays. It is important to select an appropriate confirmatory assay taking account of the characteristics of the screening assay. The most common approach for this is to include an incubation step with the mAb product in the assay to show that this results in a significantly diminished signal when assaying real antibody positive samples.

7.4. Controls

Generation of positive control sera is in general a critical issue for immunogenicity studies for mAbs. The chosen positive control serum affects sensitivity and specificity of the immunogenicity assay. If human sera are not available (as is likely during early phases of product development) then use of animal sera is the only option. Choice of species for this has important consequences. Non-human primates produce primarily anti-CDR responses against human or humanized mAbs, which may closely mimic human responses. However, non-primate species usually produce antibodies primarily against the constant regions of the mAb, which is unlike human responses. Use of an anti-idiotypic antiserum or mAb can, in some cases, provide a useful positive control. Selection of appropriate negative controls

is important. For confirmatory assays, spiking samples with an irrelevant mAb or (better) with a mAb with the same Fc but different CDRs as the product can be used to confirm specificity.

8. Assessing the neutralising capacity of antibodies induced against mAbs.

Antibodies which neutralize the biological activity of biological products may diminish clinical efficacy of the product. It is normally expected that the neutralizing capacity of any antibodies induced is measured. Deviations need to be justified. For most biological products, the most appropriate neutralizing antibody assay is a bioassay which measures the neutralization of the bioactivity of the product by antibodies. However, the nature of the clinical mode of action of mAbs implies that induced antibodies which block mAb binding to target are those which are mostly associated with reduced clinical efficacy. Therefore, competitive ligand binding assays are often the neutralizing assays of choice for mAbs rather than classic bioassays. This distinguishes mAbs from other classes of biologicals with regard to immunogenicity assessment.

MAbs exert their action by various mechanisms ranging from simple binding to antigen, which alone mediates the clinical effect, to binding antigen and mediating one or more immunobiological mechanisms which combine to produce the overall clinical response. Therefore, although simple binding may seem to be the only mechanism operating to achieve clinical efficacy, other effects may also play a role in this. In some cases multiple functions of the mAb may be involved in an additive or synergistic manner to produce an overall combined clinical affect and this may be hard to dissect experimentally to allow a clear understanding of how the mAb mediates its clinical potency. Therefore, if intact mAbs are used, care must be taken not to assume that the Fc mediated immunobiological effects of the product are not involved in clinical efficacy, even when simple antigen binding is considered to be the primary mode of action. In such cases a thorough biological characterization of the mAb must be undertaken, using appropriate biological and immunological assays. Following this, the properties of the mAb need to be assessed to allow selection of an appropriate neutralizing assay strategy.

9. Risk-based Approach

Every therapeutic mAb needs to be evaluated for immunogenicity individually and all immunogenicity strategies should be adapted for each mAb development programme.

A risk-based approach can provide a starting point from which the further concept of immunogenicity testing can be designed, but due to the diversity of risk factors, as discussed in this guideline, and the variety of mAbs and mAb-related products, the recommendations given here cannot be generalized. Assessment is based on the identification of risk factors inherent to the particular mAb in question, the final drug product and the treated patient population. The mechanism of action and the basic structure (chimaeric, humanized, fully human) are not sufficient for deciding on the attribution of risk level. For a risk-based approach, applicants need to define what “risk” in this context means.

9.1. Risk of mounting an unwanted immune response

This will depend on various factors that can be divided into three different subgroups, i.e. product-, process- and patient-related risk factors (see general immunogenicity guideline). These risk factors should be identified, specified and comprehensively estimated. The relative significance of each factor needs to be taken into account and comprehensively discussed individually for each product on a case-by-case basis. Applicants should thoroughly justify their overall concept for the design and extent of immunogenicity testing of their development programme, and should carefully plan this concept early

in product development. During development of a novel mAb, the overall assumed immunogenic risk profile is composed of the total of all risk factors, whereas the most prominent factors usually determine the extent of data necessary to allow for an assessment of immunogenicity. If, based on such an evaluation, the risk of unwanted immunogenicity is perceived to be high, more safety measures may have to be implemented in clinical trials to study immunogenic potential and measures implemented to potentially handle the clinical consequences of these. For products that are claimed to exhibit a particular advantage as regards immunogenic potential, (e.g. a claim in the Summary of Product Characteristics) appropriate data is usually required to support such a claim.

MAbs are produced in various cell lines that can be of mammalian or non-mammalian origin. Non-mammalian products may contain proteins acting as adjuvants which may lead to development of antibodies thereby influencing the product's safety and efficacy. The same applies to other process related impurities and these need to be reduced to the lowest levels possible during the purification steps. In addition, product isoforms, as well as product related impurities and degradation products should be considered in the risk assessment.

At the beginning of clinical development applicants may have to assign a high risk level, although the mechanism of action may per se not necessarily suggest a higher risk. The risk level may, depending on the results of larger clinical trials, need to be re-considered following the trials. This should be fully justified by Applicants at the time of marketing authorisation.

Treatment modalities such as dosing, schedule of administration and concomitant treatments can impact on the immunogenicity profile and should be carefully considered. The route of administration can be classified as potentially lower (IV), medium (IM) or higher (SC) risk. For mAbs that are developed as subcutaneous products, often intended for patient self-administration, the risk should be carefully justified, taking into account that for such a clinical scenario, the mAb will, after initial physician supervision, be administered by patients in a home setting with less physician surveillance. In general, short-term treatment is usually associated with a lower risk of inducing an unwanted immune response than long-term treatment. For the latter, the optimal time period between repeated administrations should be determined.

Patient-related risk factors include age, genetic background and the underlying disease. Children may have higher protein metabolism and a different immune status than adults, and cases are known where data suggest a considerably higher immunogenicity of mAbs. In this patient group immunogenicity should be evaluated separately as for adults. Extrapolation of immunogenicity data from a previously conducted clinical study in adults is not sufficient. The genetic background or underlying disease and concomitant therapy of the patient can influence its immune status impacting on the immunogenicity of the product. The immune status of the patients should be taken into account for risk estimation.

The risk perception may be higher if the methodology to either detect anti-drug antibodies or to detect clinical consequences is not sensitive.

9.2. The severity of clinical consequences of an immune response

A pivotal aspect of risk assessment is the evaluation of clinical consequences of an unwanted immune response. Therefore, the mode of action of the mAb (e.g., lytic, apoptotic), and especially the nature of the target molecule (e.g., immunosilencing, immunostimulating), needs to be adequately characterized and comprehensively investigated.

Antibody responses which target the idiotype of a mAb usually result in diminished efficacy. However, it needs to be considered that based on the knowledge of the signalling cascade mediated by the mAb target, a potential cross-reactivity of an anti-idiotypic immune response with certain agonistic

structures might be possible. Non-idiotypic antibodies to mAbs can be clinically important by positively or negatively affecting the bioavailability of the product. Alternatively non-idiotypic antibodies against mAb may react with endogenous serum immunoglobulins and trigger clinical effects similar to those mediated by rheumatoid factors. Furthermore, the detection of binding, non-neutralizing antibodies may be an early indication of the development of neutralizing antibodies due to epitope spreading.

Depending on the class and subclass of the mAb (which affects immunobiological functions e.g. binding to Fc receptors) or the mechanism of action, individual mAb products may not all have the same risk associated with an unwanted immune response. For example, mAbs can be neutralized by antibodies resulting in a reduced efficacy, or result in adverse events such as infusion reactions. Such infusion reactions can be severe, but can be potentially handled by appropriate clinical measures such as the use of pre-medication. Antibodies can cross-link immunomodulatory mAbs with agonistic properties. Such cross-linking can lead to enhanced activation of the immune system and potentially threatening cytokine release syndromes, and maybe difficult to predict at the individual patient level. For agonistic mAbs or for mAbs where cross-linking could on theoretical considerations lead to immunoactivation, applicants should consider careful observation of patients in early clinical trials to see if such events occur.

Another possible, though relatively rare, scenario is the occurrence of immediate hypersensitivity reactions that usually appear after re-exposure to the mAb. The incidence of such unwanted immune responses is also dependent on the time interval between doses and usually reduces with longer time periods. Hypersensitivity reactions should be monitored and administration of high risk mAbs should be conducted under conditions where life-threatening events can be mitigated. Close surveillance of patients on the second and subsequent dosing with mAb is necessary, since sensitization can occur de-novo upon first exposure.

9.3. Consequences with regard to different risk classes

For all mAbs a validated screening and confirmatory assay should be performed followed by a validated neutralizing assay in case of positive results in the confirmatory assay. Distinguishing between neutralizing and non-neutralizing antibodies is essential for all mAbs regardless of their risk level as lack of, or even reduced efficacy due to the neutralizing activity of the antibodies may result in a discontinuation of treatment with the mAb.

Correlation of antibody development with clinical outcome is important and has to be thoroughly evaluated. However, the frequency of sampling and analysis could vary depending on the individual risk level. Depending on the risk level attributed to a mAb it may be possible to reduce sampling frequency in later stages of development, provided that no adverse events or reduced efficacy is observed. Nevertheless, banking of samples should be undertaken on a routine basis over the whole development programme. For high risk mAbs sampling should be frequent during the whole clinical development. In this situation it is advisable to analyze samples in real time.

The approach outlined above should enable appropriate immunogenicity testing and assessment of mAbs on the basis of scientific data underpinning identified immunogenicity risks. It is recommended that the applicants address these risks adequately in a Risk Management Plan (RMP) ensuring not only risk identification and characterisation but also risk monitoring, minimization and mitigation strategies.

References

- Directive 2001/83/EC, as amended.
- Part III of the Annex I of Directive 2001/83/EC, as amended.
- Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins (EMEA/CHMP/BMWP/14327/2006)