EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 September 2015
EMEA/CHMP/BMWP/14327/2006 Rev. 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins

Draft

Draft agreed by Biosimilar Medicinal Products Working Party (BMWP)	August 2015
Adopted by CHMP for release for consultation	24 September 2015
Start of public consultation	01 October 2015
End of consultation (deadline for comments)	31 January 2016

This guideline replaces 'Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins' (EMEA/CHMP/BMWP/14327/2006).

Comments should be provided using this template. The completed comments form should be sent to BMWP.secretariat@ema.europa.eu

Keywords	***Immunogenicity, therapeutic proteins, anti-drug antibodies (ADA), assays, assay strategy, binding antibodies, neutralising antibodies, risk factors, safety, efficacy, pharmacokinetics, risk management, integrated summary of immunogenicity***

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

Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins

Table of contents

Executive summary

The number of biological/biotechnology-derived proteins used as therapeutic agents is steadily increasing. These products may induce unwanted immune responses, which can be influenced by various factors, including patient- and disease-related factors as well as product-related factors. This document is a revision of the guideline EMEA/CHMP/BMWP/14327/2006 on the basis of experience from marketing authorisation applications and other new information. It contains, among others, more specific requirements for assays for immunogenicity and integrated analysis of the clinical significance of immunogenicity. The risk of immunogenicity varies between products and product categories, on one hand, and between individuals and patient groups, on the other hand. In order to facilitate the risk analysis, the guideline contains a list of issues to be considered, a multidisciplinary summary of immunogenicity, including risk assessment. This summary allows a risk-based approach to immunogenicity which means that the extent and type of pre-authorisation immunogenicity studies and post-marketing risk management program are tailored according to the risk of immunogenicity and the severity of its potential or observed consequences.

From a regulatory point of view, the predictive value of non-clinical studies for evaluation of immunogenicity of a biological medicinal product in humans is low due to differences between human and animal immune systems and to immunogenicity of human proteins in animals. While non-clinical studies aimed at predicting immunogenicity in humans are normally not required, novel models may, for example, be of value in selecting lead compounds for development and unravelling cellular mechanisms.

The development of adequate screening and confirmatory assays to measure immune responses against a therapeutic protein is the basis of the evaluation of immunogenicity. Assays capable of distinguishing neutralizing from non-neutralizing antibodies are normally needed. Assays that are used in pivotal clinical trials as well as in post-authorisation studies need to be validated.

In the clinical setting, the investigation of immunogenicity should be based on integrated analysis of immunological, pharmacokinetic and clinical efficacy and safety data in order to understand the clinical consequences. The sampling schedule for each product should be determined on a case-by-case basis, taking into account the potential risks associated with unwanted immune responses to patients and the timing of pharmacokinetic and clinical evaluations. Immunogenicity issues should be further addressed in the Risk Management Plan (RMP).

Considering the scope of this guideline is wide, the recommendations might have to be adapted on a case-by-case basis to fit into an individual development program. Applicants should consider the possibility to seek Scientific Advice from EMA or from National Competent Authorities.

The planning and evaluation of immunogenicity is a multidisciplinary exercise. Therefore, it is recommended that the Applicant will present an integrated summary of the immunogenicity program, including a risk assessment to justify the selected approach.

1. Introduction

Most biological/biotechnology-derived proteins induce an immune response. This immunological response to therapeutic proteins is complex and, in addition to antibody formation, T cell activation or innate immune responses could contribute to potential adverse effects.

The consequences of an immune reaction to a therapeutic protein range from transient appearance of antibodies (anti-drug antibodies, ADAs) without any clinical significance to severe life-threatening

conditions. Potential clinical consequences of an unwanted immune response include loss of efficacy of the therapeutic protein, serious acute immune effects such as anaphylaxis, and, for therapeutic proteins used for substitution, cross-reactivity with the endogenous counterpart.

Many patient-, disease-and product-related factors may influence the immunogenicity of therapeutic proteins. Patient-related factors that might predispose an individual to an immune response include the genetic background, pre-existing immunity, immune status, including immunomodulating therapy, as well as dosing schedule and route of administration. Product-related factors that influence the likelihood of an immune response include the manufacturing process, formulation, and stability characteristics.

Although data on possible unwanted immune reactions to therapeutic proteins are required before marketing authorisation, problems may still be encountered in the post-authorisation period. Depending on the immunogenic potential of the therapeutic protein and the rarity of the disease, the extent of immunogenicity data before approval might be limited. Controlled clinical trials cannot be used to study rare adverse effects. Thus, further systematic immunogenicity testing is often necessary after marketing authorization, and may be included in the risk management plan.

2. Scope

The general principles adopted and explained in this document mainly apply to the development of an unwanted immune response against a therapeutic protein in patients and to a systematic evaluation of it. The guideline applies to proteins and polypeptides, their derivatives, and products of which they are components, e.g. conjugates. These proteins and polypeptides are mainly produced by recombinant or non-recombinant expression systems. Throughout this guideline, the term "therapeutic protein" is used.

For coagulation factors, please, refer to the specific CHMP guidelines in this area (see chapter 3).

3. Legal basis and relevant guidelines

This guideline has to be read in conjunction with the introduction and general principles (4) and parts II and III of the Annex I to Directive 2001/83 as amended. This guideline should be read in conjunction with other relevant guidelines, e.g.:

- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (EMEA/CHMP/BMWP/42832/2005 Rev. 1)
- Guideline on Comparability of biotechnology-derived medicinal products after a change in the manufacturing process - non-clinical and clinical issues (EMEA/CHMP/BMWP/101695/2006)
- Guideline on Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use (EMA/CHMP/BMWP/86289/2010)
- Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev.1 Corr.*)
- Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products (EMA/CHMP/BPWP/144533/2009)
- Clinical investigation of recombinant and human plasma-derived factor IX products (superseded by EMA/CHMP/BPWP/144552/2009 rev 1)
- ICH S6 (R1) Harmonised Tripartite Guideline on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals

- ICH Harmonised Tripartite Guideline on Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process Q5E, 2004

4. Factors that may influence the development of an immune response against a therapeutic protein

4.1. Patient- and disease-related factors

Patient-related factors, which might influence the immune response to a therapeutic protein, include genetic factors, age of the patient, disease-related factors including other treatments, and pre-existing antibodies (protein therapeutic -reactive antibodies) due to previous exposure to the product or products containing substances with structural similarity; as well as sensitisation of patients due to process-and product-related impurities and excipients.

- *Genetic factors modulating the immune response*

Genetic factors may alter immune responses to a therapeutic protein and lead to inter-patient variability. Genetic variation at the level of MHC molecules- and T-cell receptor will modify the immune recognition whereas genetic variation at the level of the modulating factors, such as cytokines and cytokine receptors, may influence the intensity of the response.

- *Genetic factors related to a gene defect*

When the therapeutic protein is used for substitution of an endogenous protein (e.g. factor VIII; enzyme replacement) where the patient is deficient of the natural counterpart, the physiological antigen may represent a neo-antigen and the immune system will interpret the therapeutic protein as foreign or non-self.

- *Age*

Data on immunogenicity from one age group cannot necessarily be projected to others, since immune response to therapeutic proteins can be affected by patient age. Among children, different levels of maturation of the immune system are seen depending on age, and discrepant immune responses to a biological product may be expected.

If the product is indicated for children, clinical studies are usually expected to be carried out in this age group. In that case, immunogenicity data should be gathered in these studies as well. If indicated for elderly, consideration should be given to a potentially altered immune response, including autoimmunity.

- *Disease-related factors*

A patient's underlying disease can be an important factor in the context of developing an unwanted immune response. Patients with activated immune systems (for example those suffering from chronic infections, allergies and autoimmune diseases may be more prone to immune responses to therapeutic proteins. In other conditions (e.g. malnutrition, advanced malignant disease, advanced HIV disease, organ failure), an immune response might be less likely to occur due to an impaired immune system.

For some products, it has been reported that the development of an antibody response can be different for different therapeutic indications or different stages of the disease. In principle, immunogenicity needs to be addressed in all clinical indications unless justified.

- *Concomitant treatment*

Concomitant therapies may either decrease or increase the risk of an immune response to a therapeutic protein. Typically, the immune reaction against a therapeutic protein is reduced when immunosuppressive agents are used concomitantly. However, an immune response against a therapeutic product is a result of many factors and thus, conclusions on potential impact of the concomitant immuno-modulating medication are not straightforward.

Consideration should also be given to previous treatments that can influence the immune reaction to a therapeutic protein and may have a long-term impact on the immune system. If clinical trials of a product with a new active substance are performed in combination with immuno-suppressants, a claim for use of the protein drug in monotherapy must be accompanied by adequate clinical data on the immunogenicity profile in the absence of immuno-suppressive agents.

- *Posology-related factors*

Factors which may increase the immune response to a therapeutic protein include dosage, dosing schedule and route of administration. Products given intravenously may be less immunogenic than drugs given subcutaneously or intramuscularly. Inhalational and intradermal administration may also enhance immune responses towards the therapeutic protein.

Short-term treatment is usually less likely to be associated with a harmful immune response than long-term treatment, and products given continuously are usually less immunogenic than those given intermittently. Intermittent treatment or re-exposure after a long treatment-free interval may be associated with an enhanced immune response.

ADA formation against protein therapeutics can be either transient (disappear over time) or sustained.

- *Pre-existing antibodies*

Previous exposure to similar or related proteins can lead to pre-sensitisation that may modify the response to the new therapeutic protein, e.g. proteins being used for replacement therapy. In addition, sensitisation to excipients in the formulation, as well as to impurities/contaminants from the manufacturing process, may also lead to the generation of pre-existing immunogenicity to the product.

Pre-existing reactivity towards the therapeutic proteins (cross-reacting ADAs, rheumatoid factors, antibodies to non-human carbohydrate moieties etc.) at baseline may influence the production of ADAs. Pre-existing antibodies against a variety of protein therapeutics (e.g. monoclonal antibodies, fusion proteins) are frequently encountered, especially in the context of autoimmune diseases. Pre-existing antibodies may also be found in treatment-naïve patients. While the impact of pre-existing antibodies on safety and/or efficacy of biologics is poorly understood, consequences could be severe for e.g. patients receiving 'replacement products' like blood clotting factors, if the previous antibodies are cross-reacting with the newly introduced protein product. Therefore, potential cross-reactivity with pre-existing antibodies should be considered.

4.2. Product related risk factors

Product-related factors influencing the immunogenicity of biotechnology-derived therapeutic proteins include the origin (for instance, the expression construct) and nature of the active substance (structural homology, post-translational modifications), major modifications of the therapeutic protein (e.g. pegylation, fusion proteins, bispecific antibodies, conjugates between a protein and a chemical drug/moiety), product-related (e.g. degradation products, impurities, aggregates) and process-related impurities (host cell proteins, lipids or DNA, bacterial contaminants), formulation (excipients) and product packaging (containers, stoppers).

- *Protein structure and immunological tolerance*

T cell-dependent antibody responses involve a complex interplay among antigen presenting cells, T cells, secreted cytokines and B cells, subsequently leading to B cell activation, somatic hypermutation and antibody production and sometimes to immunoglobulin class-switch. Within this cascade, there are central and peripheral immunological factors that together form the basis of immunological tolerance to endogenous proteins.

Immune tolerance to endogenous proteins is variable; in general tolerance is weaker for low-abundance proteins than for high-abundance proteins. Thus, levels of cytokines and growth factors are low whereas autoantibodies towards cytokines and growth factors in healthy individuals are not uncommon.

Biotechnology-derived analogues to human endogenous proteins may trigger an immune response due to variations in the amino acid sequence or changes to the protein structure as a result of post-translational modifications or other changes during all steps of the manufacturing process, storage and administration.

The key driver of mature immunological reactions is the activation of T cells. T cell epitopes are linear peptides. Thus, a difference in the amino acid sequence between an endogenous and a therapeutic protein may modify T cell epitopes.

T cell-independent antibody responses may be generated when B cells recognise a repeated pattern in the biological product (polysaccharides, lipopolysaccharides, possible moieties in the therapeutic protein, aggregates), that elicits low-affinity IgM antibodies. However, switching to IgG classes including clonal expansion, may also take place, and the mechanism behind this evolution of the immune reaction is still not fully understood.

Glycosylation can influence both the physico-chemical and biological properties of a protein. The presence and structure of carbohydrate moieties may have both a direct or indirect impact on the immunogenicity of therapeutic proteins; the glycan can induce an immune response itself (e.g. glycans of non-human origin), or its presence may affect the conformation of the protein in such a way that the protein becomes immunogenic.

Fusion proteins may contain neo-epitopes due to the introduction of foreign peptide sequences, e.g. in linkages/fusion junctions. Antibodies generated specifically against the polyethylene-glycol part of pegylated proteins have been identified. However, pegylation and glycosylation may also decrease immunogenicity by shielding the immunogenic epitopes, while maintaining the native conformation of the protein.

Fusion proteins composed of a foreign and self-protein may be of concern, especially because of the potential of the foreign moiety to provoke an immune response to the self-protein (epitope spreading). Thus, identification of the antigenic moiety of a fusion protein is useful.

- *Formulation and packaging*

The composition of a formulation is chosen in order to increase the stability of the product, i.e. to best maintain the native conformation of therapeutic proteins. A successful, robust formulation depends on the understanding of the physical and chemical nature of the active substance and the excipients alone and their interaction. The formulation and the source of excipients may influence the immunogenicity of therapeutic proteins. This should be taken into account when introducing variations to the formulation.

In addition, interactions between the protein substance, excipients in the chosen formulation, and the primary packaging (e.g. leachables and impurities from stoppers and syringes derived from their manufacturing process; like tungsten) as well as the conditions for clinical use (e.g. dilution of infusion solutions and infusion devices of different materials), may impact on product quality and generate negative effects like adherence to walls, denaturation and aggregation. Both denaturation and aggregation of the protein therapeutic may potentially trigger an immune response.

- *Aggregation and adduct formation*

Aggregation and adduct formation of proteins may either reveal new epitopes or lead to the formation of multivalent epitopes, which may stimulate the immune system. Aggregation can enhance a protein-specific immune response and lead to the formation of ADAs. Removal of aggregates (present as visible or sub-visible particles) has been associated with strongly reduced immunogenicity in preclinical in vivo studies.

Factors which may contribute to aggregate or adduct formation include formulation, purification processes, viral inactivation procedures (low pH), packaging material and storage conditions of intermediates and finished product. The use of proteins as excipient, e.g. albumin, may lead to the formation of more immunogenic aggregates. It is important to monitor the aggregate and adduct content of a product throughout its shelf life.

Higher-molecular weight (MW) aggregates are more prone to elicit immune responses than lower-MW aggregates, and the repetitive ordered epitopes (multimeric epitopes) that are often displayed by protein aggregates (e.g. viral-like arrays) may involve T cell independent mechanisms and activate B cells directly. Extensive crosslinking of B cell receptors by higher order structures can activate B cells to proliferate and produce antibodies not only to the aggregated, but also to the monomeric form of the protein.

- *Impurities*

There are a number of potential impurities in the drug substance of therapeutic proteins, which potentially can serve as adjuvants. Host cell proteins from the cell substrate co-purified with the active substance, could induce immune responses against themselves, as well as to the active substance. Bacterial proteins, contaminants from the manufacturing process, host cell-derived lipids or DNA could also function as adjuvants triggering immune responses against the therapeutic protein.

5. Potential clinical consequences of immunogenicity

Immunogenicity of a protein therapeutic may have profound effects on the efficacy and safety of the product. Factors which determine whether antibodies to a therapeutic protein will have clinical significance include the epitope recognised by the antibody, the affinity and class of the antibody, the amount of antibodies generated, as well as the pharmacological properties of the biotechnological medicinal product. In addition, the ability of immune complexes to activate complement may have an impact on the clinical outcome.

5.1. Consequences on Efficacy

ADAs can influence efficacy by eliminating the pharmacological action of the product or altering its pharmacokinetic profile.

"Neutralising" antibodies can cause a reduction or loss of efficacy by binding to or near the active site, or by inducing conformational changes. Usually, 'non-neutralising' antibodies are expected to be

associated with less clinical consequences. However, as discussed below, such antibodies may reduce the exposure to the therapeutic protein and thereby influence efficacy indirectly.

The effects of ADAs on therapeutic proteins may vary from zero to complete loss of efficacy. Sometimes, the efficacy is reduced gradually over time without a clear correlation to ADA titres.

5.2. Consequences on Safety

In general, most adverse effects of therapeutic proteins are related to their pharmacological effects. The main exception is their potential immunogenicity. Immune-based adverse effects may be both acute and delayed.

Less severe immune-based adverse effects include infusion-site reactions. Non-allergic (not involving IgE-generation) infusion reactions are typically seen during the first infusions and can be mitigated by appropriate pre-medication.

- *Hyper acute / acute reactions*

Acute infusion-related reactions including anaphylactic / anaphylactoid reactions (type I), may develop within seconds or during a few hours following infusion.

All infusion-related reactions involve the immune system; however, some (anaphylactic) are allergic in nature and are usually mediated by immunoglobulin E (IgE), whereas others (anaphylactoid) are not true allergic reactions and are not mediated by IgE. Although infusion-related reactions can be allergic or non-allergic, the clinical manifestations are the same. Acute reactions can cause severe hypotension, bronchospasm, laryngeal or pharyngeal oedema, wheezing and/or urticaria. The term anaphylaxis should be restricted to such situations and represent a strict contraindication to further exposure to the drug.

Usually, patients who develop antibodies are more prone to suffer from infusion-related reactions.

A thorough assessment concerning a products' potential to inducing hyper acute / acute infusion – related reactions, as well as the identification of all cases meeting the clinical diagnostic criteria for anaphylaxis regardless of presumed pathophysiology, is important.

- *Delayed reactions*

In addition to acute reactions, delayed type (T cell mediated) hypersensitivity and immune complex-mediated reactions have to be considered. The risk of such reactions may be higher with an increasing drug free interval. Delayed hypersensitivity reactions should be clearly delineated from infusion-related reactions. Applicants should ensure the systematic collection of non-acute clinical sequelae following application of the therapeutic protein. Clinical signs can include myalgia, arthralgia with fever, skin rash, and pruritus, but other, less obvious clinical symptoms should be systematically collected as well.

- *Autoimmunity: Cross-reactivity to an endogenous counterpart*

A possible life-threatening clinical consequence of ADA formation against a therapeutic protein is cross-reactivity with an endogenous protein when this protein has a non-redundant role in key physiological functions. For example, ADAs cross-reacting with endogenous erythropoietin have caused pure red cell anaemia in epoetin alfa-treated patients with kidney failure. Novel constructs, like hybrid molecules fused to physiological functional molecules, should be carefully investigated for ADAs cross-reacting with relevant endogenous (or self) proteins.

6. Non-clinical assessment of immunogenicity and its consequences

Therapeutic proteins show species differences in most cases. Thus, human(ised) proteins will be recognised as foreign proteins by animals. For this reason, the predictivity of non-clinical studies for evaluation of immunogenicity in humans is considered low. Non-clinical studies aiming at predicting immunogenicity in humans are normally not required.

However, ongoing consideration should be given to the use of emerging technologies (novel *in vivo*, *in vitro* and *in silico* models), which might be used as tools during development or for a first estimation of risk for clinical immunogenicity. *In vitro* assays based on innate and adaptive immune cells could be helpful in revealing cell-mediated responses.

It is expected that (non-)clinical studies are supplied with material sufficiently representative of the medicinal product that is going to be placed on the market. Since immunogenicity concerns may arise from the presence of impurities or contaminants, it is preferable to rely on purification processes to remove impurities and contaminants rather than to establish a preclinical testing program for their qualification (refer to ICH S6 (R1) Harmonised Tripartite Guideline on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals).

Measurement of anti-drug antibodies in non-clinical studies may be needed as part of repeated dose toxicity studies, in order to aid in the interpretation of these studies (as discussed in "ICH S6 (R1) Harmonised Tripartite Guideline on preclinical safety evaluation of biotechnology-derived pharmaceuticals."). Blood samples should be taken and stored for future evaluations if then needed. The assays used should be validated (see also chapter 7.3). In toxicology studies, where usually higher concentrations of therapeutic protein are present in the samples, the interference of the therapeutic protein in the ADA assays needs to be considered.

In the development of similar biological medicinal products (biosimilars), the comparison of the anti-drug antibody response to the biosimilar and the reference product in an animal model is not recommended as part of the biosimilar comparability exercise, due to the low predictivity for the immunogenicity potential in humans.

An immune response to a therapeutic protein representing a counterpart to an endogenous protein may result in cross-reactivity, directed to the endogenous protein in cases where endogenous protein is still produced. Any relevant experience on the consequences of induction of an immune response to the endogenous protein or its absence/dysfunction in animal models should be discussed in the integrated summary of immunogenicity. Both humoral and cellular immune responses (where relevant) should be considered. Usually, safety risks would be predictable, based on existing knowledge on the biological functions of the endogenous protein and animal studies would not be required to confirm these safety risks. Only in absence of sufficient knowledge, and if theoretical considerations are suggestive of a safety risk, animal immunisation 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.

7. Development of assays for detecting and measuring immune responses in humans

Developing an integrated analysis strategy relevant for the intended treatment plan is critical for elucidating the clinical relevance of immunogenicity data. It is very important to select and/or develop assays and assay strategies for assessment of immune responses. While most effort is usually focused on antibody detection and characterisation as this is often related to clinical safety and efficacy, cell-mediated responses are also important and their assessment may be considered by applicants on a case by case basis.

Although assays will be refined during product development and assay suitability reassessed as per their use, the applicant is expected to provide data supporting full assay validation for marketing authorisation.

7.1. Strategy and Antibody Assays

Adopting an appropriate strategy which includes use of sensitive and valid methods for immunogenicity assessment is essential. Typically, a multi-tiered approach should be employed. This includes a screening assay for identification of antibody positive samples/patients, a procedure for confirming the presence of antibodies and determining antibody specificity followed by functional assays for the assessment of the neutralizing capacity of antibodies. Tests for determining antibody isotype and epitope specificity may also be considered on confirmed antibody positive samples. In some cases, testing samples for cross-reactivity with other products based on the same protein and the endogenous protein is important as it may have implications for clinical efficacy and safety.

In addition, assays for measuring the level of the product and for assessing clinical relevance to products e.g., assays for relevant biomarkers or pharmacokinetic measurements are required to evaluate the clinical impact of induced antibodies if these are detected (see Annex 1).

Evaluation of the kinetics of antibody development and the duration as well as the magnitude of the antibody response is important as it may correlate with clinical consequences.

If antibodies are induced in patients, serum or plasma samples need to be characterised in terms of antibody level (titre), neutralizing capacity and possibly other criteria determined on a case-by-case basis according to the biological product, the type of patients treated, the aim of the study, clinical symptoms and possibly other factors. These may include antibody class and subclass (isotype), affinity and specificity. The degree of characterization required will differ depending on the study purpose and stage of development of the product. The assays used should be qualified for their intended purpose.

- *Screening assays*

Screening assays are the first step in immunogenicity evaluation. They should be sensitive and capable of detecting all antibodies (including IgM and IgG subclasses) induced against the product in all antibody positive patients. A low false positive rate is desirable but false negative results are unacceptable.

Screening is performed using immunoassays which are based on a variety of formats and detection systems. All screening procedures detect antigen-antibody interaction (binding) but may differ in their underlying scientific/ technical principles. These assays are configured to have moderate throughput and appropriate automation, however each assay has its own attributes and inherent limitations which need to be considered (see chapter 10).

Assays need to be developed, optimized and selected according to and taking account of their intended use. The importance and requirements of assay characteristics depends on the use of the assay. Adoption of the simplest assay suitable for all requirements is normally a valid approach to assay selection. However, care with this is necessary to ensure that it does not compromise other stages of immunogenicity assessment. For example direct binding ELISAs, with antigen directly immobilized on plate well surfaces are often the simplest assay approach, but may be associated with a very high incidence of false positivity. They may also be associated with a high incidence of false negatives for samples containing low affinity antibodies. It is often necessary to adopt a more suitable assay, e.g. bridging assays, electrochemiluminescence or surface plasmon resonance methods. Epitope masking can give false negative results in screening assays and a strategy to avoid this may be necessary e.g. by labelling detecting reagents using procedures that avoid masking of particular epitope(s).

In this respect the reagents (e.g. blocking reagents) should be considered carefully. Blocking reagents like BSA and milk contain non-human glycans that are sometimes found on proteins produced in non-human animal cells. Thus, antibodies against these glycans may be missed.

Samples (normally serum or plasma) may contain substances that interfere with the assays i.e. matrix effects which produce false positive or negative results and/or incorrect assessment of antibody content. Examples include complement components or complement receptors, mannose binding protein, Fc receptors, soluble target molecules, and rheumatoid factors. The influence of such matrix components on assay results should be considered and measured. To mitigate the potential influence corrective measures should be implemented. Applicants need to justify the suitability of the chosen approach, taking into consideration the limitations of the respective methods.

Additionally, residual therapeutic product present in patients' blood can complex with induced antibody and hence reduce the amount of antibody detectable by assays. This may affect assays differently, depending on the assay, assay format or type and the antibody characteristics. If this occurs, it may be circumvented/resolved by using a number of approaches e.g. by dissociating the immune-complexes with acid, removing excess biological by solid-phase adsorption, use of long incubation times and/or using an assay which allows sufficient sample dilution to avoid this problem. Such approaches must themselves be validated for effectiveness and adopted on a case-by-case basis according to needs. In some cases this problem can be overcome by appropriate spacing of the timing between administration of product and sampling for antibody assessment i.e. allowing time for the product to be cleared from the circulation before sampling. However this latter approach must not significantly compromise the detection of antibodies or the treatment of the patient. In any case, the Applicant has to demonstrate that the drug-tolerance of the assay exceeds the levels of the therapeutic protein in the samples for ADA testing.

- *Assays for confirming the presence of antibodies*

Confirmatory assays are necessary for eliminating any false positive results following the initial screen. Assay selection should take account of the limitations and characteristics of the screening assay. A common approach for confirming antibodies is addition of an excess of antigen to the sample followed by a comparison of spiked and unspiked sample in the binding assay. This should result in a reduction of positive signal for true positives in the spiked sample.

Antibodies present in confirmed positive samples need to be examined for specificity for the active protein and, in relevant cases, distinguished from antibodies which bind to product-related and process-related components (e.g., host cell proteins). It has been shown that antibodies can be induced against all or any of these.

- *Neutralization assays*

Determination of the neutralizing potential of the induced antibodies is an essential element of immunogenicity assessment. Deviation from this concept needs a strong justification. Neutralizing antibodies (NAbs) inhibit the biological activity of a therapeutic by binding to epitope(s) within or close to the active site(s) of the molecule or by causing conformational changes. Because NAbs can trigger clinical effects, specific and sensitive *in vitro* methods are needed for detection. Two types of NAb assays are mainly used - cell-based and non-cell-based assays.

An assay must be selected or developed which responds well to the biological product. Bioassays used for potency estimation can often be adapted to assess neutralising antibodies. However, they frequently require refining if they are to perform optimally for measuring the neutralizing capacity of antibodies.

Understanding the mode of action, the target and effector pathways of the therapeutic are critical for identification of a suitable NAb assay format. Additionally, the risk of developing NAbs and the impact on clinical sequelae also needs to be considered. While cell-based assays are often employed for agonistic therapeutics, non-cell-based CLB assays are often considered for antagonistic molecules with humoral targets. For products that do not have intrinsic biological activity (e.g., some MAbs), competitive ligand binding assays (CLB) or other alternatives may be suitable. However, when these are used it must be demonstrated that they reflect neutralizing capacity/potential in an appropriate manner. For antagonists such as monoclonal antibody therapeutics with effector functions for clinical efficacy, cell-based assays are recommended as the mechanism of action cannot be adequately reflected in a non-cell-based CLB assay.

The neutralising capacity of antibodies present in positive samples needs to be established as this often correlates with diminished clinical responses to biological product. Usually a single concentration of biological is chosen for the assay and dilutions of each sample assessed for their inhibitory effect on the assay response. This allows a neutralizing dose response to be determined and calculation of neutralizing capacity ('titre') for each sample

As for screening, inclusion of a step confirming that the neutralization is truly related to the antibodies and not due to other inhibitory components in sample matrix is useful. Approaches for showing specificity such as antibody depletion, use of alternative stimuli (if assay responds to multiple stimuli) can be considered.

It should be noted that neutralizing activity does not necessarily correlate with binding antibody content, i.e. samples containing significant or high amounts of binding antibodies may fail to neutralize biological activity whereas samples containing lower amounts of binding antibodies can neutralize variable (sample dependent) amounts. This may depend on product, but must be determined empirically.

- *Immunogenicity Assessment strategy –design and interpretation*

Immunogenicity studies need to be carefully and prospectively designed to ensure all essential procedures are in place before commencement of clinical assessment. This includes the selection, assessment, and characterisation of assays, identification of appropriate sampling points including baseline samples for determination of pre-existing antibodies, adequate sample volumes and sample processing/storage and selection of statistical methods for analysis of data.

This applies to assays used to measure and characterise antibodies and to methods employed for assessing clinical responses to antibodies if they are induced. Much of this needs to be established on a case-by case basis, taking account of product, patients, and expected clinical parameters.

7.2. Assay Controls and Reagents

The identification and/or development of appropriate well characterized positive and negative controls are crucial. These reagents are essential for assay calibration and validation. They are intimately associated with assay interpretation and with distinguishing antibody positive from antibody negative samples.

Ideally, an antibody positive control should be a human preparation with a significant antibody content which is available in sufficient quantity for continued use. However, sufficient human serum is often not available to serve as a positive control preparation. In such cases, use of an animal serum raised against the product as a reference is the only option. However, its use is more limited than a human preparation e.g. immunochemical procedures, which involve the use of an anti-human immunoglobulin reagent, will not reliably respond to non-human antibodies and the response in all assays may differ in characteristics from responses to human antibodies in human samples.

Use of the positive control for estimating antibody levels in binding assays in mass units is problematical as the immunoglobulin present in standards and samples is heterogeneous in structure, specificity and avidity. This makes direct valid comparison between samples and positive control difficult, if not impossible. An option is to report immunoassay data as a titre based on a standard procedure for calculating this value.

The positive control antibodies for neutralization assays should have significant neutralizing activity, but it is also useful to include a non-neutralizing antibody preparation in assays, at least in validation studies. Biological assays used to assess the neutralizing capacity of antibodies may be calibrated using International Standards/Reference Preparations where these are available. This would allow expression of neutralizing activity in terms of meaningful units of biological activity of product/ preparation and also provide information relevant to assay validation. If such standards are not available, appropriate in-house preparations can be established. In many cases, it is useful to express the neutralizing capacity of samples in terms of the volume of sample required to neutralize a constant biological activity of product e.g. ml of serum/defined unit of bioactivity of biological. Using the sample dilution or titre required to neutralize the biological activity of the product is also an option.

It is also very useful to prepare a panel of reference materials containing different amounts of antibodies and antibodies with different characteristics e.g. neutralizing/non-neutralizing, which can be used to characterize/validate assays and act as assay performance indicators. If possible this should include one or more preparations with low antibody content (close to the minimum detection limit) and containing low avidity antibodies.

Negative controls are needed to establish assay baselines and characterize/validate the assays. Assay baseline for normal (healthy) individuals is clearly fairly easily determined by measuring the assay response using samples derived from an appropriate number of such individuals and analysing this to provide a statistically valid background value. However, this may not be representative of the baseline response of samples derived from the patient population, which would therefore need to be established separately, using pre-treatment samples from patients or drug naïve disease patients. Some individual's/patient's samples may contain pre-existing (pre-treatment) antibodies or possibly other substances which produce significant positive responses in assays, and so screening patients for this is necessary to ensure that post-treatment data can be interpreted correctly in terms of treatment emergent antibodies.

Reagents used in assays need to be qualified and acceptance specifications set, at least for those which are most important. They should be stored appropriately (lyophilized or frozen at a suitable temperature) and characterized.

7.3. Assay validation and interpretation of results

Assay validation is an ongoing process throughout product development. Assays used for the pivotal clinical trials need to be validated for their intended purpose. Validation studies must be conducted to establish that the assays show appropriately linear, concentration dependent responses to relevant analytes as well as appropriate accuracy, precision, sensitivity, specificity and robustness. Inclusion of data supporting minimal required dilution of samples is important. For pivotal clinical trials, the use of a central laboratory to perform the assays is helpful to avoid inter-laboratory variability. In the post-approval setting, it is also important to consider inter-laboratory variability. Assays must also be validated to show that matrix effects caused by reagents or substances present in samples do not adversely affect the results obtained. This is normally addressed by 'recovery' investigations conducted by observing the effects of such substances present in the matrix on the response obtained in their absence. This needs to be investigated for the full range of dilutions of samples, which are to be used in assays, and, at least in some cases, limits the dilutions, which can be validly assessed.

It is essential to establish clear criteria for deciding how samples will be considered positive or negative, and also how positive results will be confirmed. Approaches to these can differ according to assay etc. and need to be decided accordingly. A common procedure for establishing positive cut-off for immunoassays is to establish assay background using samples from normal healthy controls and or diseased individuals (see above). A statistical approach should be used to establish the assay cut-off value. Alternatively, real data (e.g. double background value) can be used to determine what will be considered the lowest positive result. For antibody positive samples, a titre needs to be determined using a standard approach and reporting the reciprocal of the highest dilution at which the sample gives a positive result. Another option is to report in mass units using a positive antibody control but this has caveats as explained above.

7.4. Assays for comparative immunogenicity

Comparative immunogenicity studies are always needed in the development of biosimilars (see Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues, EMEA/CHMP/BMWP/42832/2005 Rev1) but rarely for a change of the manufacturing process of a given biological product (for changes to the manufacturing process of the drug substance see ICH Harmonised Tripartite Guideline on Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process Q5E, 2004).

Immunogenicity testing of the biosimilar and the reference product should be conducted within the biosimilar comparability exercise by using the same assay format and sampling schedule which must meet all current standards. Analytical assays should be performed with both the reference and biosimilar molecule in parallel (in a blinded fashion) to measure the immune response against the product that was received by each patient. The analytical assays should preferably be capable of detecting antibodies against both the biosimilar and the reference molecule but should at least be able to detect all antibodies developed against the biosimilar molecule. Usually, the incidence and nature (e.g. cross-reactivity, target epitopes and neutralising activity) of antibodies and antibody titres should be measured and presented and should be assessed and interpreted in relation to their potential effect on clinical efficacy and safety parameters.

When comparative immunogenicity studies are required in the context of a manufacturing change of a given product, assays to compare the pre- and post-change products need to be developed. Ideally, there should be two assays, one using the pre-change protein and the other with the post-change protein as the target antigen.

7.5. Immunogenicity assessment of conjugated proteins and fusion proteins

Elicitation of an antibody response with multiple specificities and variable affinity towards different epitopes resulting in varying degrees of clinical impact is expected for novel biotherapeutic molecules such as engineered fusion proteins and chemically conjugated proteins. The evaluation of this response, in particular, the characterization of the specificity of the induced antibodies is challenging and may require multiple assays for measuring immune responses to various moieties. Alternatively, a strategy based on the competitive inhibition principle of the confirmatory assay to dissect the specificities of the antibodies to individual moieties can be used. For example, for a pegylated protein, the assessment strategy would comprise a screening assay using the pegylated therapeutic and testing of any positive samples using the whole therapeutic, the non-pegylated protein and the PEG moiety in a confirmatory assay.

7.6. Characterisation of antibodies to a therapeutic protein

Normally, the incidence and titre, persistence and neutralizing capacity of the ADAs are required. In certain circumstances, it may be feasible to further characterize the ADA response, e.g., in case of anaphylactoid reactions and follow up of the maturity of emerging immune response. In these cases, determination of the isotype and IgG-subclasses or even T cell reactivity may be useful. Cross-reactivity of the ADAs with relevant endogenous proteins should be investigated if emerging autoimmunity is suspected.

8. Immunogenicity and Clinical Development

Testing of immunogenicity should be included in all pivotal clinical trials of a new biological medicinal product targeting patient populations that have not been exposed to the product previously. The aim is not only to demonstrate an immune response to the product but also to investigate correlations between binding and neutralising ADAs, on one hand, and pharmacokinetics and –dynamics as well as efficacy and safety, on the other hand. Therefore, assessment of immunogenicity should be included in the planning of the clinical trials, including the synchronization of sampling for ADAs and relevant biomarkers as well as evaluation of efficacy and safety (see chapter 10).

8.1. Rationale for sampling schedule and kinetics of the antibody response

Immunogenicity should be systematically tested in patients by scheduled routine repetitive sampling as well as in a symptom-driven manner with additional samples, when the occurrence of an unwanted immune response is suspected.

Several product-related factors will influence the development of an immune response against a therapeutic protein (see chapter 4). Therefore, the sampling schedule for detection of an immune response should be adapted and selected individually for each product, also taking into account its pharmacokinetics (e.g. elimination half-life) and the drug tolerance of the ADA-assay(s). Baseline samples should always be collected.

Applicants should endeavour to standardise, assays as well as terminology and definitions of potential immune-mediated adverse effects taking into account also experience with comparable products and relevant regulatory and scientific publications (see also chapter 10). During treatment, samples should also be taken before administration of the product, since residual levels of the active substance in plasma can interfere with the assay (see chapter 7).

The frequency of sampling and the timing and extent of analyses will also depend on the risk assessment for a particular drug (as described in the integrated summary of immunogenicity, chapter 10). Sampling schedules should be designed to distinguish patients being transiently positive from patients developing a persistent antibody response. The post-treatment follow up sampling should be long enough to allow conclusions on the persistence of the immune response triggered by the therapeutic protein and uncover an immune reaction that was suppressed by the therapeutic protein itself. The timing of post-treatment sample(s) is determined by the half-life of the protein and the drug tolerance of the ADA assay. The first post-treatment sample should not be taken earlier than four weeks after the last dose.

More frequent sampling is necessary in the earlier phase of treatment, where patients are normally most at risk of antibody development. Since longer-term treatment is more likely to result in an immune response, routine, less frequent sampling later in the treatment course should be implemented in clinical trials. In case of continuous chronic treatment, immunogenicity data for one year of treatment should become available pre-authorisation but shorter follow up is possible with a proper justification.

The immunogenicity associated with intermittent treatment should be considered on the basis of a risk assessment, e.g. experience from other similar products, risks associated with potential immunogenicity, persistence or appearance of antibodies after the exposure.

If used for different routes of administration, Applicants should justify their approach as regards immunogenicity assessment for each route at the time of Marketing Authorisation Application (see integrated summary on immunogenicity).

The risk of immunogenicity and its possible consequences should be described in the relevant chapters of the SmPC in a concise way and taking account the fact that a comparison of results from different sources and or by different assays is unreliable. The feasibility of and possibilities for routine monitoring of immunogenicity, including the usefulness of drug concentration measurements, should also be included in the SmPC, if applicable.

8.2. Consequences on pharmacokinetics of the product

Antibodies recognising epitopes outside the active sites of the protein (non-neutralising) may be associated with fewer clinical consequences than the neutralising antibodies. However, such antibodies can influence pharmacokinetics, especially the elimination phase. Non-neutralizing, "binding" antibodies, may sometimes also increase, rather than decrease, the efficacy of a product by prolonging the half-life, or stimulating a pathway or mechanism. A change in pharmacokinetics may be an early indication of antibody formation. Thus, the Applicants are encouraged to incorporate concomitant sampling for both pharmacokinetics and immunogenicity into all repeat dose studies.

8.3. Impact of immunogenicity on safety and efficacy

The presence of ADAs may or may not have clinical consequences. It is essential that the clinical development is based on an analysis of potential risks and possibilities to detect and mitigate them. The planning of the analysis of immune-mediated adverse effects should be based on risk analysis, including previous experience of the product (class), presence of potentially immunogenic structures in the protein and patient population (see integrated summary). Patients with pre-existing antibodies may exhibit a different safety profile and should be analysed as a subgroup. The analysis plan should define symptom complexes that might be associated with acute or delayed hypersensitivity and

autoimmunity as well as with the loss of efficacy (see chapter 10). Potential immunological adverse effects should be addressed in the risk management plan (see chapter 9).

When ADAs have been demonstrated, further characterization beyond the titre and neutralizing capacity of the antibodies may be useful, e.g. immunoglobulin class in case of acute hypersensitivity. It may also be possible to determine a "threshold" level of ADAs beyond which there is a significant impact on efficacy and/or safety.

8.4. Methodological aspects to assess comparability of immunogenicity potential as part of a comparability exercise

Comparative immunogenicity studies are always required in the development biosimilar products (Similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues, EMEA/CHMP/BMWP/42832/2005 Rev. 1) and occasionally after changes to the manufacturing process of a given product, before or after marketing authorisation. When changes to the manufacturing process of a licensed product are made, the comparability exercise is a stepwise process (see ICH Q5E). If the initial physicochemical and biological testing indicates a difference between the pre- and post-change versions of the product, the potential consequences to safety and efficacy need to be considered, including altered immunogenicity.

The type of immunogenicity studies, if required, should be justified on the basis of the observed difference(s), route of administration, dose-response curve and therapeutic window, the potential clinical impact, and knowledge gained with this product and product class before (see ICH Q5E). The target population needs to be sensitive for differences in immunogenicity and its consequences and be representative for the population(s) for whom the product is indicated. In high risk situations, the samples should be analysed on ongoing basis.

In the cases when a manufacturing process change needs to be supported by a clinical trial, investigation of immunogenicity should be integrated with the pharmacokinetic, safety, and efficacy testing. Immunogenicity evaluation as part of a clinical trial for a comparability exercise in the context of a manufacturing change should preferably involve head-to-head study of pre- and post-change product.

Increased immunogenicity as a result of a change in the manufacturing process will question the comparability. If the observed difference is uncertain, a specific risk management strategy and an update of the risk management plan (see chapter 9) may be required. If there is a risk of rare immune-mediated adverse effects, this may be addressed after the implementation of the change in a post-marketing setting. Decreased immunogenicity should be discussed and justified, including potential root cause and impact on exposure.

8.5. Management of immunogenicity

The presence of an immune reaction to a therapeutic protein may lead to clinical consequences in spite of the efforts by the Applicants to select compounds that have a reduced immunogenic potential (see chapter 6). In this case, the Applicant needs to explore possibilities to reduce the adverse impact of immunogenicity observed during the clinical development.

Applicants should provide guidance to the prescriber as part of the Summary of Product Characteristics on how to mitigate the effects of immunogenicity. An evidence based recommendation is needed to guide prescriber as when to stop the treatment of a patient with loss of efficacy or side effects or when an immunosuppressive co-medication, an increase of dose or a reduced dosing interval might be helpful. In some cases, as with coagulation factors, it may be possible to re-establish the

immunological tolerance by tolerisation, e.g. by administration of large doses of the therapeutic protein or normal intravenous immunoglobulin. Certain hypersensitivity symptoms may be prevented or mitigated by appropriate pre-medication.

9. Pharmacovigilance

Within the authorisation procedure, the applicant should present a risk management plan (RMP) in accordance with current EU legislation and pharmacovigilance guidelines. Immunogenicity should always be considered in the safety specification section of the RMP of biotechnology-derived therapeutic proteins and the need for additional pharmacovigilance activities should be evaluated. For changes in the manufacturing process, implications of this change on the immunogenic potential also have to be addressed in the RMP. Again, it should be emphasized that evaluation of immunogenicity is a multidisciplinary approach, at best providing input of quality, non-clinical and clinical experts.

The extent of data on immunogenicity that can be obtained during the clinical development program of a biotechnology-derived product before approval depends on the event rate, driven both by the immunogenic potential of the protein and the rarity of the disease. The availability of data on immunogenicity at time of approval might, therefore, be limited. In addition, knowledge obtained for the product class and/ or the reference product (in the case of biosimilar development) should be discussed in the RMP. The potential for immunogenicity should be fully evaluated based on the available evidence with appropriate conclusions drawn on whether or not a product may pose such a (potential) risk. If this is the case, immunogenicity should be included in the RMP as either a potential or identified risk. Immunogenicity should always be related to the clinical consequence, e.g. drug neutralizing antibodies resulting in a lack of efficacy, hypersensitivity and/ or infusion reactions and/ or development of antibodies directed towards an endogenous available protein resulting in a serious adverse event. If no particular concern or uncertainty arises from the evaluation, inclusion by default of immunogenicity as a potential risk is not required.

Since systematic sampling of antibodies might not be feasible in a post-marketing setting, it is important to conclude on potential unwanted immune responses based on suspicious safety and/ or (loss of) efficacy signals, including changes in relevant biomarkers.

Within the pharmacovigilance plan of the RMP, the need for additional pharmacovigilance studies should be evaluated and assessed. In case additional studies on immunogenicity are considered necessary the most suitable design should be evaluated based on the aim of the study. At this moment, drug-neutralizing antibodies are not routinely measured in clinical practice. Additional clinical trials or extensions of ongoing clinical trials in the post-marketing setting might therefore be necessary to obtain additional data on the incidence and titres of drug-neutralizing antibodies. Such a trial might also be necessary during biosimilar development in case additional immunogenicity data should be collected in a comparative manner in the post-marketing setting, e.g. immunogenicity data for a chronically administered product has only been collected for 6 months pre-authorisation and additional 6 months data is considered necessary by the regulatory authorities.

Follow-up of patients treated with a biopharmaceutical during routine clinical practice, e.g. patient registries, has been shown a valuable tool to collect data on the safety of these products. These data sources can also be used for the collection of drug-neutralizing antibodies and adverse events related to immunogenicity, e.g. infusion related reactions. The use of other pharmacoepidemiological data sources should also be explored.

Collection of spontaneously reported adverse events should always be done as laid down in the pharmacovigilance legislation. In relation to immunogenicity, spontaneous reporting has been shown

an important tool in case of serious safety problems resulting from immunogenicity, e.g. pure red cell aplasia during use of erythropoietins. Other signals related to immunogenicity, e.g. lack of efficacy and hypersensitivity reactions, might also be triggered from spontaneous reporting and should be described in the RMP.

The need for additional risk minimisation activities in relation to immunogenicity should be discussed in the RMP and, if considered needed, these activities should be described. Risk minimization activities related to immunogenicity might, among others, consist of guidance in the Summary of Product Characteristics how to measure neutralizing antibodies and deal with the development of neutralizing antibodies.

Identification of the product responsible for an adverse event, traceability, is important for biopharmaceuticals. This is especially important for adverse events related to immunogenicity. Traceability is important for both routine pharmacovigilance (collection of spontaneously reported adverse events) and additional pharmacovigilance activities. Appropriate measures to improve traceability, collection of brand name and batch number, should be taken.

10. Summary of the immunogenicity program

Both the planning and the evaluation of immunogenicity studies of a biological product are multidisciplinary exercises. Data that are relevant to the assessment of immunogenicity are dispersed to numerous locations of the marketing authorization application. Therefore, it is recommended that the applicant will include an integrated summary of immunogenicity in the application, including a risk assessment to support the selected immunogenicity program. It is recommended that this summary is placed in chapter 2.7.2.4 Special Studies of the CTD. The summary should be concise and contain links to the appropriate chapters of the application.

This summary with risk assessment can evolve through the lifecycle of the product and may be used to support post-authorisation applications.

The risk assessment may suggest a low risk. Nevertheless, it is expected that immunogenicity is studied with validated assays according to the scheme in Annex 1. Deviation from this scheme, e.g. omission of the testing for neutralizing ADAs, must be justified. The risk assessment may have an impact on additional characterization of the immune response (e.g. isotyping and epitope mapping), frequency of sampling, timing of the analysis, and selection of the target population.

The summary should include at least the following topics when applicable:

Analysis of risk factors

1. Previous experience of the product/product class
    a. does the product have an endogenous counterpart
    b. do animal models provide useful data of potential consequences of immunogenicity (e.g. elimination of an endogenous protein)
    c. are there known antigenic sites of the molecule
    d. attempts to reduce the immunogenicity of the product before and during clinical trials

2. Physicochemical and structural aspects
    a. Are there potentially immunogenic structures, e.g. sequences that are foreign to human
    b. Expression construct and the posttranslational profile e.g. non-human glycosylation patterns/glycans
    c. Stability and impurities (e.g. presence of aggregates (as visible or sub-visible particles)
    d. Formulation and packaging, e.g. potential impurities and leachables

3. Does the route and/or the mode of administration raise concerns
4. Patient- and disease-related factors
    a. State of the immunological tolerance
        i. prone to autoimmune reactions
        ii. lack of immunological tolerance, e.g. defects in genes coding for endogenous proteins
        iii. concomitant immunomodulative therapy
    b. Pre-existing immunity
        i. "natural" antibodies
        ii. cross-reactive antibodies, e.g. due to previous therapy with related substances

The risk-based immunogenicity program

5. Assay strategy
    a. Rational for the choice of assays
        i. screening and confirmation
        ii. neutralizing
        iii. other, e.g. immunoglobulin class, sub-class
    b. Specificity and sensitivity of the selected assays in the context of the particular product
        i. selection of the positive control(s)
        ii. determination of the threshold for ADA-positivity
    c. Drug tolerance of the assay at therapeutic concentrations

6. Approach to immunogenicity in clinical trials
    a. Sampling for immunogenicity testing
    b. Justification for the length of the follow up
        i. on-treatment
        ii. off-treatment, post-exposure
    c. Pharmacokinetics
    d. Pharmacodynamics, efficacy and safety trials
        i. how the program aims to reveal the incidence, persistence and clinical significance of potential ADAs
        ii. antigen tolerance of the ADA assay and the trough concentrations
        iii. loss of efficacy, hypersensitivity, autoimmunity
            1. definitions and symptom complexes[1]
            2. analysis of clinical correlations of ADAs
7. Impact on the risk assessment on the immunogenicity program

Immunogenicity results

8. Immunogenicity in clinical trials (relative immunogenicity in case of manufacturing changes and biosimilars)
    a. (Relative) incidence of ADAs, including neutralising ADAs
    b. (Relative) titres and persistence over time
    c. Further characterisation if appropriate, e.g. immunoglobulin classes, cross-reactivity with related therapeutic or endogenous proteins
    d. Impact of ADAs on pharmacokinetics
    e. Impact of ADAs on pharmacodynamics, efficacy and safety
    f. Impact of pre-existing antibodies on pharmacokinetics, safety and efficacy

Conclusions on the risk(s) of immunogenicity

9. Impact of the immunogenicity on the benefit/risk
10. Tools to manage the risk
    a. Identification of risk groups

b. Is there a safe level or type of immunogenicity
c. Pre-medication
d. De-immunisation
e. Risk detection and mitigation tools

11. How to link adverse events to immunogenicity post-marketing

[1] The Applicant should systematically use terminology and definitions to characterise potentially immune-mediated symptoms according to relevant publications (e.g. Kang P and Saif M. Infusion-Related and Hypersensitivity Reactions of Monoclonal Antibodies Used to Treat Colorectal Cancer—Identification, Prevention, and Management. Journal of Supportive Oncology, 5, 451–457)

Annex 1: An example of a strategy for immunogenicity assessment