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GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

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GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

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EXECUTIVE SUMMARY

The number of biological/biotechnology-derived proteins used as therapeutic agents is steadily increasing. 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. This document contains background information concerning the potential causes and impacts of immunogenicity and provides general recommendations for the performance of a systematic immunogenicity assessment from a marketing authorisation perspective.

The predictive value of non-clinical studies for evaluation of immunogenicity of a biological medicinal product in humans is low due to inevitable immunogenicity of human proteins in animals. While non-clinical studies aimed at predicting immunogenicity in humans are normally not required, animal models may for example be of value in evaluating the consequences of an immune response.

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 may need to be capable of distinguishing neutralizing from non-neutralizing antibodies, and for use in pivotal clinical trials as well as in post-authorisation studies to be validated.

In the clinical setting, careful planning of immunogenicity evaluation should include data systematically collected from a sufficient number of patients. For a given product, sampling should preferably be standardized across studies (e.g., sampling at baseline, under treatment and follow up samples). The sampling schedule for each product is determined on a case-by-case basis, taking into account also the risks associated with an unwanted immune response to patients. 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.

The scope of this guideline covers a wide applicability. Thus, the concepts might have to be adapted on a case-by-case basis to fit an individual development programme. Applicants should consider the possibility to seek Scientific Advice from EMEA or from National Competent Authorities.

1. INTRODUCTION

Most biological/biotechnology-derived proteins induce an unwanted immune response that is triggered by more than one single factor. This immunological response is complex and, in addition to antibody formation, other events such as T cell activation or innate immune response activation could contribute to potential adverse 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 of an unwanted immune response are a loss of efficacy of the therapeutic protein, serious general immune effects such as anaphylaxis, and, for therapeutic proteins used for substitution, a potential cross-reactivity with the endogenous counterpart in case it is still produced.

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 an individual to an immune response include: underlying disease, genetic background, immune status, including immunomodulating therapy, and dosing schedule. Product-related factors also influence the likelihood of an immune response, e.g. 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. In the marketing authorisation application, the applicant should include a summary of investigations of immunogenicity in the respective overview sections with full cross-reference to the data in the relevant modules. 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. Further systematic immunogenicity testing might become 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 how to systematically evaluate this. 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 from recombinant or non-recombinant expression systems. Throughout this guideline, the term "therapeutic protein" is used. This guideline should be read in conjunction with other relevant guidelines, e.g.:

- Guidelines on similar biological (biosimilar) medicinal products;
- Guidelines on comparability of biotechnology-derived medicinal products after a change in the manufacturing process.

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

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

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 have to be taken into account, since these can also influence the clinical presentation of immunogenicity. Therefore, immunogenicity evaluation needs to be studied individually for each indication/patient population.

Evaluation of immunogenicity should be a multidisciplinary task, encompassing joint efforts of quality, non-clinical and clinical experts.

This document gives general recommendations and principles for developers and assessors of biotechnology-derived therapeutic proteins of how to approach immunogenicity evaluation from a marketing authorisation perspective. The scope of this guideline covers a wide applicability. Thus, the concepts might have to be adapted on a case-by-case basis to fit an individual development programme. For the justification of their approach to immunogenicity testing, Applicants should take into consideration both the risk for developing an unwanted immune response, and the potential clinical consequences as outlined below. The approach taken for the design of the immunogenicity development concept should be fully justified, e.g. when omitting assays or immune response measurements proposed in this guideline. Applicants should consider the possibility to seek Scientific Advice from EMEA or from National Competent Authorities.

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

4.1.1 Patient- and disease-related 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 modulating the immune response*

Genetic factors can alter the immune response to a therapeutic protein and lead to inter-patient variability. Allelic polymorphism in the major histocompatibility complex (MHC), impacting on affinity and stability of the interaction between MHC molecules and antigenic peptides, and genes

encoding the T cell receptor of helper T cells may influence immune responses and immunological tolerance induction.

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

Other genetic factors influencing immunogenicity could be 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 of an endogenous protein, reduced levels or even the lack of this protein may 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 (see section 4.5.4). If indicated in elderly, consideration should be given to a potentially altered immune response.

- *Disease-related factors*

The patient's underlying disease itself can be an important factor in the context of developing an unwanted immune response.

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

In other conditions (e.g. malnutrition, advanced metastatic disease, advanced HIV 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 development of an antibody response can be different for different therapeutic indications or different stages of the disease. Therefore, immunogenicity normally needs to be studied separately for each disease or stage of the disease as part of the clinical studies.

- *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 absence of immunosuppressants, i.e. immunogenicity data from the combination with immunosuppressants are not relevant for the monotherapy setting.

- *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.

Short-term treatment only is usually less likely to be associated with 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.

- *Previous exposure to similar or related proteins*

Previous exposure to similar or related 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 or immunological memory that affects 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. breakdown products, aggregates and host cell proteins, lipids or DNA), and formulation.

- *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 (epitope-spreading). Identification of the antigenic moiety of the fusion protein 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. Therefore, when the same protein is manufactured under different conditions (e.g. change in cell culture process) there might be changes in the pattern of post-translational modifications and the immunogenic potential of the protein. This means also that antibodies induced by one product may react differently with the analogous product manufactured under modified conditions. This might have to be considered for evaluation of immunogenicity.

- *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. The formulation and the source of excipients may alter immunogenicty of therapeutic proteins and should be considered as a possible cause of such events. This should be considered when variations to the formulation are made.

Impact of the primary packaging material and the conditions of clinical use e.g. dilution in infusion solutions and infusion devices of different materials could also influence the immunogenic potential of a therapeutic protein.

- *Aggregation and Adduct Formation*

Aggregation or adduct formation of proteins may either reveal new epitopes or lead to the formation of multivalent epitopes, which may stimulate the immune system. Factors which could be considered to contribute to aggregate or adduct formation include formulation, purification processes, viral inactivation procedures, and storage conditions of intermediates and finished product. The use of proteins, e.g. albumin, as excipient 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.

- *Impurities*

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 against themselves. But it is also possible that these HCPs, host cell-derived lipids or DNA function as adjuvants for the protein of interest.

4.2 Non-clinical assessment of immunogenicity and its consequences

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 non-clinical studies for evaluation of immunogenicity 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.

Measurement of antibodies in non-clinical studies are however requested as part of repeated dose toxicity studies, in order to aid in the interpretation of these studies (as discussed in "Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals." ICH S 6).

Also, the comparison of the antibody response to the reference product in an animal model may be part of the comparability exercise both for similar biological medicinal products (see Guideline on Similar biological medicinal products containing biotechnology-derived proteins as active substance: Non-clinical and clinical issues CHMP/42831/05 and product-specific annexes) and for changes in manufacturing processes (see Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process – Non-clinical and clinical issues CHMP/BMWP/202695/06).

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. Both humoral and cellular immune responses (where relevant) should be considered. In absence of such data, 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.

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

Unwanted immunogenicity induced by biologicals can comprise humoral and cellular immune responses. It is therefore very important to select and/or develop assays and 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 could play an important role and their assessment may be considered by applicants on a case by case basis.

4.3.1 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 assays for the assessment of the neutralizing capacity of antibodies. In addition, non-antibody assays e.g., assays for relevant biomarkers or pharmacokinetic measurements 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 where appropriate.

Annex 2 shows an example of a possible strategy for antibody detection and characterisation.

4.3.2 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 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). To confirm specificity, it is not normally sufficient or appropriate to simply repeat the screening assay in its original form.

- *Assays for dissecting the specificity of antibodies*

Assays which provide information concerning the specificity of the antibodies detected may be useful in some cases. This data 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. If neutralising cell-based assays are not feasible/available, competitive ligand binding assays 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.

4.3.3 Assay validation

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. For pivotal clinical trials, the use of a central laboratory to perform the assays may be 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.

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, 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.

- *Standardisation and controls*

Assays must be standardised and this requires the identification and/or development of appropriate reference materials, i.e. the use of relevant biological standards and/or well characterized positive and negative controls. These reagents function as critical assay reagents and 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.

4.3.4 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 non antibody assays as part of *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), 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, specificity. The degree of characterization required may differ depending on the study purpose and stage of development of the product. The assays used should be qualified for their intended purpose.

Antibodies present in confirmed positive samples need to be examined for specificity for the active protein and, where applicable, 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. In some cases, 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. 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.

- *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, 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. Such studies can provide valuable information concerning significant immunogenicity of biological products, its characteristics and potential clinical consequences. They can be valuable for 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 conducted at a pre-approval stage, due to the restricted number of patients normally available for study. In view of this, it is often necessary to continue assessment of unwanted immunogenicity and its clinical significance post-approval, usually as part of pharmacovigilance surveillance. In some cases, post-approval clinical studies may be needed to establish the risk associated with an unwanted immune response.

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

4.4 Potential clinical consequences of immunogenicity

4.4.1 Consequences on Efficacy

Factors which influence whether antibodies to a therapeutic protein will induce clinical consequences include the epitope recognised, affinity, class of the antibody, the amount of antibodies generated, and the pharmacological properties of the biotechnological medicinal product. In addition, the ability of immune complexes to activate complement or be cleared may be a factor that impacts clinical outcome. Usually, antibodies recognising epitopes on the therapeutic protein not linked to activity are expected to be 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. Determination of neutralizing antibodies from confirmed positives, and the assays used, should be appropriate (see section 4.3). Most importantly, neutralizing antibody assays should be capable of detecting clinically relevant neutralizing antibodies. 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, or 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, e.g. biomarkers/pharmacodynamic markers. The choice of such markers should be justified. *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.

4.4.2 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. However, acute reactions can be true allergic, namely IgE-mediated type I reactions (anaphylactic reactions), including hypotension, bronchospasm, laryngeal or pharyngeal oedema, wheezing and/or urticaria. The term "anaphylaxis" should be restricted to such situations and represents a strict contraindication to further exposure to the drug. However, the majority of infusion reactions are characterized by more non-specific symptoms, for some products more frequently occurring on initial exposure and sometimes less frequent/severe reactions are observed on re-exposure. An infusion might not represent a contraindication to further exposure. A range of symptoms including headache, nausea, fever or chills, dizziness, flush, pruritus, and chest or back pain have been described in relation to infusions. It is acknowledged that the distinction between an infusion reaction and anaphylaxis can be challenging, but nevertheless such distinction in necessary due to the different clinical consequence.

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

- *Non-acute consequences*

Delayed hypersensitivity and immune complexes

In addition to acute reactions, delayed type (T-cell mediated) hypersensitivity and immune complex mediated reactions have to be considered. The risk of these reactions may be higher with an increasing drug free interval. Such delayed hypersensitivity reactions should be clearly delineated from infusion 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, 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.

Cross-reactivity with an endogenous counterpart

Antibodies developing against therapeutic proteins with endogenous counterparts can cross-react with this endogenous counterpart in cases where it 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 endogenous (or self) components.

4.5 Immunogenicity and Clinical Development

4.5.1 Rationale for sampling schedule and kinetics of the antibody response

Immunogenicity assessment should be part of the clinical trials, since the correlation to clinical efficacy and safety is important. 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). However, further to scheduled routine repetitive sampling, patients should also be evaluated in a symptom-driven manner with additional samples, when the occurrence of an unwanted immune response is suspected.

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, taking into account also its pharmacokinetics. Baseline samples should always be collected. The overall incidence of immunogenicity should be evaluated for a given product in all indications, thus sampling schedules should preferably be comparable between different trials in order to enable for direct comparison of the incidence of anti-drug antibodies. Deviations from this concept should be justified. Applicants should endeavour to standardise sampling schedules, assays, definitions etc. taking into account also experiences with comparable products. 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). Adequate conditions for storage and shipment of samples need to be established to ensure appropriate quality of the test material.

The frequency of sampling and the timing and extent of analyses will also depend on the risk identified for a particular drug and the clinical consequences, and has to be justified. Sampling schedules should include repetitive sampling and be designed to clearly distinguish patients being transiently positive from patients developing a persistent antibody response. Both transient and persistent antibody responses should be combined to determine the overall immunogenicity of a product in a given condition. In particular, persistent antibodies are 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.

For products intended for chronic use, it may be necessary to study the evolution and persistence of an observed immune response. 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. On a case-by-case basis, e.g. when required according to a risk assessment, potential long-term consequences of an unwanted immune response should be considered when planning the clinical programme of immunogenicity evaluation. More frequent sampling will usually be employed 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 sampling later in the treatment course should be implemented in clinical trials. In case of continuous chronic treatment, usually immunogenicity data for one year of treatment should be available pre-authorisation. Deviations should be fully justified, e.g. shorter exposures or differences as regards the extent of data for different routes of administration. 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. Depending on the medicinal product and the potential risks associated with the occurrence of an unwanted immune response, it might become important to cover a sufficient number of exposures.

If feasible, sampling should also be done after completion of the treatment regimen to determine persistence of response. 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.

Where feasible and possible, Applicants should 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.

4.5.2 Consequences on pharmacokinetics of the product

Antibodies recognising epitopes on the therapeutic protein not linked to activity are associated with fewer clinical consequences. However, such antibodies can influence pharmacokinetics and, as such, influence efficacy. "Clearing" antibodies may be neutralizing or non-neutralizing, and reduce efficacy by removing the therapeutic protein from circulation. 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." Neutralizing antibodies may inactivate the drug with or without clearance. The loss of efficacy may be characterized through the Assay Strategy described in Section 4.3 as needed. A change in pharmacokinetics may be an early indication of antibody formation. If antibodies are detected during the clinical programme, their possible interference with the pharmacokinetics should be studied (see also Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins).

4.5.3 Methodology aspects to assess comparability of immunogenicity potential as part of a comparability exercise

It has been reported that variations to the production process may induce alterations of the immunogenic properties of the product. When variations to the manufacturing process of a licensed product are made, the comparability exercise is a stepwise approach (see Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process). If the initial physicochemical and biological testing indicates a difference between the pre- and post-change product, the potential consequences to safety and efficacy need to be considered including altered immunogenicity. Even when initial physicochemical and biological testing do not indicate a difference, the potential for altered immunogenicity undetected by such tests needs to be considered. The extent of immunogenicity studies, if required, should be based on risk analysis, taking into consideration the nature of the observed difference, the potential clinical impact, and knowledge gained with this product and product class before. The determination of the appropriate target population will be selected to where best detect differences, not restricted to immunogenicity only. Applicants should make an effort to select a homogeneous and clinically relevant patient population

that allows for such comparisons. Due to expected differential susceptibility, immunogenicity data from healthy volunteers are not suitable substitutes. For most products, immunogenicity is studied in previously unexposed patients, and integrated in the clinical study to establish that the change in the manufacturing process has had no adverse impact on efficacy and safety. Immunogenicity evaluation as part of a clinical trial for a comparability exercise should preferably involve head-to-head studies of pre- and post-change product. The same assays should be used.

Changes in immunogenicity as a result of a change in the manufacturing process might require a specific risk management strategy and an update of the risk management plan (see section 4.6). 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.

4.5.4 Immunogenicity in paediatric indications

Therapeutic proteins are increasingly used in children. It has to be considered that children may differ from adults in their immune response.

When studying the product in a paediatric indication, posology and treatment schedules should be selected and justified accordingly. If applicable and feasible, results should be analysed by age groups, and immunogenicity data should be evaluated and presented separately for each age group to potentially identify vulnerable age groups.

As regards substitution therapy, 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 including the CHMP Guideline on Risk Management Systems for Medicinal Products for Human Use (EMEA/CHMP/96268/2005). Immunogenicity should always be addressed in the Risk Management Plan (RMP), taking into account risks identified during product development, and potential risks and consequences of an unwanted immune response to patients. The risk specifications and minimization should follow the principles outlined in this guideline. 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 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.
- Seriousness of the immune reaction.

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.

Immunogenicity can be further studied in a post-marketing setting e.g. by enhanced reporting of possibly immune-related adverse events (including loss of efficacy), or by pharmacoepidemiological studies.

Since systematic sampling might not be feasible in a post-marketing setting, it is important to conclude on potential unwanted immune responses based on suspicious safety and (loss of) efficacy signals. This requires that the evaluation of such events is defined prospectively in the RMP. The MAH should establish a standardized algorithm on how to further investigate those cases of suspected immune responses, including how to confirm the development of antibodies in a given case.

The RMP should include:

- Risk Identification & Characterisation (e.g. case definitions, antibody assays);
- Risk Monitoring (e.g. specific framework to associate risk with product);
- Risk Minimization & Mitigation strategies (e.g. plans to restrict to intravenous use where necessary, actions proposed in response to detected risk etc.);
- Risk communication (e.g. minimization and mitigation messages for patients and physicians, communication to physicians of how to access specific investigation tools like antibody testing assays);
- Monitoring activities to ensure effectiveness of risk minimization.

Applicants should respond to evolving data on immunogenicity by taking adequate measures, e.g. changes in the Product Information, update of the RMP, and other risk minimization activities (e.g. educational programmes etc.).

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

For changes in the manufacturing process, implications of this change on the immunogenic potential might have to be addressed in the RMP.

REFERENCES

- Note for guidance on pharmaceutical development (ICH Q8 Step 4)
- Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (ICH S6)
- Similar biological medicinal products containing biotechnology-derived proteins as active substance: Non-clinical and clinical issues. (CHMP/42831/05)
- Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process. Non-clinical and clinical issues. (CHMP/BMWP/101695/2006)
- Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins (CHMP/EWP/89249/2004).
- Clinical Investigation of Human Plasma Derived Factor VIII and IX Products (CPMP/BPWG/198/95)
- Clinical Investigation of Recombinant Factor VIII and IX Products (CPMP/BPWG/1561/99).

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 confirming antibody positivity*

Different assays can be used for this purpose and high sample throughput may be less important than for screening assays due to the smaller number of samples requiring analysis. To achieve confirmation of specificity, it is necessary to include an assay which evaluates specificity. For example, addition of an excess of antigen to the sample prior to evaluation in binding assays, which should result in a adsorption of antibody and therefore reduction of positive signal for true positive samples. Identification of immunoglobulin as the analyte in some assays e.g., by using specific anti-immunoglobulin reagents can also aid in identifying genuine antibody positive samples.

In certain problematical cases, it may be useful to include an assay based on a different scientific/technical rationale than that used for the screening assay but the characteristics of the assays e.g., different sensitivities need to be considered.

- *Assays for dissecting the specificity*

Analytical immunoassays such as immunoblotting and radioimmunoprecipitation analysis can be used to dissect the specificity of the detected antibodies.

- *Neutralization assays*

Bioassays or other functional assays need to be selected using a product-based approach.

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.

- *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. Cell-mediated cytotoxicity assays may be useful in some cases.

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. They may also be associated with a high incidence of false negatives for samples containing low affinity antibodies and certain isotypes or subclasses. In such cases, it is often necessary to adopt a more suitable assay, e.g. 'bridging' assays, electrochemiluminescent 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, well characterized controls 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 (lyophilized or frozen at a suitable temperature) 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 serum 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. An option is to report immunoassay data as a titre based on a standard procedure for calculating this value. An alternative to this is to calculate the relative antibody concentration of samples and positive controls.

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/defined unit of bioactivity of biological. In other cases, 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, 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.

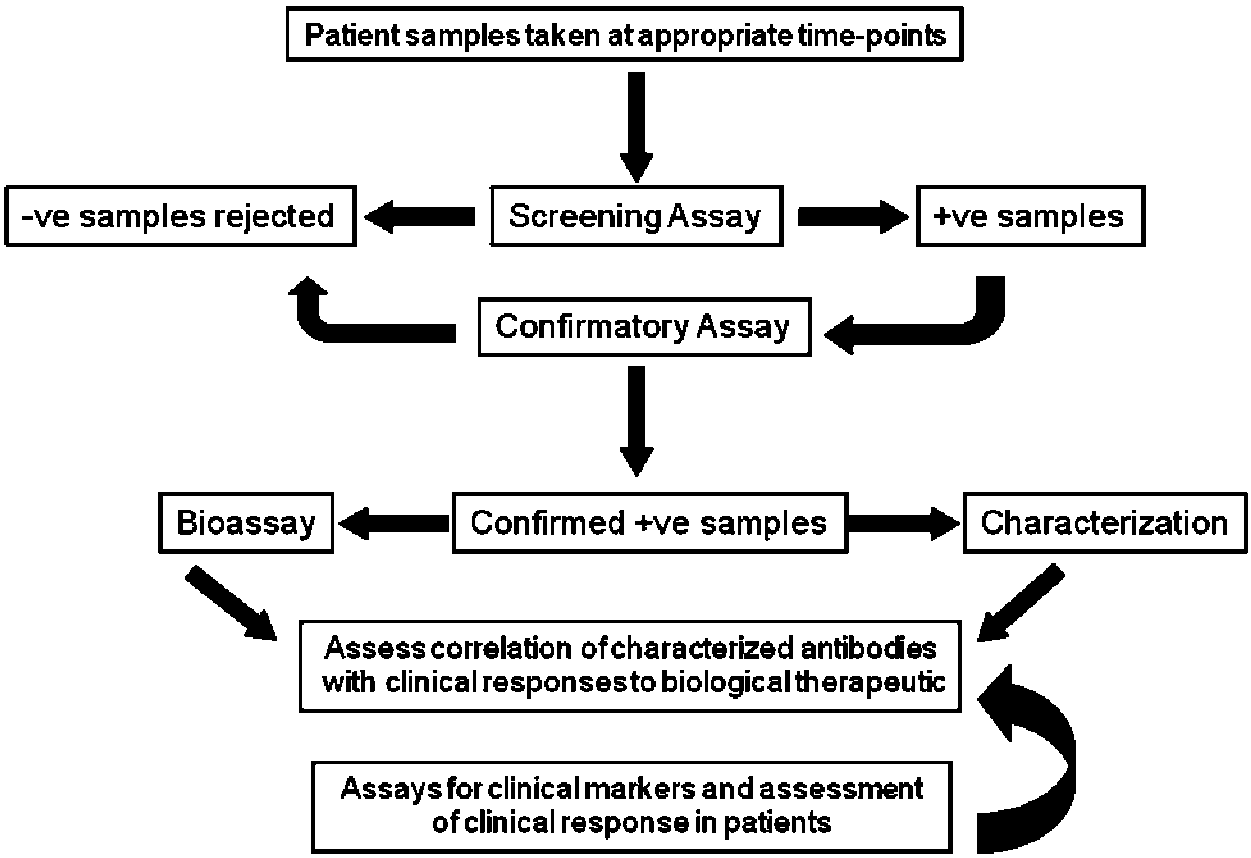
Negative standards/controls are needed to establish assay baselines and characterize/validate the assays. Assay baseline for normal (healthy) individuals is clearly fairly easily determined by measuring the assay response using samples derived from an appropriate number of such individuals and analysing 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). A statistical approach should preferably 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.

ANNEX 2 An example of a strategy for antibody detection and characterisation



-ve denotes negative; +ve denotes positive