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DRAFT

Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins

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Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins

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This guidance document should be read in conjunction with Directive 2001/83/EC, as amended, and all other pertinent elements outlined in current and future EU and ICH guidelines and regulations especially those on:

- Pharmacokinetic Studies in Man (Notice to Applicant, Vol. 3C C3A, 1987)
- Guideline on similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Non-clinical and Clinical Issues" (EMEA/CHMP/42832/2005 Draft)
- Pharmacokinetic Studies in Patients with Renal Impairment (CPMP/EWP/225/02)
- Evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function (CPMP/EWP/2339/02)
- The Investigation of Drug Interaction (CPMP/EWP/560/95)
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- Structure and Content of Clinical Study Reports (ICH topic E3)
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- Preclinical Safety Evaluation of Biotechnology Derived Pharmaceuticals (ICH topic S6)

1 INTRODUCTION

Therapeutic proteins include different molecules ranging from peptides to large proteins such as coagulation factors. Historically, the pharmacokinetic evaluation of such products has suffered essentially from limitations in the assay methodology and the derived pharmacokinetic parameters, limiting the usefulness of such studies.

One of the main objectives of the pharmacokinetic documentation is to ensure efficacy and safety in all patients, including sub-populations not represented in the Phase III trials. Development of therapeutic proteins and small molecules share the same comprehensive goals of safe human investigation, leading to a knowledge that provides compelling information on efficacy and safety in the patient population. Thus, the pharmacokinetics of protein products (in this document also referring to polypeptides) should be evaluated based on the same scientific grounds as conventional products. However, due to the specific features of proteins, special considerations should be given when designing pharmacokinetic studies as compared with conventional molecules. Hence, the objective of this document is to address points to consider when evaluating the clinical pharmacokinetics of proteins used in therapeutics.

It is the objective of this document to:

- underline the specific problems related to the pharmacokinetics of protein products that needs careful consideration during drug development
- draw attention to dissimilarities in pharmacokinetic characteristics between proteins and conventional molecules affecting the content of the development program
- give recommendations concerning the pharmacokinetic development program for proteins

2 BIOANALYSIS

One of the key elements of a pharmacokinetic study is the analytical method and its capability to detect and follow the time course of a given analyte (the parent compound and/or metabolites) in a complex biological matrix that contains many other proteins and with satisfactory specificity, sensitivity and a range of quantification with adequate accuracy and precision. The ability to distinguish the therapeutically applied protein from endogenously produced equivalents should be

considered in selecting the analytical method. In this document, the term metabolite encompasses *in vivo* degradation products and other truncated forms of the protein.

2.1 General considerations

The most frequently used analytical methods for assaying therapeutic proteins in biological samples are i) immunoassays, which estimate the amount of test compound that binds to a target antibody, and ii) bioassays, which measure the activity of the compound in a specific process. Due to the different characteristics of the methods and the entities being detected and quantified, a combination of immunological assays and bioassays is recommended for the clinical development. Indeed, whereas immunoassays are able to detect structurally-related compounds, active or not, bioassays detect only active compounds, be they the parent product or its metabolites or any other structurally-related compounds, including endogenous proteins (see below). Other methodologies, such as LC-MS, may be used but are not specifically addressed here. If possible, it is preferable to develop a specific assay early in the development and use the same assay(s) during the entire development program. The difficulty of developing such an assay at an early stage is, however, recognised. Methods should be adequately validated pre-study and within-study according to standard practice. Difficulties may arise in the bio-analysis due to e.g. lack of specificity and some aspects important for the pharmacokinetic evaluation are highlighted in the section below.

Validation of the analytical assay should comprise two distinct phases, (i) the pre-study phase in which the compliance of the assay with respect to (1) stability of the analyte in relevant matrix, (2) specificity, (3) accuracy, (4) precision, (5) limit of quantification and limit of detection, (6) dose-response relationship is determined and (ii) the within-study phase in which the method is applied to samples from a bio-study and control samples (QC and calibration standards) are used to confirm the correct performance of the run.

2.2 Methodological problems

Several possible weaknesses have been identified and may result in erroneous characterisation of drug disposition and of the formation of antibodies. The following issues should be considered by the Applicant.

<u>Immunoassay</u>

Drug assay:

(i) interference by other immuno-reactive product related substances more or less biologically active, e.g. isoforms, product degradation species formed during manufacture or storage, or in vivo (metabolites), complexes of active substance and complementary molecules (such as binding proteins), which the capture antibody cannot distinguish from the active analyte. Chromatographic methods may be used to separate different components for further analysis of the separate fractions.

(ii) different immuno-reactive components (see (i)) may vary in their response to the assay due to differences in their binding capacity or affinity (e.g. ELISA developed for rhG-CSF is less sensitive to pegylated rhG-CSF and may also vary in its affinity for positional isomers of PEG- rhG-CSF)

(iii) interference by endogenous substances

(iv) interference by anti-drug antibodies binding to the analyte inhibiting the complementary binding to capture antibody

Anti-drug antibody assay:

(v) presence of the active substance may affect the ability to detect the anti-drug antibody since the antibody “is captured” by the active substance. Thus, when quantifying the anti-drug antibody the active substance should preferably be eliminated from the circulation

<u>Bioassay</u>

(i) bioassays may not be specific for the analyte

(ii) may demonstrate low sensitivity and low precision (compared with immunoassays)

(iii) presence of plasma components, e.g. binding proteins, inhibitors, drug antibodies may alter the activity of the analyte

(iv) bioassays developed for the native protein may give deviating results when being used for the corresponding recombinant protein, leading to e.g. erroneous dose calculations

Reference material

Contrary to conventional molecules, a pure reference material that can serve as a calibration standard is either difficult or sometimes impossible to obtain for this class of compounds.

Therefore extreme care should be taken in order to ensure that the reference material used in the different analytical calibration processes is representative of the material used in clinical trials, including clinical pharmacokinetics.

Early in the development process of a new therapeutic protein, in house standards have to be developed and, as knowledge on the biochemical analytical as well as pharmacokinetic properties and purity of the compound increases, the different isolated materials should always be referred to the initial and previous intermediates developed.

Traceability of the different purity or isoforms of the compound, between the marketed product and the material used in clinical trials should always be available.

2.3 Endogenous concentrations

For some administered proteins there are measurable endogenous concentrations that may be pulsatile or produced continuously, exhibit chronotropic variability or are released following specific signals. Knowledge of the concentration time profile of the endogenous component will facilitate the understanding of the exogenous component, since the pharmacodynamic effect will be related to the total concentration. Endogenous concentrations may be systemically present to an extent that the concentration-time profile of the administered exogenous protein is substantially affected. The Applicant should describe and motivate the approach chosen to handle influential endogenous concentration. Possible differences in endogenous time-concentration profiles between healthy volunteers and patients and between sub-populations should be addressed by the Applicant.

3 PHARMACOKINETIC STUDIES

Generally, the requirements for therapeutic proteins with respect to evaluating the pharmacokinetics of the product are the same as for conventional products, but specific considerations are needed related to the inherent characteristics of proteins. The pharmacokinetics (absorption, distribution and elimination) should be characterised during single-dose and steady-state conditions in relevant populations. However, the pharmacokinetic requirements may differ depending on the type of protein.

If part of the pharmacokinetic information is gathered in healthy volunteers, the validity of extrapolation of that information to the target population needs to be addressed. Since elimination for some products is largely dependent on target receptor uptake, differences in receptor density between healthy volunteers and target population (e.g. over-expression of receptors in tumours or inflamed tissues) can create important pharmacokinetic difference in e.g. half-life, which should be considered when using healthy volunteer data for predictions to target population.

3.1 Absorption

Appropriate *in vivo* studies should be conducted in healthy volunteers or patients to describe the absorption characteristics of the compound, i.e. the extent and rate of absorption, unless the intravenous route is exclusively used. Single-dose studies are generally sufficient to characterise absorption and to e.g. compare different administration routes.

The majority of approved products are administered parenterally through IV, SC or IM administration. Following SC administration, the drug passes through the lymphatic system, which usually results in pre-systemic elimination and consequently a bioavailability of less than 100% is obtained. The recovery in lymph is correlated to MW (molecular weight). Small proteins may undergo proteolytic degradation in tissues as a first-pass mechanism. The bioavailability might differ between

administration sites e.g. thigh, abdomen, and relative bioavailability with respect to each administration site should be determined in clinical studies. Other factors that might be considered in relation to their effect on the bioavailability are depth of the injection, concentration of the solution for injection and volume of the injection.

Changes in formulation or in the manufacturing process of the drug substance may alter the pharmacokinetics and the immunogenicity of a compound (Section 3.4). Sometimes, physico-chemical and *in vitro* biological analyses of the original and the modified version are not sufficient to exclude an impact on safety and efficacy thorough information about the pharmacokinetics and the relationship between concentrations and efficacy and safety (PK/PD) might in some situations reduce the need for clinical studies.

Changes in the route of administration may alter the pharmacokinetics and the immunogenicity of a compound (Section 3.4). Alternative routes proposed for delivery of proteins are e.g. nasal and pulmonary administration, bypassing the interstitial subcutaneous or intramuscular environment. Oral delivery of proteins for systemic effects is still rare due to low bioavailability.

3.2 Disposition

The main elimination pathway should be identified, which for therapeutic proteins could be predicted, to a large extent, from the molecular size. Catabolism of proteins occurs, usually, by proteolysis. Small proteins of MW<50 000 D are eliminated through renal filtration (renal filtration becomes increasingly important the lower the molecular weight) followed by tubular re-absorption and subsequent metabolic catabolism. For larger protein molecules, hepatic elimination through receptor mediated endocytosis followed by catabolism is more important relative to renal filtration. Also, receptor mediated uptake followed by elimination in other tissues and/or in target cells contributes to the elimination e.g. G-CSF. For some proteins (e.g. recombinant tissue-type and urokinase-type plasminogen activator), receptor mediated uptake by the hepatocytes is extensive resulting in perfusion-limited elimination.

Mass-balance studies are not useful for determining the excretion pattern of the drug and drug-related material. Excreted proteins are not necessarily recovered in urine or faeces as intact substance, but are instead metabolised and reabsorbed as amino acids and incorporated in the general protein synthesis.

Pre-clinical studies on metabolism of the product should focus on those proteins for which the metabolism is likely to differ from the normal protein catabolism. The potential for hepatic microsomal metabolism should be considered especially for smaller proteins. If microsomal metabolism has been demonstrated, identification of metabolites *in vitro,* in e.g. human hepatocytes, should be evaluated.

Metabolites that have pharmacodynamic activity should preferably be measured, e.g. through chromatographic separation, collection and further *in vivo* bioassay quantification. The metabolites may have different pharmacokinetic profile compared with the parent compound. Also, measurement of complexes between the protein and other components present in plasma should be considered (Section 2.2). For some protein drugs, the activity is not only related to the unbound fraction in plasma but also to bound fraction. Thus, when interpreting the data it is important to understand what fraction is detected in the bioanalysis. Bioassays should be considered, especially if selective immunoassays for metabolites are lacking. Lack of relevant methods should be justified.

There is an inverse correlation between steady state volume of distribution (Vss) and MW. A comparable relationship is seen also for permeability and MW. For larger proteins, Vss is similar to the distribution of albumin (approximately 0.1L/kg). Unlike conventional molecules, distribution to tissues (i.e. cellular uptake) is often part of the elimination process and not part of the distribution process and thus contributing to the small distribution volumes. Thus, a small Vss should not necessarily be interpreted as low tissue penetration and adequate concentrations may be reached in a single target organ due to receptor mediated uptake.

3.2.1 Dose-and time dependency

The dose-concentration relationship may be non-proportional, depending on the relative impact of capacity-limited barriers to distribution and elimination involved, for a particular product. For example, a saturable elimination pathway may be dominating at a lower dose range, as has been

shown for some antibodies. The dose-proportionality should be evaluated in single- or multiple dose studies and the clinical consequences discussed.

Time-dependent changes in pharmacokinetic parameters may occur during multiple-dose treatment, e.g. due to down- or up-regulation of receptors responsible for (part of) elimination of the compound or formation of anti-drug antibodies (Section 3.4). Apparent time-dependency may originate from metabolites that are immunologically active but are slowly accumulating due to long half-lives. It is recommended that the pharmacokinetics is determined at several dose levels and at several occasions during long-term studies.

3.2.2 Binding to blood components

Soluble receptors, e.g. shed antigens, may bind to the protein resulting in altered pharmacokinetics through changed clearance or volume. Binding to soluble receptors may increase the inter-subject variability in pharmacokinetic parameters due to differences in circulating receptor levels between individuals. Altered levels of the soluble receptor over time may also result in time-dependent pharmacokinetics. Using appropriate methods, soluble receptors may be measured before treatment and during treatment, differentiating between free and bound receptors. The effect on the pharmacokinetics should be evaluated and the clinical relevance discussed.

The binding capacity to plasma proteins (albumin, α-acid glycoprotein) should be studied when considered relevant. Other specific binding proteins may influence the pharmacokinetics of several proteins as exemplified by growth hormone (GH) bound to GH binding proteins and insulin like growth factor (IGF-I) bound in plasma to carrier proteins. Binding proteins may also cause difficulties when quantifying the drug substance in blood or plasma (see Section 2.2).

3.2.3 Chemical modification of proteins

Chemical modification of a protein structure has been used intentionally to alter the pharmacokinetic profile of the protein, usually to prolong the half-life e.g. through pegylation, sometimes resulting in several isoforms exhibiting different pharmacokinetic and pharmacodynamic characteristics. Likewise, changes in a manufacturing process that modify the glycosylation pattern and/or sialic acid content have the potential to change the pharmacokinetics and/or dynamics of the product. Due to different pharmacokinetic behaviour (e.g. some isoforms being eliminated faster than others), the relative concentrations of isoforms within an individual might change over time. The activity of any isoforms that can be identified should be explored *in vitro* and, if large differences in activity is suspected, the pharmacokinetic profile in man should be described for each isoform, if possible. A combination of immunoassay and bioassay is recommended.

3.2.4 Variability

The inter-subject variability should be estimated and if possible the important sources of the variability identified e.g. demographic factors as weight and age. Based on the results individualised dosing should be considered if necessary from safety and efficacy perspectives. Potential sources of inter-subject variability specific to therapeutic proteins are formation of antibodies, absorption variability (e.g. differences in site of injection), variable levels of binding components in blood, variability in target burden (e.g. tumour load), variability in degradation rate (e.g. of de-pegylation) or in degradation pattern.

The variability within an individual should be quantified. For products intended for multiple-dose administration, knowledge about the variability between occasions is valuable especially for products for which titration is recommended. The, sometimes, low precision of e.g. the bioassays are likely to contribute to large estimates of intra-individual variability, if the latter is confounded with bioassay precision.

3.2.5 Sub-populations

The clinical development program should involve studies to support the approval in sub-populations as in patients with organ dysfunction. Whether a study is necessary or not should be based on the elimination characteristics of the compound. If no study is conducted this should be justified by the Applicant. An understanding of the influence of intrinsic factors, such as age and body weight should be provided. Such information might arise from conventional studies in a specific population or from population pharmacokinetic analysis of Phase II/III data.

Renal impairment: For proteins with MW lower than 50 000 D, renal excretion is of importance for the elimination (increasing importance with lower MW) and consequently for the half-life of the protein. Thus, for these products pharmacokinetic studies in patients with renal impairment are recommended (see CPMP/EWP/225/02).. It is advantageous if both immunoassay and bioassay are used. If the activity is generated by several species (e.g. metabolites, isoforms), each with different activity, their relative content might change with the degree of renal function due to different renal clearance (CL). With similar affinity to the immunoassay, a bioassay would help in a more relevant interpretation of the data.

Hepatic impairment: Reduced hepatic function may decrease the elimination of a protein for which hepatic degradation is an important elimination pathway. The lack of studies should be justified by the Applicant (see guideline CPMP/EWP/2339/02).

3.2.6 Interaction studies

The requirements for *in vivo* drug-drug interaction studies with respect to CYP-enzymes are generally lower than for conventional products. However, some therapeutic proteins (e.g. immunomodulators such as cytokines….) have shown a potential for inhibiting or inducing CYP-enzymes and thus *in vitro* or *in vivo* studies may be considered on a case by case basis. It should be recognised that an interaction might be time-dependent due to up – or down regulation of enzymes e.g. α-interferons and CYP1A2, and thus requiring a multiple-dose *in vivo* design to appropriately quantify the interaction.

Interaction studies should be considered when the protein induces changes to elimination pathways (receptors) also involved in the elimination of other drugs or when suppression of the immunological system is likely. An example of the latter is methotrexate, significantly decreasing the clearance of co-administered antibodies. The Applicant should also consider the possible interaction with endogenous proteins.

Since elimination of proteins usually involves capacity-limited steps like drug-receptor binding (e.g. transport proteins) inhibitions or induction of these proteins might cause altered pharmacokinetics. However, today we lack knowledge about suitable tools to explore such interactions. Development within this area is encouraged.

3.2.7 Data analysis

As for conventional products the pharmacokinetics may be analysed through compartment- or non-compartment methods. Mean (median) and individual results should be submitted. Population pharmacokinetic analysis of Phase II/III using a sparse sample approach is recommended for characterising the pharmacokinetics and possible explanatory covariate relationships.

3.3 PK/PD-relationship

It is recommended that the relationship between drug concentration and pharmacodynamic response (PK/PD) is evaluated. If possible, established surrogate markers for both efficacy and safety should be measured preferably in the same study. Given that the pharmacodynamic response as well as the pharmacokinetics may be altered due to modifications of the molecule or the expression system for its production, binding to blood components or formation of anti-drug antibodies, evaluation of the exposure-response relationship is considered an important tool in the drug development. Early pre-clinical and clinical data can be evaluated using appropriate models for a mechanistic understanding of the disease and the PK/PD relationship. Models may be developed accounting for the time-delay between plasma concentrations and measured effect. Such models may allow extrapolation from volunteers to target population given that suitable assumptions have been made, e.g. regarding pathological factors, and provide guidance for dose selection. The models are helpful when interpreting changes in the pharmacokinetics in important sub-populations (Section 3.2.6) or for comparability reasons (Section 2.2). Effort to explore relevant biomarkers and their link (surrogacy) to safety and efficacy endpoints is encouraged.

3.4 Immunogenicity

For many proteins and peptides, a number of patients develop clinically relevant anti-drug antibodies. The immune response against therapeutic proteins differs between compounds and the immunogenic potential (neoantigenicity) is influenced by many factors, such as the expression system in which the protein is produced, the purification system or its final formulation, although many others are still

unknown and unpredictable. In general, the antibody response in man cannot be predicted from animal studies. The immune response may depend on the dose and route of administration (SC more immunogenic than IV). Considerable heterogeneity in antibody response may be observed since an individual may form multiple antibodies with different affinities, epitopes and binding capacities. Thus, data should be collected from a sufficient number of patients to characterise the variability in antibody response.

Since anti-drug antibodies may alter the pharmacokinetics and pharmacodynamics of a protein, testing for antibody response is always necessary when developing a new protein. It is especially important for new drugs intended for multiple-dose or long-term treatment. The timing of sampling for antibody response should be carefully evaluated and justified. For example, a sufficient interval between the last dose and the time-point for antibody detection is crucial, since the drug molecule needs to be eliminated from the circulation otherwise interference with the antibody-assay is likely (Section 2.2). Thus, samples should be collected when drug concentration is low enough not to interfere with the analysis, i.e. after 6-7 half-lives, and when anti-drug antibodies have developed. When measuring antibodies during drug treatment any possible analytical interference should be investigated and discussed (Section 2.2). Information on antibody formation should preferably be gathered already in Phase I/II (Phase II likely to have longer exposure time) to guide planning of Phase III.

Although the pharmacodynamic effect is directly altered only by neutralising antibodies, the pharmacokinetics may be affected irrespective of the neutralising capacity. Thus, altered effect due to anti-drug antibody formation might be a composite of both pharmacokinetic and pharmacological changes and likewise non-neutralising antibodies might induce effect changes if the pharmacokinetics is altered to a significant extent. Antibody formation can cause increased or decreased (CL) of the therapeutic protein, although the former effect is the most common.

In case of a relevant antibody response to the drug, the effect of anti-drug antibodies on the pharmacokinetics of a protein should be studied unless justified by the Applicant. Due to variability between individuals, it is important that samples are collected within the same subjects pre- and post dosing. Pharmacokinetic sampling in Phase III studies is important in the assessment of anti-drug antibody effects due to the generally prolonged exposure of the drug and the increased number of patients in the study. As a minimum, plasma samples for pharmacokinetic analysis should be collected after the first and last dose to compare the plasma concentrations and degree of accumulation in antibody positive and negative subjects. Special consideration should be given to patients withdrawing from a trial. The onset and degree of the antibody response should be correlated to the drug exposure or relevant pharmacokinetic parameters. If possible the antibody production over time should be evaluated and retention of plasma samples should be considered.

Needless to say, the overriding question to address is the impact of antibodies on the efficacy and/or safety of the drug. This includes how to treat patients with a decreasing response to the drug due to antibodies as well as the safety and efficacy of repeated treatment after a significant period of ”drug holiday”. As outlined above, adequate pharmacokinetic data are of value to address these issues.

3.5 Comparability

Comparability between biotechnological products is thoroughly discussed in the draft CHMP “Guideline on similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Non-clinical and Clinical Issues” (EMEA/CHMP/42832/2005).

Demonstration of comparability between two products is most often a step-wise procedure where pharmacokinetic data are an important part. The design of the pharmacokinetic study should be based on the question to be addressed by pharmacokinetic data. Often comparative pharmacokinetics is needed to establish equivalence between two products. Since not only similarity in terms of absorption/bioavailability is of interest, the standard bioequivalence design may not be optimal. In fact, the risk of differences in elimination rate may be more likely, requiring the demonstration of equivalence on clearance and/or half-life.

The choice of single dose design, steady-state studies, or repeated determination of PK parameters with a treatment period in between should be justified by the applicant. The ordinary cross-over design is not appropriate for therapeutic proteins with a long half-life, e.g. therapeutic antibodies and

pegylated proteins, or for proteins for which formation of anti-drug antibodies is likely. In the parallel design, effort should be made to reduce the risk for potential imbalance between the groups.

The acceptance range to conclude equivalence with respect to any pharmacokinetic parameter should be based on a clinical judgement, taking all available efficacy and safety information on the reference and test products into consideration. Hence, the criteria used in standard bioequivalence studies may not be appropriate and the equivalence limits should be defined and justified prior to conducting the study.

It is important to note that for proteins, not only the pharmacokinetics but also the concentration-response relationship may differ between products. Hence, adequate PK/PD data may be used as additional support to clinical data.