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DRAFT

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

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7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 86 13
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

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EXECUTIVE SUMMARY

Biological/biotechnology-derived proteins are increasingly used as therapeutic agents. These products may induce an unwanted immune response in treated patients, which can be influenced by various factors, including patient-/disease-related factors and product-related factors.

The consequences of such immune reactions to a therapeutic protein, range from transient appearance of antibodies without any clinical significance to severe life threatening conditions.

The predictive value of animal models for evaluation of immunogenicity is low due to inevitable immunogenicity of human proteins in animals. Non-clinical studies may contribute to the interpretation of comparability of the immunogenicity potential and of repeat dose toxicity studies.

It is essential to adopt an appropriate strategy for the development of adequate screening and confirmatory assays to measure an immune response against a therapeutic protein. Assays should be capable of distinguishing neutralizing from non-neutralizing antibodies, be validated and standardised.

In the clinical setting, careful planning of immunogenicity evaluation should include data systematically collected from a sufficient number of patients. The sampling schedule for immunogenicity evaluation should be standardized, adapted for each product on a case-by-case basis and taking a risk-based approach. Data on the impact on efficacy and safety should be collected in order to fully understand the clinical consequences of the immune response. Immunogenicity issues should be further addressed in the Risk Management Plan.

1. INTRODUCTION

Biological/biotechnology-derived proteins are increasingly used as therapeutic agents. It has been recognised that these proteins may induce humoral and cellular immune responses.

The consequences of an immune reaction to a therapeutic protein range from transient appearance of antibodies without any clinical significance to severe life threatening conditions. Potential clinical consequences are severe hypersensitivity-type reactions, decrease in efficacy and induction of autoimmunity, including antibodies to the endogenous form of the protein.

Many factors may influence the immunogenicity of therapeutic proteins. They can be considered to be patient-, disease- or product-related. Patient-related factors that might predispose to an immune response include: underlying disease, genetic background, immune status, including immunomodulating therapy. Product-related factors also influence the likelihood of an immune response, e.g. intensity of treatment (route of administration, source of protein, manufacturing process (impurity profile, contaminants), formulation and stability characteristics (degradation products, aggregates) of a given protein and dose, dosing interval and duration of treatment).

Although data on possible unwanted immune reactions to therapeutic proteins are required before authorisation, problems may still be encountered in the post-authorisation period. In the marketing authorisation application, the applicant should include a summary of investigations of immunogenicity in the overview with full cross-reference to the data in the relevant modules.

2. SCOPE

The principles adopted and explained in this document apply proteins and polypeptides, their derivatives, and products of which they are components, e.g., conjugates. These proteins and polypeptides are produced from recombinant or non-recombinant cell-culture expression systems.

For coagulation factors, please, refer to the specific CHMP guidelines in this area.

3. LEGAL BASIS

This guideline has 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. MAIN GUIDELINE TEXT

4.1 Risk factors for developing an immune response against a therapeutic protein

4.1.1 Patient- and disease-related risk factors

Patient-related factors, which might influence the immune response to a therapeutic protein, may include, genetic factors, age of the patient, disease related factors including other treatments and previous exposure to similar proteins.

Genetic factors

- *Genetic factors modulating the immune response*

Genetic factors can alter the immune response to a therapeutic protein and lead to inter-patient variability. Certain allelic loci in the major histocompatibility complex and strength or time of the MHC interaction influence immune responses and tolerance induction.

Immune responses may occur even if the amino acid sequence of the therapeutic protein is fully human.

Other genetic factors influencing immunogenicity are gene polymorphisms for cytokines that play a role in the fine-tuning of the immune response (e.g. interleukin-10, TGF-beta etc.).

- *Genetic factors related to a gene defect*

If the therapeutic protein is used for substitution, reduced levels or even lack of endogenous protein influence immunological tolerance, since for these patients the physiological antigen may represent a neo-antigen.

Age

The data from one age group cannot necessarily be projected to others since immune response against a therapeutic protein can be an age-related phenomenon. Children may possibly have a different immune response to these proteins. If the product is indicated in children studies on immunogenicity should be carried out in this age group.

Disease-related factors

The patient's underlying disease itself can be an important factor in the context of immunogenicity. It is possible that patients with autoimmune disease, i.e. with an immune system prone to altered immunological tolerance to self-proteins, may react differently.

Some patients with chronic infections may be more prone to an immune response, since their immune system is in an activated state.

In severe conditions (i.e. malnutrition, advanced metastatic disease, organ failure), an immune response against a therapeutic protein might be less likely to occur due to an impaired immune system.

For some products, it has been reported that the susceptibility to an antibody response can be different for different indications. Therefore, immunogenicity may need to be studied separately for each disease.

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. Consideration should also be given to previous treatments, that can modulate the immune reaction to a therapeutic protein and that have a long-term impact on the immune system. If clinical trials are performed in combination with immunosuppressants, a claim for use of the therapeutic protein in monotherapy must be accompanied by adequate clinical data on the immunogenicity profile in monotherapy.

Concomitant therapies can also promote the immune response to a therapeutic protein.

- *Duration, route of administration, treatment modalities*

Factors, which may increase the immune response to a therapeutic protein, may be the route of administration, dose, and the schedule of administration.

Products given intravenously may be less immunogenic than those given subcutaneously or intramuscularly.

Treatment for a short time only is usually less likely to be associated with formation of antibodies 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 increase in immunogenicity.

- *Previous exposure to similar proteins*

Previous exposure to similar proteins can lead to pre-sensitisation and cause an immune response. For certain proteins being used for replacement therapy, previous therapies may have induced cross-reacting antibodies that affect subsequent therapies.

4.1.2. Product related risk factors of immunogenicity

Product-related factors influencing the immunogenicity of biological/biotechnology-derived therapeutic proteins include the origin and nature of the active substance (structural homology, post translational modifications), modification of the native protein (e.g. pegylation), product and process related impurities (e.g. break down products, aggregates and host cell proteins) and formulation. Substances activating molecular or cellular elements of the innate immune system are likely to be strongly immunogenic and/or may enhance immunogenicity of concomitantly administered proteins (e.g. GM-CSF).

Protein structure

Biotechnology derived analogs 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, physical, chemical or enzymatic degradation and/or modification e.g. deamidation, oxidation and sulfatation during all steps of the manufacturing process and during storage. Fusion proteins composed of a foreign and self-protein are of particular concern because of the potential of the foreign moiety to provoke an immune response to the self-protein (antigen-spreading). Identification of the antigenic site is advisable. Glycosylation is a frequent posttranslational modification of biotechnology-derived therapeutic proteins. These modifications may differ in the number and position of glycosylation sites as well as sequence, chain length and branching of the attached oligosaccharide. Because glycosylation is species and cell-specific and also depends on cell culture conditions, it is likely that the endogenous and recombinant proteins exhibit different glycosylation patterns. When the same protein is manufactured under different condition there might be changes in pattern of post-translational modifications. Consequently, antibodies induced by one product may or may not cross-react with another product. It is also important to consider this aspect for immunogenicity testing.

Formulation

The composition of a formulation is chosen in order 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. Therefore, critical properties of excipients should be identified and characterised. The stability of the formulation and the composition and the source of excipients may alter immunogenicty of therapeutic proteins and should be considered as possible cause of such events. This should be considered when variations to the formulation are made after marketing e.g. removal of albumin

Impact of the condition of clinical use e.g. dilution in infusion solutions, use of diverse immediate containers, infusion devices of different material could be the link to increased immunogenicity.

Aggregation

Aggregation of proteins may either reveal new epitopes or leads to the formation of multivalent epitopes, which may stimulate the immune system. Factors, which could be considered to contribute to aggregate formation, include formulation, purification processes, viral inactivation procedures and storage conditions of intermediates and finished product. The use of other proteins e.g. albumin as excipient may lead to the formation f more immunogenic aggregates. It is important to monitor the aggregate content of a product throughout its shelf life.

Excipients and impurities

Excipients, or even removal of an excipient, may have significant impact on the immunogenicity of a therapeutic protein. For example, an increase in the polysorbate concentration above the critical micelle concentration has been reported to be possibly one of the multifactorial aspects contributing to the increased immunogenicity of a product by inducing the assembly of protein molecules at the surface of polysorbate micelles and forming large multivalent structures.

There are a number of impurities of therapeutic proteins, which potentially can serve as adjuvants. Host cell proteins (HCPs) from the cell substrate co-purified with the active substance could induce immune responses. But it is also possible that these HCPs, host cell-derived lipids or DNA and leachables function as adjuvants for the protein of interest. There is even evidence that silicon oil used in the siliconisation of primary packaging material can stimulate antibody response. Therefore, excipients and stabilisers should always be well characterised and be considered a possible cause of antibody formation.

Since various factors impact on immunogenicity, Applicants should take the above considerations into account when designing their development strategy. Careful planning of immunogenicity evaluation should be exercised, and data should be systematically collected from a sufficiently large number of patients to characterise the variability in antibody response. A suitable risk management strategy should be devised to address above risk factors.

4.2 Predictivity of non-clinical models

Therapeutic proteins show species differences in most cases. Thus, human proteins will be recognised as foreign proteins by animals. For this reason, the predictivity of animal models for evaluation of immunogenicity is considered low. Nevertheless, immunogenicity endpoints should be included in repeated dose toxicity studies, in order to aid in the interpretation of these studies.

There are some other situations where immunogenicity studies in animal models should be considered.

- In the development of the production process, formulation and route of administration, studies in animal models may aid in reducing the potential for immunogenicity
- During a comparability exercise, the comparison of immunogenicity between the reference product and the comparator product in an animal model should be considered. The absence of these studies should be justified, in particular in cases where immunogenity of the reference compound has been observed clinically. A clear difference in immunogenicity in the animal model would indicate non-comparability.
- An immune response to the therapeutic protein may result in autoimmune reactions, directed to the endogenous protein. 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. Both humoral and cellular immune responses should be considered. In absence of such data, and if theoretical considerations suggest a potential safety risk, animal studies involving immunisation with the animal homolog of the therapeutic protein may be considered

Evolving *in vitro* and *in vivo* technologies e.g. transgenic mouse models may be useful for evaluating the potential immunogenicity of a given protein product.

4.3 Development of assays for humoral and cellular immune response

Unwanted immunogenicity induced by biologicals can comprise humoral and cellular immune responses. It is therefore very important to select and/or develop assays & assay strategies for

assessment of such immune responses. Most effort is usually focused on antibody detection and characterisation, as this is technically feasible and often related to clinical safety and efficacy. However, cell-mediated responses are clearly important and their assessment also needs to be considered.

Assay strategy

Adopting an appropriate strategy for assessment of unwanted immunogenicity of biological products is essential. This should usually include a screening assay for identification of antibody positive samples/patients, analytical immunochemical procedures for confirming the presence of antibodies and determining antibody specificity and functional bioassay(s) for the assessment of the neutralizing capacity of antibodies. In addition, assays will be required which assess and characterize the clinical impact of antibodies (and possibly other components of immune responses) if these are detected/induced. It is important to include baseline data from all patients were appropriate.

For further details on the proposed strategy for antibody detection and characterisation see Annex 2.

Types of antibody assays

- *Screening assays*

A screening assay should be capable of detecting antibodies induced against the biological product in all antibody positive samples/patients. This implies that detection of some false positive results is inevitable as absolute screening-assay specificity is normally unattainable and false negative results must be avoided. The desirable characteristics of screening assays are sensitivity, specificity, precision, reproducibility and robustness.

- *Assays for dissecting the specificity and confirming the presence of antibodies*

These assays are necessary for elimination of false positive samples/patients following the initial screen. Various approaches can be adopted for this purpose but it is necessary to select assays taking account of the limitations and characteristics of the screening assay(s). It is usually advisable to use a different assay format from that used for the screening assay. For this, it is often possible to use an assay, which provides information concerning the specificity of the antibodies, detected and this contributes to confirmation of the specificity of the immune response.

- *Neutralization assays*

Assessing the neutralizing capacity of antibodies usually requires the use of bioassays. An assay must be selected or developed which responds well to the biological product. Bioassays used for measuring the potency of biological products e.g. for lot release purposes 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.

- *Assay validation*

Assays need to be validated for their intended purpose. Validation studies must be conducted to establish that the assays show appropriately linear responses to relevant analytes as well as appropriate accuracy, precision, sensitivity, specificity and robustness.

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.

Residual biological 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 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 if these are induced or the treatment of the patient.

- *Standardisation and reference materials*

Assays must be standardised and this requires the identification and/or development of appropriate reference materials and the use of relevant biological standards. Reference materials and standards are essential for assay calibration and validation. This is especially important for assays used in unwanted immunogenicity investigations/studies, as it is intimately associated with assay interpretation and with distinguishing antibody positive from antibody negative samples.

Characterisation of antibodies to a therapeutic protein

If antibodies are detected in patients undergoing therapy, these need to be characterized to establish their clinical significance. This normally involves an immunological and/or biological assessment of antibody characteristics and investigation of effects of the antibodies (or other induced immune responses) on the product. Some of this can be addressed by *in vitro* studies but it may also require clinical assessment of the patients receiving therapy.

- *Antibody Characteristics*

If antibodies are induced in patients, serum or plasma samples need to be characterised in terms of antibody content (concentration/titre) and other criteria, which need to be considered on a case-by-case basis according to the biological product, the type of patients treated, the aim of the study and possibly other factors. These may include antibody class and subclass (isotype), affinity, specificity,

Antibodies present in confirmed positive samples need to be examined for specificity for the active protein and distinguished from antibodies, which bind to product-related and process-related components. It has been shown that antibodies can be induced against all and or any of these. It is also useful to screen for cross reactivity with other products based on the particular protein as well as (if possible and relevant) its endogenous counterpart.

The neutralising capacity of antibodies present in positive samples needs to be established as this often correlates with diminished clinical responses to biological product. 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 dependant) amounts. This may depend on product, but must be determined empirically. Screening neutralizing samples for cross-neutralization of other products based on the same protein and the endogenous protein is important as it may have implications for clinical efficacy and safety.

- *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. This includes the selection, assessment, characterisation and validation of all assays, identification of appropriate sampling points, 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, expected clinical parameters. Such studies can provide valuable information concerning significant immunogenicity of biological products, its characteristics and potential clinical consequences. They can be valuable for preliminary comparative immunogenicity studies for biosimilar products or following production/process changes introduced for established products. However, unwanted immunogenity can occur at a level, which will not be detected by such studies when conduced at a pre-approval stage, due to the restricted number of patients normally available for study. The clinically significant immunogenicity problems now widely

acknowledged for EPO could not have been revealed even by relatively large, well-planned studies, which are possible to conduct at this stage. In view of this it is usually necessary to continue assessment of unwanted immunogenicity and its clinical significance post-approval, usually as part of pharmacovigilance surveillance.

For further details on methods for assessment and characterisation of immunogenicity see Annex 1.

4.4 Potential clinical consequences of immunogenicity

The consequences of an immune reaction to a therapeutic protein range from transient appearance of antibodies without any clinical significance to severe life threatening conditions. As a rule, therapeutic proteins should be seen as individual products, and experience from related proteins can only be considered supportive. Also in this respect, concomitant medications and other patient-related factors like the underlying disease (section 1.1) have to be taken into account, since these can also influence the clinical presentation of immunogenicity. Therefore, the risk of immunogenicity needs to be considered individually for each indication/patient population.

Consequences on Efficacy

Factors, which influence whether antibodies to a therapeutic protein will induce clinical consequences, include the epitope recognised, affinity and class of the antibody. Usually, antibodies recognising epitopes on the therapeutic protein not linked to activity are associated with less clinical consequences. However, as discussed below, such antibodies can influence pharmacokinetics and, as such, influence efficacy indirectly. “Neutralising” antibodies, interfering with biological activity by binding to or near the active site, or by induction of conformational changes, can induce loss of efficacy. Discrimination between neutralizing and non-neutralizing antibodies is of great importance, and the assays used should be able to discriminate accordingly (see section 4.3). Correlation of antibody characteristics with clinical responses requires a comparison of data generated in assays assessing antibody responses (see above) with results generated using patients’ samples and assays designed to assess clinical responses. Most of the latter are product specific, e.g. assessing expansion of leukocyte populations by colony-stimulating factors, increased reticulocyte numbers by erythropoietin. Such assays need to be selected according to product and need. In many cases, it might be difficult to identify a clinical endpoint, which is sensitive enough to establish the impact on clinical outcome directly, and adoption of a surrogate measure of response may be an option. *In vivo* comparison of patient’s clinical responses to product before and following antibody induction can provide information on the correlation between antibody development (and antibody characteristics) and clinical responses. This can be done either by intra-group analysis (response in patients before and after occurrence of antibodies), or by comparison with patients within the study who do not show an immune response.

Consequences on Safety

Loss of efficacy and alteration of the safety profile are not necessarily linked. Safety issues, like infusion-related reactions, can occur even when there is no loss of efficacy.

- *Acute consequences*

Usually, patients who develop antibodies are more likely to show infusion-related reactions. Acute infusion reactions including anaphylactic reactions may develop during (within seconds) or within a few hours following infusion. Applicants should differentiate between the terms “infusion reaction” and “anaphylaxis” and carefully define which symptoms to label as “infusion-related reaction”. “Infusion reactions” usually represent symptoms occurring in a close timely relationship to an infusion and are not necessarily linked to anaphylaxis or even hypersensitivity. The term “anaphylaxis” should be restricted to typical anaphylactic symptoms such as laryngeal or pharyngeal oedema, urticaria, hypotension, bronchospasm etc., since anaphylaxis represents a strict contraindication to further exposure to the drug.

Applicants should not only focus on anaphylactic symptoms since the consequence of immunogenicity is product-specific and can elict unexpected clinical symptoms.

- *Non-acute consequences*

Delayed hypersensitivity and immune complexes

In addition to acute reactions, delayed hypersensitivity reactions have been reported, possibly mediated by immune complexes. The risk of these reactions may be higher with an increasing drug free interval. Delayed hypersensitivity reactions should be clearly delineated from infusion reactions. The 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, pruritus etc., but also other, less obvious clinical symptoms should be systematically collected.

Besides consequences on pharmacological characteristics, immune-complexes can potentially be deposited in tissues. The underlying disease and the potential consequences of immune complexes on the further clinical course should be considered and critically evaluated, e.g. potential worsening of renal involvement in patients with underlying autoimmune disease.

Autoimmunity

Antibodies developing against therapeutic proteins can cross-react with the endogenous protein in cases where endogenous protein is still produced (e.g., erythropoietins). In-depth characterization of the antibody response including cross-binding and close surveillance of the clinical consequences should be part of the pre-approval development programme. Experiences with similar products can be supportive, but are not sufficient per se.

Applicants developing novel constructs like hybrid molecules fused to physiological functional molecules should carefully consider the potential consequences of cross-reactivity of antibodies against all components.

4.5 Clinical Safety

Pre-authorisation signal detection in clinical setting

- *Rationale for sampling schedule and kinetics of the antibody response*

Several factors such as dose, schedule and treatment modalities influence the development of an immune response against a therapeutic protein (see 4.1). Therefore, the sampling schedule for detection of an immune response should be adapted and selected individually for each product. Baseline samples should always be collected.

For products intended for chronic use, more frequent sampling will be employed in the earlier phase of treatment, where patients are usually most at risk of antibody development. Sampling schedules should include repetitive sampling and be designed to clearly distinguish patients being transiently positive from patients developing a persistent antibody response. The latter is of high importance, since patients with persistent antibodies are more likely to experience clinical sequelae in terms of safety and efficacy, while a transient antibody response can resolve without further consequence. Since longer-term treatment is more likely to result in an immune response, routine sampling later in the treatment course for a sufficient number of patients should be implemented in clinical trials. In case of continuous chronic treatment, immunogenicity data in general for one year should be available pre-authorisation.

Efforts should be engaged to collect data on potential changes in the character of the antibody response over time, e.g. change from non-neutralizing to neutralizing in a given patient, where applicable. During treatment samples should always be taken before administration of the product, since residual levels of the active substance in plasma can interfere with the assay (see section 4.3). To enable intra-product comparison, Applicants should endeavour to standardise sampling schedules, assays, definitions etc. However, for some therapeutic proteins, different timings for antibody formation have been reported depending on the underlying disease. Applicants should consult relevant bibliographical data relating to other products to identify the appropriate timing of measurements in relation to the underlying disease, and scheduling might have to be adapted accordingly. If feasible, sampling should be done after completion of the treatment regimen to determine persistence of response. Adequate follow-up of patients for measuring an immune response after discontinuation of treatment should be implemented to evaluate immunogenicity in absence of the therapeutic protein. Sampling should take into account both the half-life of the therapeutic protein and the duration of

pharmacodynamic effects. While a decrease of anti-drug antibodies might occur over time in patients initially positive for such antibodies, also a rise in such antibodies might occur, e.g. if the therapeutic protein has immunosuppressive properties and by its mechanism of action suppresses an immune response against itself.

Impact on pharmacokinetics of the product

Both neutralizing and non-neutralizing antibodies can impact on the pharmacokinetics of the product. Immune complexes can be formed that are taken up by the reticuloendothelial system, which will reduce bioavailability and enhance clearance. However, reduced clearance and longer half-life have also been described. If antibodies are detected during the clinical programme, the Applicant should investigate the impact on the pharmacokinetics in the individual patient. The binding characteristics (binding vs. neutralizing) should be linked to this evaluation. The half-life may be prolonged, but not necessarily associated with the prolonged therapeutic effect. A change in pharmacokinetics may be an early indication of antibody formation.

Methodology aspects to assess comparability of immunogenicity potential

This section applies to comparability testing either for changes in the manufacturing process or to a reference product in case of the development of a similar biological medicinal product. Immunogenicity evaluation should be part of clinical efficacy and safety studies. Studies should be carefully planned and data should be systematically collected from a sufficiently large number of patients to characterise the variability in antibody response. Since the comparative evaluation of immunogenicity both inter-product (i.e., similar biological medicinal products or products in the same class) and intra-product (i.e., between different versions of the product, indications or different patient populations for a given product) is of relevance, Applicants should make an effort to select a homogeneous patient population that allows for such comparisons. A patient population should be chosen that is representative of the target population intended for clinical practice. Due to expected differential susceptibility, immunogenicity data from healthy volunteers are not suitable substitutes. For most products, immunogenicity is studied in previously unexposed patients. Children should be studied separately, if applicable, stratified by age. A sufficient washout period for previous treatments potentially influencing the immune response should be included, taking into account not only elimination, but also reversal of the pharmacodynamic effect, where appropriate.

Variations to the production process, even of well-characterised products, have also been reported to considerably alter their immunogenic properties. If intra-product comparative immunogenicity is analysed when changes in the production process have been made, a population should be chosen in an indication where differences can best be detected (i.e. due to susceptibility to immunogenicity). Caution should be exercised when using surrogate parameters, e.g. pharmacodynamic parameters, instead of efficacy (or safety) endpoints. Such parameters should correlate with clinically relevant endpoints and have to be fully justified.

Changes in immunogenicity as a result of a change in the manufacturing process might require a specific risk management and pharmacovigilance plan (see section 4.8)

Recommendations for routine monitoring of changes in clinical response and linking immunological findings to clinical events

Antibody testing should be considered as part of all clinical trial protocols. For a clinical trial, Applicants are encouraged to evaluate immunogenicity in all patients and not only in a symptom-driven manner (i.e. only for patients when a change in safety or efficacy profile is suspected).

For a new active substance, the overall incidence of immunogenicity should be evaluated for a given product in all indications, thus sampling schedules should be comparable between different trials in order to enable for direct comparison of the incidence of anti-drug antibodies.

However, further to scheduled routine repetitive sampling patients should also be evaluated in a symptom-driven manner, when the occurrence of an antibody is suspected. Applicants should collect data and provide guidance for the prescriber as part of the marketing authorisation application on how a patient with loss of efficacy should be handled over time, e.g. by an increase of dose or a reduced dosing interval or cessation of treatment.

The results of the immunological studies should be included in the relevant sections of the SPC.

Immunogenicity in paediatric indications

Therapeutic proteins are increasingly used in children who may differ in their immune response.

When studying the product in a paediatric indication, posology and treatment schedules should be selected and justified accordingly. Patients should be stratified by age, and immunogenicity data should be evaluated and presented separately for each age stratum.

Recombinant technology has allowed the development of proteins for use in genetic disorders where previous substitution treatment has not been available. Children are the most likely subjects exposed to these products and may be at high risk for antibody development.

4.6 Risk management Plan

Within the marketing authorisation application, the applicant should present a risk management plan in accordance with current EU legislation and pharmacovigilance guidelines. This should take into account risks identified during product development and potential risks. The risks of immunogenicity should be addressed according to the principles outlined in this guideline.

The extent of data on immunogenicity that can be obtained during the clinical development programme 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. Therefore, further systematic immunogenicity testing might become necessary after marketing authorization, and may be included in the risk management plan.

The extent of immunogenicity data to be collected in the post-marketing setting will depend on various factors including:

- Disease-related factors like its prevalence, the vulnerability of the patients, availability of alternative therapies, duration of treatment, etc.
- Pre-authorization immunogenicity findings including impact on efficacy and safety
- Experience on immunogenicity with similar proteins or related members from that class of proteins, including proteins manufactured with similar production processes.

However, biotechnology-derived proteins should be considered individually, and therefore the possibility for extrapolation from other related proteins is limited and needs to be fully justified.

For planning immunogenicity assessment in the post marketing setting, the same recommendations apply as discussed in previous sections of this guideline.

REFERENCES

- Note for guidance on pharmaceutical development (ICH Q8 Step 4)
- Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (ICH S6)

ANNEX 1 Further details on methods for assessment and characterisation of immunogenicity

Types of antibody assays

- *Screening assays*

The need to accommodate screening of relatively large numbers of samples necessitates use of an assay with high throughput and appropriate automation. Screening methods include immunoassays, radioimmunoprecipitation assays and surface plasmon resonance assays. All procedures detect antigen-antibody interaction (binding) but may differ in their underlying scientific/ technical principles.

Immunoassays constitute a large group of assays and are based on a variety of formats and detection systems. These include direct binding assays, bridging assays, capture (sandwich) assays and competitive immunoassays using radioligand, enzymatic, fluorescent, chemiluminescent or electrochemical luminescence detection systems.

- *Assays for dissecting the specificity and confirming antibody positivity*

Different assays can be used for this purpose and high sample throughput maybe less important than for screening assays due to the smaller number of samples requiring analysis. To achieve confirmation of specificity, it is advisable to select an assay based on a different scientific/technical rationale than that used for the screening assay.

Competitive immunoassays and surface plasmon resonance assays can be used. Analytical immunoassays such as immunoblotting and radioimmunoprecipitation analysis offer the advantage that they can be used to dissect the specificity of the detected antibodies as well as confirming antibody positivity.

- *Neutralization assays*

Bioassays need to be selected using a product-based approach.

Usually a single concentration of biological is chosen for the assay & dilutions of each sample assessed for their inhibitory effect on the assay response. This allows a neutralizing dose response to be determined & calculation of neutralizing capacity (‘titre’) for each sample.

In some cases neutralizing bioassays can be used directly as ‘confirmatory’ assays following screening, but this must be determined on a case-by-case basis.

- *Assays for assessing cell-mediated immune responses*

The strategy for assessing cell-mediated immune responses induced by biologicals is generally less clear than for humoral responses. Assays need to be developed or selected on a case-by-case basis if these are required. In most cases, development of a mature IgG response implies underlying antigen specific helper T-cell involvement.

Examples of assays of use for detecting/assessing cell-mediated responses are T-cell stimulation/proliferation assays and cytokine (e.g. IL2, IL4, IFN-gamma) production/release methods. These involve the use of T-cell preparations sometimes co-cultured with preparations of other cell types e.g. dendritic cells.

Elispot and flow cytometry procedures are commonly used for these assays.

In some cases more detailed studies involving assessment of cell-mediated immune responses may be useful. Memory B-cell (and sometimes memory T-cell) assays can provide useful information regarding the nature of the immune response and may contribute to prediction of development of immunogenicity problems. Studies using peptides or full-length protein (depending on the assays and purpose of the assays) and Elispot methodologies can be used for these. In some cases more complex investigations of cell-mediated immunity e.g. involving study of regulatory T-cells may be valuable. The need for such investigations must be decided on a case-by-case basis depending on the aims and purpose of the studies.

Assay characteristics

Assays need to be selected, optimized and analysed according to and taking account of their intended use. The importance and requirements of assay characteristics (see above under *screening assays* for a list of some of these) depends on the use of the assay. For example great sensitivity may not be required for an assay if this is not needed for detection of the amounts of antibodies, which are induced by a particular biological product in patients receiving therapy. Developing unnecessarily sensitive assays for such antibodies would be inappropriate especially if this sensitivity can only be achieved by sacrificing other desirable characteristics e.g. specificity, robustness.

Adoption of the simplest assay suitable for all requirements is normally a valid approach to assay selection (particularly when high throughput is important e.g. for screening assays). 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. In such cases, it is often necessary to adopt a more complex assay eg 'bridging' assays, ECL or SPR methods to avoid this. False negative results in screening assays due to epitope masking can be encountered and a strategy to avoid these may be necessary e.g. by using assays that avoid specific masking of particular epitope(s).

Standardisation, reference materials and assay validation

An antibody positive standard/reference material/control is clearly needed for all assays. This is used to demonstrate assay response and can be used for calibration purposes. If possible this should be a human preparation with a significant antibody content which is available in sufficient quantity for continued use. It should be stored appropriately (normally lyophilized) and well characterized. Reference preparations for neutralization bioassays should have significant neutralizing activity, but it is also useful to include a non-neutralizing antibody preparation in assays, at least in validation studies. However, in several cases, sufficient human antiserum may not be available to allow preparation of an appropriate reference preparation. In such cases, pooling of samples is usually the best approach and this may also avoid problems due to the specific characteristics of a single donor sample. In some cases human serum is unavailable in the quantities required either as a pool or even at all e.g. early in product development/trials and in such cases use of an animal serum as a reference is the only realistic option. However, this needs to be selected carefully and its use is more limited than for human reference preparations 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. Calibration of immunoassays 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 reference materials, especially on a mass basis difficult, if not impossible. This implies that calibration of such assays should be carried out using an acceptable, valid approach, which is clearly described. Often the best option is to report immunoassay data as a titre based on a standard procedure for calculating this value.

Biological assays used to assess the neutralizing capacity of antibodies should be calibrated using International Standards/Reference Preparations where these are available. This allows expression of neutralizing activity in terms of meaningful units of biological activity of product/preparation and also provides information relevant to assay validation. If such standards are not available, appropriate in-house preparations need to 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/ IU of biological.

It is also very useful to prepare a panel of reference materials containing different amounts of antibodies and antibodies with different characteristics, 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 standards/controls are needed to establish assay baselines and characterize/validate the assays. Assay baseline for normal (healthy) individuals is clearly fairly easily determined by

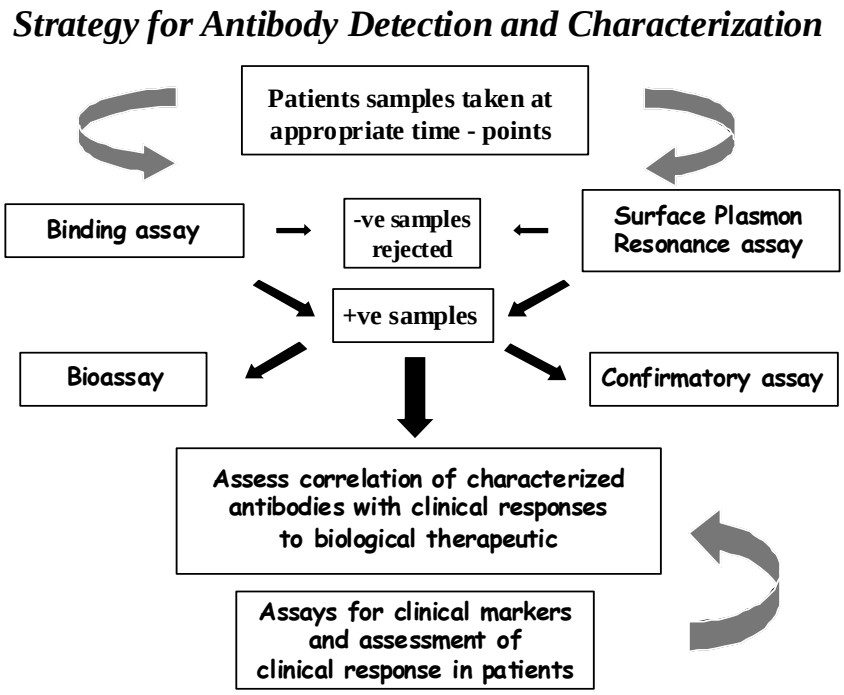
measuring the assay response using samples derived from an appropriate number of such individuals and analysing this to provide a statistically valid background value. However, this may not represent the baseline response of the assay to samples derived from the patient population, which would therefore need to be established separately, using pre-treatment samples from patients, or from some other valid, relevant population. In any case, 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.

Reagents used in assays need to qualified and acceptance specifications set, at least for those, which are most important.

Interpretation of Results

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 (see above) and decide on a statistical (e.g. 3 SD above background value) or real data (e.g. double background value) basis of what will be considered the lowest positive result. Confirming positivity normally requires repeating assays, often using a different assay method(s).

ANNEX 2 Proposed Strategy for antibody detection and characterisation



-ve denotes negative; +ve denotes positive