WHO Guidelines on the Quality, Safety, and Efficacy of Biological Medicinal Products Prepared by Recombinant DNA Technology

Proposed guidelines

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed WHO Guidelines on the Quality, Safety, and Efficacy of Biological Medicinal Products Prepared by Recombinant DNA Technology to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received by 19 April 2013 in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Quality Safety and Standards (QSS). Comments may also be submitted electronically to the Responsible Officer: Dr Hye-Na Kang at email: kangh@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide" (WHO/IMD/PUB/04.1).

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Recommendations and guidelines published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If a NRA so desires, these Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Guidelines made only on condition that modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the recommendations set out below.
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Introduction

These guidelines are intended to provide national regulatory authorities and manufacturers with guidance on the quality, safety and efficacy of biological medicinal products prepared by recombinant DNA technology (rDNA-derived BMP) and intended for use in humans. They are based on experience gained over the past 25 years or so in this technically demanding field and replace “Guidelines for assuring the quality of pharmaceutical and biological products prepared by recombinant DNA technology” (1).

Part A sets out updated guidelines for the manufacture and quality control of rDNA-derived BMP, including consideration of the effects of manufacturing changes and of devices used in delivery on the product and its stability. Part B is new and provides guidelines on nonclinical evaluation: Part C, also new, provides guidance on clinical evaluation.

Background

Developments in molecular genetics and nucleic acid chemistry have enabled genes encoding natural biologically active proteins to be identified, modified and transferred from one organism to another so as to obtain highly efficient synthesis of their products. This has led to the production of new biological medicines using a range of different cells such as bacteria, yeast, transformed cell lines of mammalian origin, insect and plant cells, as well as transgenic animals. Chimeric or humanized monoclonal antibodies, or fully human monoclonal antibodies, can also be produced using rDNA technology.

There has also been enormous progress in the ability to purify and characterize biologically active macromolecules. Separation and analytical technologies have improved tremendously since the early days of biotechnology and many biological macromolecules can now be characterized in exquisite detail using a range of analytical techniques for protein, lipid and polysaccharide components.
Together these technologies have enabled the production of large quantities of medicinal products that are difficult to prepare from natural sources or were previously unavailable. Nevertheless, it is still not possible to fully predict biological properties and clinical performance of these macromolecules from physicochemical characteristics alone. In addition, the production processes are biological systems which are known to be inherently variable, a feature which has important consequences for the safety and efficacy of the resulting product. A pre-requisite, therefore, for introducing such biologicals into the clinic is to ensure consistency of quality from lot to lot and for this purpose in-process laboratory based controls are developed. Production consistency is critical since it is known that slight changes can occasionally lead to major adverse effects, such as immunogenicity, with serious safety implications.

As with many other new technologies, a new set of safety issues for consideration both by industry and national regulatory authorities (NRAs) was generated by these particular biotechnologies. Potential safety concerns arose from the novel processes used in manufacture, from product and process related impurities and from the complex structural and biological properties of the products themselves. Factors that have received particular attention include the possible presence of contaminating oncogenic host cell DNA in products derived from transformed mammalian cells (2), and the presence of adventitious viruses (2). Since the nature and production of these products are highly sophisticated, they require similar sophisticated laboratory techniques to ensure their proper standardization and control. Although comprehensive characterization of the drug product is expected, considerable emphasis must also be given to in-process control. Adequate control measures relating to the starting materials and manufacturing process are, therefore, as important as analysis of the drug product. Thus data on the quality and purity of cell cultures and on the effectiveness of purification and test methods are required for licensing.

At a very early stage in the development of rDNA-derived medicines, the European Medicines Agency and the US Food and Drug Administration produced guidelines and points to consider, respectively, for the development and evaluation of these new
products (3, 4). Such guidelines, based as they were on long experience with traditional biologicals, set the scene for regulatory expectations both for clinical trials and for licensing. At the global level, the WHO produced a series of guidance documents on the quality, safety and efficacy of rDNA-derived products, including specific guidance for products such as interferons and monoclonal antibodies (1, 5-7). These regulatory concepts have been instrumental in establishing the quality, safety and efficacy of rDNA-derived BMP which now play a major role in today’s medical practice.

As patents and data protection measures on biotechnology products have expired, or neared expiration, considerable attention has turned to producing copies of the innovator products with the view to making more affordable products which may improve global access to these medicines. Since by definition it is not possible to produce identical biologicals, the normal method of licensing generic medicines, which relies primarily on bioequivalence data, is not appropriate for licensing non-innovative biotherapeutics and the term similar biological product, or biosimilar product, came into existence (8, 9). The concept of similar biological medicinal products was introduced first by the European Medicines Agency (8) and subsequently by other national regulatory authorities (although the actual term used has varied slightly from agency to agency). WHO guidelines on the evaluation of similar biotherapeutic products were produced in 2010 (9), and provided a set of globally acceptable principles regarding the regulatory evaluation of biosimilars, although it was recognized that they will not by themselves resolve all issues. During international consultations on the development of the biosimilar guidelines and also their implementation, it became clear that there was a need to update WHO guidance on the quality, safety and efficacy of rDNA-derived medicines and biotechnology products in general (10). In 2010, the International Conference of Drug Regulatory Authorities noted that WHO should supplement its guidance on the evaluation of similar biotherapeutic products by providing up-to-date guidelines for the evaluation of biotherapeutic products in general.

The present guidelines have been developed through international consultation and are intended as a replacement of those in Annex 3, TRS No 814, 1991. They are considered
to be a replacement and not a revision of those guidelines because they contain new
sections on nonclinical and clinical evaluation of rDNA-derived BMP which were
lacking in the original document. In addition, a section on issues related to manufacturing
changes both during development and once the product is on the market has also been
introduced since considerable improvements to the production process and to the product
itself can take place during the later stages of development and post licensure, especially
in the immediate post licensing years. These changes can unintentionally impact the
clinical performance of the product and need to be handled carefully from a regulatory
perspective.

Scope

These guidelines apply, in principle, to all biologically active protein products used in the
prevention, treatment or diagnosis of human diseases and which are prepared by rDNA
technology. They set out regulatory expectations both for clinical trials and for licensing,
as well as for changes in products already on the market. Included are biotherapeutics,
recombinant blood products, enzymes, diagnostic agents (e.g. for monoclonal antibody
products including in vivo diagnosis and ex vivo treatment, but excluding in vitro
diagnosis) and vaccines. Also included are all non-innovative products (11-13), whether
claimed to be biosimilar or not and being considered for licensing by the head-to-head
comparison of “biosimilar approach” or by a “stand alone approach”, as well as products
which have been intentionally modified e.g. by pegylation (13). In some cases highly
detailed product specific WHO guidelines and recommendations are available, for
example in the case of vaccines such as yeast derived hepatitis B vaccine or malaria
vaccine produced by rDNA technology, and these should be consulted. They can be
found in the WHO Technical Report Series over a number of years
(http://www.who.int/biologicals/vaccines/en/).
The present guidelines are not intended to apply to genetically modified live organisms
designed to be used directly in humans, such as recombinant viral vectors (14) or live
attenuated vaccines, nor to gene transfer products. A WHO guideline is available on
DNA vaccines for therapeutic as well as prophylactic use (15). Products produced in
transgenic animals are also excluded.
Glossary

The definitions given below apply to the terms used in this document. They may have different meaning in other contexts.

Acceptance criteria
Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the drug substance or drug product or materials at other stages of their manufacture should meet.

Biosimilar approach
The aim of the biosimilar approach is to demonstrate similarity of all characteristics of the similar biotherapeutic product (also called ‘biosimilar’, ‘subsequent entry biologics’) to a chosen reference product in terms of quality, safety and efficacy through head-to-head comparability exercise, as described in the WHO Guidelines (9). Any differences between biosimilar candidate and its reference product should be proven to be of no clinical relevance; however, extrapolation of clinical indications may only be acceptable if scientifically justified.

Biomarkers
A biomarker is defined as a laboratory measurement that reflects the activity of a disease process, correlates (either directly or inversely) with disease progression, and may also be an indicator of a therapeutic response. A genomic biomarker is a measurable DNA and/or RNA marker that measures the expression, function or regulation of a gene.

Biological medicinal product (BMP)
Biological medicinal product is a synonym for biological product described in WHO Technical Report Series. The definition of a medicinal substance, used in treatment, prevention or diagnosis, as a "biological" has been variously based on criteria related to its source, its amenability to characterization by physicochemical means alone, the requirement for biological assays, or on arbitrary systems of classification applied by
regulatory authorities. For the purposes of WHO, including the present document, the list
of substances considered to be biologicals is derived from their earlier definition as
"substances which cannot be fully characterized by physicochemical means alone, and
which therefore require the use of some form of bioassay". However, developments in the
utility and applicability of physicochemical analytical methods, improved control of
biological and biotechnology-based production methods, and an increased applicability of
chemical synthesis to larger molecules, have made it effectively impossible to base a
definition of a biological on any single criterion related to methods of analysis, source or
method of production. Nevertheless, many biologicals are produced using in vitro culture
systems.

Comparability exercise
The activities, including study design, conduct of studies, and evaluation of data, that are
designed to investigate whether the products are comparable.

Critical quality attribute
A physical, chemical, biological or microbiological property or characteristic that is
selected for its ability to help indicate the quality of the product. It should be within an
appropriate limit, range, or distribution to ensure the desired product quality.

Drug product
A pharmaceutical product type that contains a drug substance, generally in association
with excipients.

Drug substance
The active pharmaceutical ingredient and associated molecules that may be subsequently
formulated, with excipients, to product the drug product. It may be composed of the
desired product, products-related substances, and product- and process-related impurities.
It may also contain other component such as buffers.

Good clinical practice (GCP)
An international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible.

**Good manufacturing practice (GMP)**
That part of the pharmaceutical quality assurance process which ensures that products are consistently produced and to meet to the quality standards appropriate to their intended use and as required by the marketing authorization. In these guidelines, GMP refers to the current GMP guidelines published by WHO.

**Good laboratory practice (GLP)**
A quality system concerned with the organizational process and conditions under which nonclinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

**Immunogenicity**
The ability of a substance to trigger an immune response or reaction (e.g. development of specific antibodies, T cell response, allergic or anaphylactic reaction).

**Impurity**
Any component present in the drug substance or drug product that is not the desired product, a product-related substance, or excipient including buffer components. It may be either process- or product-related.

**in-silico modeling**
A computer-simulated model.

**in-process control**
Checks performed during production in order to monitor and, if necessary, to adjust the process to ensure that the product conforms to its specifications. The control of the environment or equipment may also be regarded as a part of in-process control.

**Manufacturer’s working cell bank (WCB)**

A quantity of cells of uniform composition, derived from one or more containers of the master cell bank and stored frozen in the vapour phase above liquid nitrogen in aliquots, one or more of which are used for production purposes.

**Master cell bank (MCB)**

Homogeneous cell suspension derived from the original cell line. It is stored frozen in the vapour phase above liquid nitrogen in aliquots of uniform composition, one or more of which are used for the production of the manufacturer’s working cell bank.

**Non-human primates (NHPs)**

Primates used as models for the study of the effects of drugs in humans, prior to clinical studies.

**Non-innovative product licensed by a “stand alone approach**

Biological medicinal product developed on its own, likely designed for abbreviated application, and not directly compared and analysed in a head to head comparability study against a licensed reference product. It may or may not have been compared clinically, however, extrapolation of clinical indications are not acceptable in any case, since clinical comparison alone usually is not sensitive enough to pick up differences of potential relevance.

**P450 (CYP) enzymes**

Indicates the family of metabolising enzymes which is the most common group.

**Pharmacodynamics (PD)**
The study of the biochemical and physiological effects of drugs on the body and the mechanisms of drug action and the relationship between drug concentration and effect. One dominant example is drug-receptor interactions. PD is often summarized as the study of what a drug does to the body, as opposed to pharmacokinetics which is the study of what the body does to a drug.

**Pharmacogenomics**
The study of the pharmacologic correlation between drug response and variations in DNA and RNA, has become of increasing importance for drug development. Such variations can have effects on the risk of developing adverse drug reactions as well as on the response to treatment; variations in drug pharmacokinetics and metabolic pathways can cause higher drug concentrations in some patients resulting in increased drug toxicity, and/or lower drug concentrations in some patients resulting in decreased drug effects.

**Pharmacokinetics (PK)**
A branch of pharmacology dedicated to the determination of the fate of substances administered to a living organism. This includes the determination of the fate of pharmaceutical agents, hormones, nutrients, and toxins. PK includes the study of the mechanisms of absorption and distribution of an administered drug, the chemical changes of the substance in the body (e.g. by metabolic enzymes such as CYP or UGT enzymes), and the effects and routes of excretion of the metabolites of the drug. PK is often studied in conjunction with PD the drug's pharmacological effect on the body.

**Pharmacovigilance (PhV)**
The activities that are carried out after a medicinal product is marketed to observe and manage in a continuous manner the safety and the efficacy of the products.

**QT/QTc (TQT)**
One of the electrocardiogram (ECG) intervals is all that can be done. A change in the QT/QTc interval indicates arrhythmogenic potential for the drug/biological.
rDNA-derived BMP
Biological medicinal products prepared by recombinant DNA technology. All biologically active protein products used in the prevention, treatment or diagnosis of human diseases and which are prepared by rDNA technology and included in recombinant protein biotherapeutics, recombinant blood products, enzymes, diagnostic agents (e.g. for monoclonal antibody products) and vaccines.

Risk management plan (RMP)
The activities that will, in a continuous manner ensure that patients continue to be safe and benefit from a medicinal ingredient. These plans include PhV plans amongst many other elements.

Source material/starting material
Any substance of a defined quality used in the production of a vaccine product, but excluding packaging materials.

Specification
A list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities.

Stand alone approach
A full licensing application contains a complete data package to support quality, safety and efficacy of a new product. There is no reliance on data from clinical use of an already licensed product for supporting the licensure. Own data will be generated and submitted for licensing. This approach does not necessarily require a comparison with an already licensed and used product of that class.
Part A. Manufacturing and quality control

A.1 Definitions

A.1.1 International name and proper name
Where an International Non-Proprietary Name (INN) for a rDNA derived product is available, it should be used (16). The proper name should be the equivalent of the INN in the language of the country of origin.

A.1.2 Descriptive definition
The description of the rDNA derived product should indicate the biological system in which it is produced (e.g. bacterial, fungal or mammalian cells) as well as whether it is a liquid or freeze dried preparation.

A.1.3 International standards and reference materials
International standards and reference preparations have been established for a wide range of biologicals prepared by rDNA technology. They are used to calibrate bioassays either directly or for calibration of secondary standards or manufacturers working standards. A list of such materials is available on WHO website (http://www.who.int/entity/bloodproducts/catalogue/AlphMay2011.pdf). Each standard/reference preparation is held by one of the WHO custodian laboratories, e.g. the National Institute for Biological Standards and Control, Potters Bar, United Kingdom.

A.2 General manufacturing guidelines
The present Guidelines cover the following three main areas:

1) Control of starting/source materials, including data both on the host cell and on the source, nature and sequence of the gene used in production.

2) Control of the manufacturing process.

3) Control of the drug substance and the drug product.
In this respect, rDNA-derived products are considered to be like biologicals produced by traditional methods, such as bacterial and viral vaccines, where the quality, safety and efficacy of the product relies heavily on adequate control of the starting/source materials and on the manufacturing process, in addition to control tests on the drug substance and drug products themselves. These guidelines therefore place considerable emphasis on the characterization and testing of cell lines and other materials used in products, in validating the ability of the purification processes to remove unwanted materials, especially possible viral contaminants and residual DNA from continuous mammalian cell lines, as well as in-process controls on the manufacturing process and comprehensive characterization and of the drug substance and the drug product.

Information should therefore be provided to adequately describe the starting/source materials, manufacturing process and in-process controls. The description of the manufacturing process should be provided in the form of a flow diagram and sequential procedural narrative and the in-process controls for each step or stage of the process should be indicated in this description. Also, an explanation should be provided of how batches of the drug substance and drug product are defined (e.g. splitting and pooling of harvests or intermediates). Details of batch size or scale and batch numbering should also be included.

The general recommendations for manufacturing establishments contained in the WHO Good manufacturing practices: main principles for pharmaceuticals preparations (17) and the Good manufacturing practices for biological products (18) as well as those in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (2) should apply to establishments manufacturing rDNA-derived BMP.

A.3 Control of starting/source materials

A.3.1 Expression vector and host cell

A description of the host cell, its source and history, and of the expression vector used in production, including source and history, should be given. This should include details of
the origin and identity of the gene being cloned as well as the construction, genetics and
structure of the expression vector. An explanation of the source and function of the
component parts of the vector, such as the origins of replication, promoters, or antibiotic
markers, should be provided as well as a restriction-enzyme map indicating at least those
sites used in construction.

Methods used to amplify the expression constructs, transform expression constructs into
host cells, and criteria used to select the cell clone for production should be fully
described. The site of the vector within the cell, whether integrated or extrachromosomal,
and copy number, should be provided. A host cell containing an expression vector should
be cloned and used to establish a MCB and the correct identity of the vector construct in
the cell bank should be established. The genetic stability of the host-vector combination
should be documented (see below).

The nucleotide sequence of the cloned gene insert and of the flanking control regions of
the expression vector should be indicated and all relevant expressed sequences clearly
delineated.

Measures to promote and control the expression of the cloned gene in the host cell during
production should be described in detail.

A.3.2 Cell bank system

Typically, rDNA-derived BMPs are produced using a cell bank system which involves a
manufacturer’s WCB derived from a MCB.

The type of banking system used, the size of the cell bank(s), it’s life expectancy at the
anticipated rate of use, the container (vials, ampoules, or other appropriate vessels) and
closure system used, the methods used for preparation of the cell bank(s) including the
cryoprotectants and media used, and the conditions employed for cryopreservation and
storage should all be documented and described in detail.
Evidence for banked cell stability under defined storage conditions should be provided. Such evidence can be generated during production of material from the banked cells and supported by a programme for monitoring stability indicating attributes over time (e.g. data on cell viability upon thawing, stability of the host-vector expression system in the cell bank). Available data should be clearly documented and the proposed stability monitoring programme described in the marketing application. Evidence for the stability of the host-vector expression in the cell bank under storage as well as under recovery conditions should be provided.

For animal cells and animal derived cell banks, reference should be made to the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (2).

A.3.2.1 Control of cell banks

The characterization and testing of banked cell substrates is a critical component of the control of rDNA-derived BMPs. Cell banks should be tested to confirm the identity, purity, and suitability of the cell substrate for the intended manufacturing use. The MCB should be characterized for relevant phenotypic and genotypic markers which should include the expression of the recombinant protein and/or presence of the expression construct. The testing program chosen for a given cell substrate will vary according to the nature and biological properties of the cells (e.g. growth requirements) and its cultivation history (including use of human-derived or animal-derived biological reagents). The extent of characterization of a cell substrate may influence the type or level of routine testing needed at later stages of manufacturing. Molecular methods or other suitable techniques should be used to analyse the expression construct for copy number, for insertions or deletions, and for the number of integration sites. Requirements for bacterial systems expressing the protein from a plasmid or mammalian epigenetic expression should be distinguished from mammalian cell systems. The nucleic acid sequence should be shown to be identical to that determined for the expression construct and should correspond to that expected for the protein sequence.
Animal cell substrates are subject to contamination with and have the capacity to propagate extraneous, adventitious organisms, such as mycoplasma and viruses. In addition, animal cells contain endogenous agents such as retroviruses that may raise safety concerns. Testing of cell substrates for both endogenous (e.g. retroviruses) and adventitious agents is critical. A strategy for testing cell banks for microbial agents should be developed and in the case of viruses takes into consideration the families of viruses and specific viruses that may be present in the cell substrate. Such testing is described in detail in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (2) and the ICH guidelines Q5A viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (19).

In general, cell substrates contaminated with microbial agents are not suitable for production. However, there are exceptions. For example, the CHO and other rodent cell lines that are widely used for the production of rDNA-derived BMPs express endogenous retroviral particles. In such circumstances, risk mitigating strategies should be implemented. These include removal of such agents and/or their inactivation by physical, enzymic and/or chemical treatment during processing of the rDNA-derived BMP.

In addition, tests of purity and limited tests of identity should be performed once on each WCB. For the WCB, a specification including test methods and acceptance criteria should be established. A protocol for establishing future WCB should be provided. Each new WCB should comply with the established WCB specification.

**A.3.2.2 Cell substrate genetic stability**

The limit of *in vitro* cell age for production use should be based on data derived from production cells expanded under pilot plant scale or commercial scale conditions to the proposed limit of *in vitro* cell age for production use or beyond. Generally, the production cells are obtained by expansion of cells from the WCB.
Specific traits of cells, which may include, for example, morphological characteristics, growth characteristics, biochemical markers, immunological markers, productivity of the desired product, or other relevant genotypic or phenotypic markers may be useful for the assessment of cell substrate stability. Indices such as, for example, oxygen or glucose consumption rates, ammonia or lactate production rates may be useful tests to support the consistency of the cell substrate characteristics.

The maximum permitted generation number or population doubling level for production should be defined and should be based on information concerning the stability of the host cell/vector system up to and/or beyond the level of production. In some cases, multiple harvests from long fermentations could lead to a drift in some quality attributes such as glycosylation, with the appearance of "new" variants with possible impact on quality, safety and efficacy of the product. This would indicate instability of the cell substrate.

A.3.3 Cell culture medium/other materials

Materials used in the manufacture of the drug substance (e.g. raw materials, solvents, reagents, catalysts) should be listed identifying where each material is used in the process. Information on the quality and control of these materials should be provided. Information demonstrating that materials (including biologically-sourced materials, e.g. media components, monoclonal antibodies, enzymes) meet standards appropriate for their intended use (including the clearance or control of adventitious agents) should be provided, as appropriate.

Media and other components should comply with current WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products (20). The latest version of the WHO Guidelines on tissue infectivity distribution in transmissible spongiform encephalopathies (21) should also be consulted. These tables are periodically updated as new data becomes available (e.g. 22).

A.4 Control of the manufacturing process
Adequate design of a process and knowledge of its capability are part of the strategy used to develop a manufacturing process which is controlled and reproducible, yielding a drug substance and drug product that meet specifications. In this respect, limits are justified based on information gained from the entire process from early development through commercial scale production.

In-process controls are performed at critical decision making steps and at other steps where data serve to confirm the appropriate performance of the manufacturing process, and to demonstrate adequate quality during the production of both the drug substance and the drug product. Those process parameters that are found to impact the quality attributes of the drug substance or drug product should be controlled by suitable acceptance limits. Where appropriate, in-process controls may alleviate the need for routine testing of some quality attribute(s) at the level of the drug substance and/or drug product.

### A.4.1 Cell culture

#### A 4.1.1 Production at finite passage

Procedures and materials used both for cell growth and for the induction of the product should be described in detail. For each production run, data on the extent and nature of any microbial contamination of culture vessels should be provided. Acceptable limits for such contamination should be set and the sensitivity of the methods used to detect it indicated. Microbial and fungal contamination should be monitored according to Part A Section 5.2 of *General requirements for the sterility of biological substances* (23) or by methods approved by the NRA.

Data on the consistency of culture conditions and culture growth and on the maintenance of product yield should be presented. Criteria for the rejection of culture lots should be established. The maximum number of cell doublings or passage levels to be permitted during production should be specified, based on information on the stability of the host-cell / vector system on serial subculture up to and beyond the level intended for use in production taking into account the limit of in-vitro cell age.
Host-cell / vector characteristics at the end of production cycles should be monitored to establish consistency, for which purpose information on plasmid copy number or degree of retention of the expression vector within the host cell may be of value, as may restriction enzyme mapping of the vector containing the gene insert. The nucleotide sequence of the insert encoding the rDNA-derived BMP should be determined at least once after a full-scale culture for each MCB. If the vector is present in multiple copies integrated into the host cell genome, confirming the rDNA sequence directly may be difficult. In such cases, alternative approaches to confirming the sequence of insert encoding the rDNA-derived BMP should be considered (e.g. ref).

**A 4.1.2 Continuous culture production**

As recommended above, all procedures and materials used for cell culture and induction of the product should be described in detail. In addition, particular consideration should be given to the procedures used in production control. Monitoring is necessary throughout the life of the culture, although the frequency and type of monitoring required depend on the nature of both the production system and product.

The molecular integrity of the gene being expressed and the phenotypic and gentotypic characteristics of the host cell after long-term cultivation should be established. Evidence should be produced to show that variations in yield or other culture parameters do not exceed-specified limits. The acceptance of harvests for further processing should be clearly linked to the monitoring schedule in use, and a clear definition of “batch” of product for further processing should be established. Criteria for the rejection of harvests or termination of the culture should also be established. Tests for microbial contamination should be performed as appropriate to the harvesting strategy.

The maximum period of continuous culture should be specified, based on information on the stability of the system and consistency of the product during and after this period. In long-term continuous culture, the cell line and product should be fully re-evaluated at intervals determined by information on the stability of the host-vector system and the characteristics of the product.
A.4.2 Purification

The methods used for harvesting, extraction and purification of the product and related in-process controls, including their acceptance criteria, should be described in detail. Special attention should be given to the elimination of viruses, nucleic acid and undesirable antigenic material.

In procedures involving affinity chromatography using biological substances, such as monoclonal antibodies, appropriate measures should be taken to ensure that these substances or any other potential contaminants arising from their use, such as adventitious viruses, do not compromise the safety of the drug product.

The ability of the purification procedure to remove unwanted product-related or host-cell derived proteins, nucleic acid, carbohydrates, viruses and other impurities, including media derived compounds and undesirable chemicals introduced by the purification process itself, should be investigated thoroughly, as should the reproducibility of the process. Particular attention should be given to demonstrating the removal and/or inactivation of possible contaminating viruses and residual DNA from products manufactured using continuous cell lines.

A 4.2.1 Residual DNA from continuous cell lines (rcDNA)

The ability of the manufacturing process to reduce the amount of rcDNA to an acceptable level, to reduce the size of the rcDNA or to chemically inactivate the biological activity of this DNA should be demonstrated.

Acceptable limits of rcDNA in a rDNA-derived BMP are discussed in WHO recommendations for the evaluation of animal cell substrates for the manufacture of biological medicinal products and for the characterization of cell banks (2). These should be set taking into consideration the characteristics of the cell substrate, the intended use and route of administration of the rDNA-derived BMP and, most importantly, the effect of the manufacturing process on the size, quantity and biological activity of the residual
host cell DNA fragments. In general it has been possible to reduce rcDNA levels in
rDNA-derived BMPs to <10 ng per dose and in many cases, to <10 pg per dose.
Quantitative PCR for short amplicons has been used to determine total residual DNA
levels as well as residual DNA fragment side distribution. Where appropriate, validation
of the key rcDNA removal or inactivating steps should be carried out.

A 4.2.2 Virus clearance
Virus clearance or inactivation processes, individually and overall, should be shown to be
able to adequately remove/inactivate any contaminating viruses and to ensure the absence
of detectable infectious agents in the drug product.

Where appropriate, validation studies (see Appendix 1) should be undertaken using small
scale studies with carefully selected model viruses to evaluate the virus
clearance/inactivation capability of selected process steps and overall, aiming at a
significant safety margins. The results will indicate the extent to which these
contaminants can theoretically be removed during purification.

The overall manufacturing process, including the testing and selection of the cells and
source materials, as well as the validation of the ability of the purification process to
adequately remove possible contaminants, should ensure the absence of detectable
infectious agents in the drug product. Nevertheless, to complement such approaches,
testing of the product itself at appropriate steps in the production process for the absence
of contaminating infectious viruses is also recommended. A sample of the unprocessed
bulk following fermentation constitutes one of the most suitable levels at which the
possibility of detecting adventitious virus contamination can be determined with a high
probability of detection. A programme of ongoing assessment of adventitious viruses in
production batches should be undertaken. The scope, extent and frequency of virus
testing on the unprocessed bulk should take into account the nature of the cell lines used,
the results and extent of virus testing performed during the qualification of the MCB and
WCB, the cultivation method, the source materials used and the results of virus clearance
studies. In vitro screening tests using one or more cell lines are generally used to test unprocessed bulk. If appropriate, a PCR test or other suitable methods may also be used.

In contamination by adventitious viruses is detected in the unprocessed bulk, the manufacturing process should be carefully checked to determine the cause of the contamination and to decide on the appropriate action to take.

Further considerations of the detection, elimination and inactivation of viruses in animal cell substrates used in the production of rDNA-derived BMPs, as well as the problem of rcDNA, can be found in the WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (2) as well as in the ICH guidelines Q5A viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (19).

A.5 Control of drug substance and drug product

A.5.1 Characterization

Rigorous characterization of the rDNA-derived BMP by chemical, physicochemical and biological methods is essential. Characterization is typically performed in the development phase to determine the physicochemical properties, biological activity, immunochemical properties, purity and impurities of the product, and following significant process changes and/or for periodic monitoring to confirm the quality of the product. Characterization allows relevant specifications to be established.

Details of the expected characterization of a rDNA-derived BMP are set out in Appendix 2. Particular attention should be given to using a wide range of analytical techniques exploiting different physiochemical properties of the molecule (size, charge, isoelectric point, amino acid composition, hydrophobicity). Post-translational modifications, such as glycosylation should be identified and adequately characterized. It may also be necessary to include suitable tests to establish that the product has the desired conformation and state of aggregation. Techniques suitable for such purposes are
discussed in Appendix 3. The rationale for selection of the methods used for characterization should be provided and their suitability should be justified. Where relevant and possible, the properties of the product should be compared with those of the naturally occurring molecule. Consideration should be given to the possibility that post-translational modifications, such as glycosylation, are likely to be different from those found in the natural counterpart and may influence the biological, pharmacological and immunological properties of the rDNA-derived BMP.

A.5.2 Routine control

It will be apparent that not all the characterization and testing described above in A 5.1 and in Appendix 2, needs to be carried out on each batch of drug substance and drug product prior release on the market. Some tests may need to be performed only initially and/or periodically to establish or verify the validity or acceptability of a product and its manufacturing process. Others may be required on a routine basis. A comprehensive analysis of the initial production batches is expected in order to establish consistency with regard to identity, purity and potency. A more limited series of tests is appropriate for routine control as outlined below and in more detail in Appendix 4. Tests for use in routine control should be chosen to confirm quality rather than to characterize the product. The rationale and justification for including and/or excluding testing for specific quality attributes should be provided.

An acceptable number, usually a minimum of three successive batches, should be fully characterized to determine consistency of analytical parameters. Any differences between one batch and another should be noted. Data obtained from such studies should be used as the basis for establishing product specifications.

The selection of tests to be included in the routine control programme will be product specific and should take into account the quality attributes (e.g. potential influence on safety, efficacy or stability), the process performance (e.g. clearance capability, content), the controls in place through the manufacturing process (e.g. multiple testing points), and the material used in relevant non-clinical and clinical studies. These tests could include
criteria such as potency, the nature and quantity of product-related substances, product-related impurities, process-related impurities, and absence of contaminants.

A.6 Filling and container

The general requirements concerning filling and containers given in the WHO Guidelines on good manufacturing practices for biological products (18) should apply.

A description of the container closure systems for the drug substance and the drug product should be provided including a specification for their component materials. Substantial evidence exists to show that formulated proteins can interact chemically with the formulation excipients and/or the container closure system, and can, for example, lead to the formation of potentially immunogenic complexes. The suitability of the container closure system should be evaluated and described for its intended use. This should cover evaluation of the compatibility of the materials of construction with the formulated product, including adsorption to the container, leaching and other chemical or physical interaction between the product and the contacting materials. The integrity of the closure and its ability to protect the formulation from contamination and maintain sterility needs to be ensured.

When a delivery device is presented as part of the drug product (e.g. prefilled syringe, single use autoinjector), it is important to demonstrate the functionality of such a combination, such as the reproducibility and accuracy of the dispensed dose under testing conditions which should simulate the use of the drug product as closely as possible. For multi-use containers such as vials or cartridges for a pen injector, proper in-use stability studies should be performed to evaluate the impact of the in-use period of the vial or the assembled device on the formulation and the functionality of the pen injector. Dose accuracy should be demonstrated for the first and last dose delivered. In addition, the effect of multiple injections/withdrawals on the closure should be evaluated.

A.7 Records, retained samples, labelling, distribution and transport
The requirements given in the WHO Guidelines on good manufacturing practices for biological products (18) should apply.

The conditions of shipping should be such as to ensure that the products are maintained at the appropriate environment. In the case of vaccines, temperature indicators should be packaged with each vaccine shipment to monitor fluctuations in temperature during transportation. Further guidance is provided in the WHO Model guidance for the storage and transport of time and temperature-sensitive pharmaceutical products (24).

A.8 Stability, storage and expiry date

A.8.1 Stability studies

For proteins, maintenance of biological activity is generally dependent on maintaining molecular conformation. Such products can be particularly sensitive to environmental factors such as temperature changes, oxidation, light exposure, ionic content, and shear stress. In order to ensure maintenance of biological activity and to avoid degradation, appropriate conditions for their storage are usually necessary.

A detailed protocol for the assessment of the stability of both drug substance and drug product in support of the proposed storage conditions and expiration dating periods should be developed. This should include all necessary information which demonstrates the stability of the rDNA-derived BMP throughout the proposed shelf life including, for example, well-defined specifications and test intervals.

Each product should retain its specification within established limits for purity, and potency throughout its proposed shelf-life. Specifications should be derived from all available information using the appropriate statistical methods. There is no single stability-indicating assay or parameter that profiles the stability characteristics of a rDNA-derived BMP. Consequently, the manufacturer should develop a stability-indicating programme that provides assurance that changes in the quality and potency of the product will be detected.
Primary data to support a requested storage period for either drug substance or drug product should be based on long-term, real-time, real-condition stability studies. Also, stability studies should include an evaluation of the impact of the container closure system on the formulated rDNA-derived BMP throughout the shelf life. In order to ensure that the formulated product is in contact with all material of the container closure system, stability studies should include samples maintained in the inverted or horizontal position (i.e. in contact with the closure), as well as in the upright position, to determine the effects of the closure on product quality. Data should be supplied for all different container closure combinations that will be marketed.

Stability information should be provided on at least 3 batches for which manufacture and storage are representative of the commercial scale and process.

When shelf-lives of 1 year or less are proposed, real-time stability studies should be conducted monthly for the first 3 months and at 3 month intervals thereafter. For products with proposed shelf-lives of greater than 1 year, the studies should be conducted every 3 months during the first year of storage, every 6 months during the second year, and annually thereafter. A minimum of 6 months data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested, unless otherwise justified. For storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis.

It is recommended that stability studies under accelerated and stress conditions, including the impact of the container closure system (see A.6), should also be conducted on the drug substance and drug product. Studies under accelerated conditions may provide useful supportive data for establishing the expiry date, provide product stability information for future product development (e.g. preliminary assessment of proposed manufacturing changes such as changes in formulation or scale-up), assist in validation of analytical methods for the stability program, or generate information which may help elucidate the degradation profile of the rDNA-derive BMP. Studies under stress conditions may also be useful in determining whether accidental exposures to conditions
other than those proposed (e.g. during transportation) are deleterious to the product and for evaluating which specific test parameters may be the best indicators of product stability.

Further guidance on both general and specific aspects of stability testing of a rDNA-derived BMP can be obtained by consulting the WHO guidelines on the stability testing of active pharmaceutical ingredients and finished pharmaceutical products (25), as well as the WHO Guidelines for stability evaluation of vaccines (26).

A.8.2 Drug product requirements

Stability information should be provided on at least 3 batches of drug product representative of that which will be used in commercial manufacture, and presented in the final container. Where possible, the drug product batches included in stability testing should be derived from different batches of drug substance.

Where one product is distributed in multiple presentations, the samples to be entered into the stability program may be selected on the basis of a matrix system and/or by bracketing. Where the same strength and exact container/closure system is used for 3 or more fill contents, the manufacturer may elect to place only the smallest and largest container size into the stability program, i.e. bracketing. The design of a protocol that incorporates bracketing assumes that the stability of the intermediate condition samples is represented by those at the extremes. In certain cases, data may be needed to demonstrate that all samples are properly represented by data collected for the extremes. Matrixing, i.e. the statistical design of a stability study in which account is taken of factors such as the tests, process characteristics, presentation characteristics and different testing time points, should only be applied when appropriate documentation is provided that confirms that the stability of the samples tested represents the stability of all samples. The differences in the samples for the same drug product should be identified as, for example, covering different batches, different strengths, different sizes of the same closure and possibly, in some cases, different container/closure systems. Matrixing should not be applied to samples with differences that may affect stability, such as
different strengths and different containers/closures, where it cannot be confirmed that
the products respond similarly under storage conditions.

For preparations intended for use after reconstitution, dilution or mixing, in-use stability
data should be obtained. The stability should be demonstrated up to and beyond the
storage conditions and the maximum storage period claimed.

In addition to the standard data necessary for a conventional single-use vial, it should be
shown that the closure used with a multiple-dose vial is capable of withstanding the
conditions of repeated insertions and withdrawals so that the product retains its full
potency, purity, and quality for the maximum period specified in the instructions-for-use
on containers, packages, and/or package inserts.

A.9 Manufacturing process changes
Changes to the manufacturing processes of a rDNA-derived BMP often occur both during
development and after approval. The reasons for such changes include improving the
manufacturing process, increasing scale, site change, improving product stability, or
complying with changes in regulatory requirements. When substantial changes are made
to the manufacturing process, a comparability exercise to evaluate the impact of the
modification(s) on the quality, safety and efficacy of the rDNA-derived BMP should be
undertaken. The demonstration of comparability does not necessarily mean that the
quality attributes of the pre-change and post-change product are identical, but that they
are highly similar and that the existing knowledge is sufficiently predictive to ensure that
any differences in quality attributes have no adverse impact upon safety or efficacy of the
rDNA-derived BMP. The reason for each significant change should be explained,
together with an assessment of its potential to impact on quality, safety and efficacy.

The extent of a comparability exercise depends on the potential impact of the process
change(s) on the quality, safety and efficacy of the product. It can range from analytical
testing alone (e.g. where process changes lead to no changes in any quality attribute) to a
comprehensive exercise requiring nonclinical and clinical bridging studies (e.g. the
establishment of a new host cell line with altered properties resulting in more pronounced changes in quality attributes). If assurance of comparability can be shown through analytical studies alone, nonclinical or clinical studies with the post-change product may not be necessary. However, where the relationship between specific quality attributes and safety and efficacy has not been established, and differences between quality attributes of the pre- and post-change product are observed, it might be appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise.

Further considerations of manufacturing changes can be found in guidelines provided by the ICH (27), the EMA (28), the US FDA (29) and other major NRAs.
Part B. Nonclinical evaluation

B.1 Introduction

The general aim of nonclinical evaluation is to determine whether new medicinal products possess the desired PD activity and have the potential to cause unexpected and undesirable effects. However, classical PD, safety or toxicological testing, as recommended for chemical drugs, may be of only limited relevance for rDNA-derived BMPs due to their unique and diverse structural and biological properties including species specificity, immunogenicity, and unpredicted pleiotropic activities. These properties pose particular problems in relation to nonclinical testing in animals, and their pharmacological and safety evaluation will have to take a large number of factors into account. Thus, certain proteins, e.g. interferons, are highly species-specific, so that the human protein is pharmacologically much more active in humans than in any animal species. Furthermore, the amino-acid sequences of human proteins will often differ significantly from those of their natural counterparts in other species, as will the carbohydrate groups of glycoproteins. Thus human proteins frequently produce immunological responses in animal species which may ultimately modify their biological effects and may result in toxicity, e.g. due to immune complex formation. Such toxicity has little bearing on the safety of the product in the intended human host.

For these and other reasons, a flexible approach will be necessary for the nonclinical evaluation of rDNA-derived BMPs. Although some safety testing will be required for most products, the range of tests that need to be carried out should be decided on a case-by-case basis, e.g. Appendix 5, in consultation with the NRA/NCL. A wide range of pharmacological, biochemical, immunological, toxicological and histopathological investigative techniques should be used, where appropriate, in the assessment of a product’s effect, over an appropriate range of doses and, in accordance with the desired clinical indication(s), during both acute and chronic exposure. However, the points made above concerning species-specificity and antibody formation should always be taken into consideration.
Further guidance can be found in for example the ICH guideline *preclinical safety evaluation of biotechnology-derived pharmaceuticals* (30) and other relevant guidelines (e.g. 31). Additional information on specific safety issues, as for example carcinogenic potential or reproductive toxicity, is provided in respective ICH safety guidelines (32, 33). Recommendations concerning timing and interplay of nonclinical and clinical studies in drug development are given in the ICH *Guidance on nonclinical safety studies for the conduct for human clinical trials and marketing authorization for pharmaceuticals* (34).

**B.1.1 Objectives of the nonclinical evaluation**

The objectives of the nonclinical studies are to define pharmacological and toxicological effects throughout clinical development, not only prior to initiation of human studies. The primary goals are to: 1) identify an initial safe dose and subsequent dose escalation schemes in humans; 2) identify potential target organs for toxicity and for the study of whether such toxicity is reversible; and 3) identify safety parameters for clinical monitoring.

Nonclinical evaluation should consider: 1) selection of the relevant animal species; 2) age of animals; 3) physiological state of animals (e.g. whether healthy/diseased animals are used, whether treatment naïve animals are used); 4) weight of animals; 5) the manner of delivery, including dose, route of administration, and treatment regimen; and 6) stability of the test material under the conditions of use. Both *in vitro* and *in vivo* studies can contribute to this characterization.

rDNA-derived BMPs that are structurally and pharmacologically comparable to a product for which there is wide experience in clinical practice may need less extensive nonclinical testing.

**B.1.2 Product development and characterization**

In general, the product that is used in the definitive pharmacology and toxicology studies should be comparable to the product proposed for the initial clinical studies. However, it is appreciated that during the course of development programs, changes normally occur in the manufacturing process in order to improve product quality and yields. The
potential impact of such changes for extrapolation of the animal findings to humans should be considered, including the impact of post-translational modifications.

The comparability of the test material should be demonstrated when a new or modified manufacturing process or other significant changes in the product or formulation are made in an ongoing development program. Comparability can be evaluated on the basis of biochemical and biological characterization (i.e. identity, purity, stability, and potency). In some cases, additional studies may be needed (i.e. PK, PD and/or safety). The scientific rationale for the approach taken should be provided.

B.1.3 Good laboratory practice

Pivotal (toxicity) studies are expected to be performed in compliance with good laboratory practice (GLP). However, it is recognized that some studies employing specialized test systems which are often needed for rDNA-derived BMP may not comply fully with GLP. Areas of non-compliance should be identified and their significance evaluated relative to the overall nonclinical assessment. In some cases, lack of full GLP compliance does not necessarily mean that the data from these studies cannot be used to support clinical trials and marketing authorization.

B.2 Pharmacodynamics

B.2.1 Primary and secondary pharmacodynamics/Biological activity

Biological activity may be evaluated using in vitro assays to determine which effects of the product may be related to clinical activity. The use of cell lines and/or primary cell cultures can be useful to examine the direct effects on cellular phenotype and proliferation. Due to the species specificity of many rDNA-derived BMP, it is important to select relevant animal species for testing (see Appendix 6). In vitro cell lines derived from mammalian cells can be used to predict specific aspects of in vivo activity and to assess quantitatively the relative sensitivity of various species to the BMP, including human. Such studies may be designed to determine, for example, receptor occupancy, receptor affinity, and/or pharmacological effects, and to assist in the selection of an appropriate animal species for further in vivo pharmacology and toxicology studies. The combined results from in vitro and in vivo studies assist in the extrapolation of the
findings to humans. *In vivo* studies to assess pharmacological activity, including defining mechanism(s) of action, are often used to support the rationale for the proposed use of the product in clinical studies. When feasible, *in vivo* pharmacology can be incorporated into general toxicity studies.

**B.2.2 Safety pharmacology**

It is important to investigate the potential for undesirable pharmacological activity in appropriate animal models. The aim of the safety pharmacology studies is to reveal any functional effects on the major physiological systems (e.g. cardiovascular, respiratory, renal, and central nervous systems). These functional indices may be investigated in separate studies or incorporated in the design of toxicity studies and/or clinical studies. Investigations may include the use of isolated organs or other test systems not involving intact animals. All of these studies may allow for a mechanistically-based explanation of specific organ effects/toxicities, which should be considered carefully with respect to their applicability for human use and indication(s).

**B.3 Pharmacokinetics/Toxicokinetics**

**B.3.1 General principles**

It is difficult to establish uniform guidelines for pharmacokinetic studies for rDNA-derived BMP. Single and multiple dose PK, toxicokinetics, and tissue distribution studies in relevant species are useful; however, routine studies that attempt to assess mass balance are not useful. Differences in PK among animal species may have a significant impact on the predictiveness of animal studies or on the assessment of dose response relationships in toxicity studies. Alterations in the PK profile due to immune-mediated clearance mechanisms may affect the kinetic profiles and the interpretation of the toxicity data (see also B.4.8.1). For some products there may also be inherent significant delays in the expression of pharmacodynamic effects relative to the pharmacokinetic profile (e.g. cytokines) or there may be prolonged expression of pharmacodynamic effects relative to plasma levels.

PK studies should, whenever possible, utilize preparations that are representative of that intended for toxicity testing and clinical use, and employ a route of administration that is
relevant to the anticipated clinical studies. Patterns of absorption may be influenced by formulation, active substance concentration, application site, and/or application volume. Whenever possible, systemic exposure should be monitored during the toxicity studies. When feasible, TK/PK evaluations can be incorporated into general toxicity studies. When using radiolabeled proteins, it is important to show that the radiolabeled test material maintains activity and biological properties equivalent to that of the unlabeled material. Some information on absorption, disposition and clearance in relevant animal models should be available prior to clinical studies in order to predict margins of safety based upon exposure and dose. Understanding the behaviour of the biopharmaceutical in the biologic matrix, (e.g. plasma, serum, cerebral spinal fluid) and the possible influence of binding proteins is important for understanding the PD effect.

B.3.2 Assays

The use of one or more assay methods should be addressed on a case-by-case basis and the scientific rationale should be provided. One validated method is usually considered sufficient. For example, quantitation of TCA-precipitable radioactivity following administration of a radiolabeled protein may provide adequate information, but a specific assay for the analyte is preferred. Ideally, the assay methods should be the same for animal and human studies. The possible influence of plasma binding proteins and/or antibodies in plasma/serum on the assay performance should be determined.

B.3.3 Distribution

Tissue cross-reactivity studies

Tissue cross-reactivity (TCR) studies are in vitro tissue-binding assays employing immunohistochemical (IHC) techniques conducted to characterize binding of monoclonal antibodies and related antibody-like products to antigenic determinants in tissues. Other technologies can be employed in place of IHC techniques to demonstrate distribution to target/binding site. A TCR study with a panel of human tissues is a recommended component of the safety assessment package supporting initial clinical dosing of these products. However, in
some cases the clinical candidate is not a good IHC reagent and a TCR study might not be technically feasible. TCR studies can provide useful information to supplement knowledge of target distribution and can provide information on potential unexpected binding. Tissue binding per se does not indicate biological activity \textit{in vivo}. In addition, binding to areas not typically accessible to the active substance \textit{in vivo} (i.e. cytoplasm) is generally not therapeutically relevant. Findings should be evaluated and interpreted in the context of the overall pharmacology and safety assessment data package.

When there is unexpected binding to human tissues, an evaluation of selected animal tissues can provide supplemental information regarding potential correlations or lack thereof, with preclinical toxicity. TCR using a full panel of animal tissues is not recommended. Since a bi-specific antibody product will be evaluated in a TCR study using a panel of human tissues, there is no need to study the individual binding components. Evaluating the tissue binding of homologous products does not provide additional value when TCR studies have been conducted with the clinical candidate in a human tissue panel, and is not recommended. TCR studies are not expected to detect subtle changes in critical quality attributes. Therefore TCR studies are not recommended for assessing comparability of the test article as a result of process changes over the course of a development program.

\textit{Studies with radiolabelled products}

Tissue concentrations of radioactivity and/or autoradiography data using radiolabeled proteins may be difficult to interpret due to rapid \textit{in vivo} protein metabolism or unstable radiolabeled linkage. Care should be taken in interpreting studies using radioactive tracers incorporated into specific amino acids because of recycling of amino acids into non-drug related proteins/peptides.

\textbf{B.3.4 Metabolism}

The expected consequence of metabolism of rDNA-derived BMP is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are
generally understood. Classical biotransformation studies, as performed for pharmaceuticals, are not needed.

B.4 Toxicity studies

B.4.1 General principles

Number/Gender of animals

The number of animals used per dose has a direct bearing on the ability to detect toxicity. A small sample size may lead to failure to observe toxic events due to observed frequency alone regardless of severity. The limitations that are imposed by sample size, as often is the case for non-human primate studies, may be in part compensated by increasing the frequency and duration of monitoring. Both genders should generally be used or justification given for specific omissions.

It is desirable to apply the “3R principles” (i.e. reduction, replacement, refinement) to minimize the use of animals for ethical reasons and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation to reduce the use of animals (35).

Administration/Dose selection and application of PK/PD principles

The route and frequency of administration should be as close as possible to that proposed for clinical use. Consideration should be given to pharmacokinetics and bioavailability of the product in the species being used, and the volume which can be safely and humanely administered to the test animals. For example, the frequency of administration in laboratory animals may be increased compared to the proposed schedule for the human clinical studies in order to compensate for faster clearance rates or low solubility of the active ingredient. In these cases, the level of exposure of the test animal relative to the clinical exposure should be defined. Consideration should also be given to the effects of application volume, active substance concentration, formulation, and site of administration. The use of routes of administration other than those used clinically may be acceptable if the route must be modified due to limited bioavailability, limitations due to the route of administration, or to size/physiology of the used animal species.
If feasible, dosage levels should be selected to provide information on a dose-response relationship, including a toxic dose and a no observed adverse effect level (NOAEL). The toxicity of most rDNA-derived BMP is related to their targeted mechanism of action; therefore, relatively high doses can elicit adverse effects which are apparent as exaggerated pharmacology.

For some classes of products which show little to no toxicity it may not be possible to define a specific maximum dose. In these cases, a scientific justification of the rationale for the dose selection and projected multiples of human exposure should be provided. To justify high dose selection, consideration should be given to the expected pharmacological/physiological effects, availability of suitable test material, and the intended clinical use. Where a product has a lower affinity for, or potency in, the cells of the selected species than in human cells, testing of higher doses may be important. The multiples of the human dose that are needed to determine adequate safety margins may vary with each class of rDNA-derived BMP and its clinical indication(s).

A rationale should be provided for dose selection taking into account the characteristics of the dose-response relationship. PK-PD approaches (e.g. simple exposure-response relationships or more complex modeling and simulation approaches) can assist in high dose selection by identifying (i) a dose which provides the maximum intended pharmacological effect in the selected animal species; and (ii) a dose which provides an approximately 10-fold exposure multiple over the maximum exposure to be achieved in the clinic. The higher of these two doses should be chosen for the high dose group in nonclinical toxicity studies unless there is a justification for using a lower dose (e.g. maximum feasible dose).

Where in vivo/ex vivo PD endpoints are not available, the high dose selection can be based on PK data and available in vitro binding and/or pharmacology data. Corrections for differences in target binding and in vitro pharmacological activity between the nonclinical species and humans should be taken into account to adjust the exposure margin over the highest anticipated clinical exposure. For example, a large relative difference in binding affinity and/or in vitro potency might suggest that testing higher doses in the nonclinical studies is appropriate. In the event that toxicity cannot be
demonstrated at the doses selected using this approach, then additional toxicity studies at higher multiples of human dosing are unlikely to provide additional useful information.

**Duration of studies**

For chronic use products, repeat dose toxicity studies of 6 months duration in rodents or non-rodents are usually considered sufficient, providing the high dose is selected in accordance with the principles above. Studies of longer duration have not generally provided useful information that changed the clinical course of development (see also B.4.3).

For chronic use of rDNA-derived BMP developed for patients with advanced cancer, see Appendix 5.

**Evaluation of immunogenicity**

Many rDNA-derived BMP intended for human use are immunogenic in animals. Therefore, measurement of antibodies associated with administration of these types of products should be performed when conducting repeated dose toxicity studies in order to aid in the interpretation of these studies (for details, see B.4.8.1).

**B.4.2 Single dose toxicity studies**

Single dose studies may generate useful data to describe the relationship of dose to systemic and/or local toxicity. These data can be used to select doses for repeated dose toxicity studies. Information on dose-response relationships may be gathered through the conduct of a single dose toxicity study, as a component of pharmacology or animal model efficacy studies. The incorporation of safety pharmacology parameters in the design of these studies should be considered. In general, single dose toxicity studies should only be pursued in cases where significant toxicity is anticipated and the information is needed to select doses for repeated dose studies.

**B.4.3 Repeated dose toxicity studies**

For consideration of the selection of animal species for repeated dose studies, see B.4.1. The route and dosing regimen (e.g. daily versus intermittent dosing) should reflect the
intended clinical use or exposure. When feasible, these studies should include toxicokinetic measurements, but interpretation should consider the formation of possible anti drug antibodies (see B.4.8.1).

Study duration
The duration of repeated dose studies should be based on the intended duration of clinical exposure and disease indication. Duration of animal dosing has generally been 1-3 months for most rDNA-derived BMP. For rDNA-derived BMP intended for short-term use (e.g. ≤7 days) and for acute life-threatening diseases, repeated dose studies up to two weeks duration have been considered adequate to support clinical studies as well as marketing authorization. For those rDNA-derived BMP intended for chronic indications, studies of 6 months duration have generally been appropriate although in some cases shorter or longer durations have supported marketing authorizations. For rDNA-derived BMP intended for chronic use, the duration of long term toxicity studies should be scientifically justified.

Recovery period
Recovery from pharmacological and toxicological effects with potential adverse clinical impact should be understood when they occur at clinically relevant exposures. This information can be obtained by an understanding that the particular effect observed is generally reversible/nonreversible or by including a non-dosing period in at least one study, at least at one dose level, to be justified by the sponsor. The purpose of the non-dosing period is to examine reversibility of these effects, not to assess delayed toxicity. The demonstration of complete recovery is not considered essential. The addition of a recovery period, for the sole purpose of assessing the potential for immunogenicity, is not required. Since antibody formation to human proteins in animal studies is usually not predictive for the clinical situation, concerns regarding antibody formation to the endogenous hormone, e.g. in case of erythropoietin orsomatropin, will have to be addressed on a clinical safety level.

B.4.4 Genotoxicity studies
The range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable to rDNA-derived BMP and therefore are not needed. Moreover, the administration of large quantities of peptides/proteins may yield uninterpretable results. It is not expected that these substances would interact directly with DNA or other chromosomal material.

With some rDNA-derived BMP there is a potential concern about accumulation of spontaneously mutated cells (e.g. via facilitating a selective advantage of proliferation) leading to carcinogenicity. The standard battery of genotoxicity tests is not designed to detect these conditions. Alternative in vitro or in vivo models to address such concerns may have to be developed and evaluated (see B.4.5).

Studies in available and relevant systems, including newly developed systems, should be performed in those cases where there is cause for concern about the product (e.g. because of the presence of an organic linker molecule in a conjugated protein product).

The use of standard genotoxicity studies for assessing the genotoxic potential of process contaminants is usually not considered appropriate. If performed for this purpose, however, the rationale should be provided.

B.4.5 Carcinogenicity studies

General principles

The need for a product-specific assessment of the carcinogenic potential for rDNA-derived BMP should be determined with regard to the intended clinical population and treatment duration. The mechanism of action of some rDNA-derived BMP might raise concern regarding potential for carcinogenicity (e.g. immunosuppressives and growth factors). Standard carcinogenicity bioassays are generally inappropriate for rDNA-derived BMP. However, product-specific assessment of carcinogenic potential may still be needed depending upon duration of clinical dosing, patient population and/or biological activity of the product (see above). When an assessment is warranted, the sponsor should design a strategy to address the potential hazard.

This strategy should be based on a review of relevant data from a variety of sources. The data sources can include published data (e.g. information from transgenic, knock-out or animal disease models, human genetic diseases), information on class effects, detailed
information on target biology and mechanism of action, *in vitro* data, data from chronic
toxicity studies and clinical data. In some cases, the available information can be
sufficient to address carcinogenic potential and inform clinical risk without additional
nonclinical studies.

**Products with expected/probable carcinogenic potential**

If a clear evidence for carcinogenic potential of a rDNA-derived BMP is identified (see
above), rodent bioassays are not warranted. In this case potential hazard can be best
addressed by product labeling and risk management practices (see “Risk communication”
below).

**Products with unclear carcinogenic potential**

For products where there is insufficient/unclear knowledge about specific product
characteristics and mode of action in relation to carcinogenic potential, a more extensive
assessment might be appropriate. In such a case a variety of approaches may be
considered to evaluate the risk.

Products that may have the potential to support or induce proliferation of transformed
cells and clonal expansion possibly leading to neoplasia should be evaluated with respect
to receptor expression in various malignant and normal human cells that are potentially
relevant to the patient population under study. The ability of the product to stimulate
growth of normal or malignant cells expressing the receptor should be determined. When
*in vitro* data give cause for concern about carcinogenic potential, further studies in
relevant animal models may be needed. Incorporation of sensitive indices of cellular
proliferation in long term repeated dose toxicity studies may provide useful information.
If the data from this more extensive assessment do not suggest a carcinogenic potential,
no additional nonclinical testing is recommended. Alternatively, if the data do suggest a
concern additional nonclinical studies that could mitigate the concern should be
undertaken or the label should clearly reflect the concern.

In those cases where the product is biologically active and non-immunogenic in rodents
and other studies have not provided sufficient information to allow an assessment of
carcinogenic potential, the utility of a study in a single rodent species should be
considered. Careful consideration should be given to the selection of doses. The use of a combination of pharmacokinetic and pharmacodynamic endpoints with consideration of comparative receptor characteristics and intended human exposures represents the most scientifically based approach for defining the appropriate doses. The rationale for the selection of doses should be provided.

Use of homologous proteins

Rodent bioassays (or short-term carcinogenicity studies) with homologous products are generally of limited value to assess carcinogenic potential of the clinical candidate.

Risk communication

The product-specific assessment of carcinogenic potential is used to communicate risk and provide input to the risk management plan along with labeling proposals, clinical monitoring, post-marketing surveillance, or a combination of these approaches.

B.4.6 Reproductive performance and developmental toxicity studies

B.4.6.1 General principles

The need for reproductive/developmental toxicity studies is dependent upon the product, clinical indication and intended patient population. The specific study design and dosing schedule may be modified based on issues related to species specificity, immunogenicity, biological activity and/or a long elimination half-life. For example, concerns regarding potential developmental immunotoxicity, which may apply particularly to certain monoclonal antibodies with prolonged immunological effects, could be addressed in a study design modified to assess immune function of the neonate.

(i) Products with expected/probable adverse effects on fertility/pregnancy outcome

When the available data (e.g. mechanism of action, phenotypic data from genetically modified animals, class effects) clearly suggest that there will be an adverse effect on fertility or pregnancy outcome, these data can provide adequate information to communicate risk to reproduction and, under appropriate circumstances, additional nonclinical studies might not be warranted.
There may be extensive public information available regarding potential reproductive and/or developmental effects of a particular class of compounds (e.g. interferons) where the only relevant species is the non-human primate. In such cases, mechanistic studies indicating that similar effects are likely to be caused by a new but related molecule, may obviate the need for formal reproductive/developmental toxicity studies. In each case, the scientific basis for assessing the potential for possible effects on reproduction/development should be provided.

(ii) Products with unclear potential for adverse effects on fertility/pregnancy outcome

The specific study design and dosing schedule can be modified based on an understanding of species specificity, the nature of the product and mechanism of action, immunogenicity and/or PK behavior and embryo-fetal exposure.

- Species selection

An assessment of reproductive toxicity of the clinical candidate should usually be conducted only in pharmacologically relevant species. When the clinical candidate is pharmacologically active in rodents and rabbits, both species should be used for embryo-fetal development (EFD) studies, unless embryo-fetal lethality or teratogenicity has been identified in one species. Developmental toxicity studies should only be conducted in non-human primates (NHPs) when they are the only relevant species. When the clinical candidate is pharmacologically active only in NHPs, there is still a preference to test the clinical candidate. However, an alternative model can be used in place of NHPs if appropriate scientific justification is provided.

- Alternative evaluation in the absence of a relevant species

When no relevant animal species exist(s) for testing the clinical candidate, the use of transgenic mice expressing the human target or homologous protein in a species expressing an ortholog of the human target can be considered, assuming that sufficient background knowledge exists for the model (e.g. historical background data).
(iii) Products for which adverse effects on fertility/pregnancy outcome are not expected

For products that are directed at a foreign target such as bacteria and viruses, in general no reproductive toxicity studies would be expected.

B.4.6.2 Fertility

For products where mice and rats are pharmacologically relevant species, an assessment of fertility can be made in one of these rodent species (32). Study designs can be adapted for other species provided they are pharmacologically relevant and should be amended as appropriate, for example to address the nature of the product and the potential for immunogenicity.

It is recognized that mating studies are not practical for NHPs. However, when the NHP is the only relevant species, the potential for effects on male and female fertility can be assessed by evaluation of the reproductive tract (organ weights and histopathological evaluation) in repeat dose toxicity studies of at least 3 months duration using sexually mature NHPs. If there is a specific cause for concern based on pharmacological activity or previous findings, specialized assessments such as menstrual cyclicity, sperm count, sperm morphology/motility, and male or female reproductive hormone levels can be evaluated in a repeat dose toxicity study.

If there is a specific concern from the pharmacological activity about potential effects on conception/implantation and the NHP is the only relevant species, the concern should be addressed experimentally. A homologous product or transgenic model could be the only practical means to assess potential effects on conception or implantation when those are of specific concern. However, it is not recommended to produce a homologous product or transgenic model solely to conduct mating studies in rodents. In absence of nonclinical information, the risk to patients should be mitigated through clinical trial management procedures, informed consent and appropriate product labeling.

B.4.6.3 Embryo-fetal development (EFD) and pre/post-natal development (PPND)

Selection of study design
Potential differences in placental transfer of rDNA-derived BMP should be considered in the design and interpretation of developmental toxicity studies (see Note 1 of Appendix 7).

For products pharmacologically active only in NHPs, several study designs can be considered based on intended clinical use and expected pharmacology. Separate EFD and/or PPND studies, or other study designs (justified by the sponsor) can be appropriate, particularly when there is some concern that the mechanism of action might lead to an adverse effect on embryo-fetal development or pregnancy loss. However, one well-designed study in NHPs which includes dosing from day 20 of gestation to birth (enhanced PPND, ePPND) can be considered, rather than separate EFD and/or PPND studies.

Enhanced pre/post-natal development (ePPND) studies

For the single ePPND study design described above, no Caesarian section group is warranted, but assessment of pregnancy outcome at natural delivery should be performed. This study should also evaluate offspring viability, external malformations, skeletal effects (e.g. by X-ray) and, ultimately, visceral morphology at necropsy. Ultrasound is useful to track maintenance of pregnancy but is not appropriate for detecting malformations. These latter data are derived from post-partum observations. Because of confounding effects on maternal care of offspring, dosing of the mother post-partum is generally not recommended. Other endpoints in the offspring can also be evaluated if relevant for the pharmacological activity. The duration of the post-natal phase will be dependent on which additional endpoints are considered relevant based on mechanism of action (see Note 2 of Appendix 7).

Developmental toxicity studies in NHPs can only provide hazard identification. The number of animals per group should be sufficient to allow meaningful interpretation of the data (see Note 3 of Appendix 7).

Study design should be justified if species other than the cynomolgus monkey are used. The developmental toxicity studies in NHPs, as outlined above, are just hazard identification studies; therefore it might be possible to conduct these studies using a
control group and one dose group, provided there is a scientific justification for the dose
level selected (see Note 4 of Appendix 7).

B.4.6.4 Timing of studies
If women of child-bearing potential are included in clinical trials prior to acquiring
information on effects on embryo-fetal development, suitable clinical risk management is
appropriate, such as the use of highly effective methods of contraception (34). For
rDNA-derived BMP pharmacologically active only in NHPs, where there are sufficient
precautions to prevent pregnancy, an EFD or ePPND study can be conducted during
Phase III, and the report submitted at the time of marketing application. When a sponsor
cannot take sufficient precaution to prevent pregnancy in clinical trials, either a complete
report of an EFD study or an interim report of an ePPND study should be submitted
before initiation of Phase III (see Note 5 of Appendix 7). Where the product is
pharmacologically active only in NHPs and its mechanism of action raises serious
concern for embryo-fetal development, the label should reflect the concern without
warranting a developmental toxicity study in NHPs and therefore administration to
women of child-bearing potential should be avoided.
If the rodent or rabbits is a relevant species, timing of reproductive toxicity/fertility
studies should follow the recommendations given (34).
For oncology products, see Appendix 5.

B.4.7 Local tolerance studies
Local tolerance should be evaluated. Ideally, the formulation intended for marketing
should be tested; however, in certain justified cases, the testing of representative
formulations may be acceptable. In some cases, the potential adverse effects of the
product can be evaluated in single or repeated dose toxicity studies, thus obviating the
need for separate local tolerance studies.

B.4.8 Other toxicity studies
B.4.8.1 Antibody Formation
Immunogenicity assessments in animals should only be conducted to assist in the interpretation of the study results and to improve the design of subsequent studies. Such analyses in animal studies are usually not relevant in terms of predicting potential immunogenicity of human or humanized proteins in humans.

Measurement of anti-drug antibodies (ADA) in nonclinical studies should be evaluated when there is: 1) evidence of altered PD activity; 2) unexpected changes in exposure in the absence of a PD marker; or 3) evidence of immune-mediated reactions (immune complex disease, vasculitis, anaphylaxis, etc.).

Since it is difficult to predict prior to study completion whether such analysis will be necessary, it is often useful to obtain appropriate samples during the course of the study, which can subsequently be analyzed when warranted to aid in interpretation of the study results.

When ADAs are detected, their impact on the interpretation of the study results should be assessed. Antibody responses should be characterized (e.g. titer, number of responding animals, neutralizing or non-neutralizing activity), and their appearance should be correlated with any pharmacological and/or toxicological changes. Specifically, the effects of antibody formation on PK/PD parameters, incidence and/or severity of adverse effects, complement activation, or the emergence of new toxic effects should be considered when interpreting the data. Attention should also be paid to the evaluation of possible pathological changes related to immune complex formation and deposition.

Characterization of neutralizing potential is warranted when ADAs are detected and there is no PD marker to demonstrate sustained activity in the in vivo toxicology studies. Neutralizing antibody activity can be assessed indirectly with an ex vivo bioactivity assay or an appropriate combination of assay formats for PK-PD, or directly in a specific neutralizing antibody assay.

The detection of antibodies should not be the sole criterion for the early termination of a nonclinical safety study or modification in the duration of the study design, unless the immune response neutralizes the pharmacological and/or toxicological effects of the rDNA-derived BMP in a large proportion of the animals. In most cases, the immune response to rDNA-derived BMP is variable, similarly to that observed in humans. If the
interpretation of the data from the safety study is not compromised by these issues, then no special significance should be ascribed to the antibody response.

Anaphylaxis tests
The occurrence of severe anaphylactic responses to rDNA-derived BMP is uncommon in humans. In this regard, the results of guinea pig anaphylaxis tests, which are generally positive for protein products, are usually not predictive for reactions in humans. Therefore, such studies are considered of little value for the routine evaluation of these types of products.

B.4.8.2 Immunotoxicity studies
One aspect of immunotoxicological evaluation includes assessment of potential immunogenicity (see B.4.1 and B.4.8.1). Many rDNA-derived BMP are intended to stimulate or suppress the immune system and, therefore, may affect humoral as well as cell-mediated immunity. Inflammatory reactions at the injection site may be indicative of a stimulatory response. However, such reaction may cause or contribute to by injection trauma and/or specific toxic effects caused by the formulation vehicle. The expression of surface antigens on target cells may be altered, with implications for autoimmune potential. Immunotoxicological testing strategies may require screening studies followed by mechanistic studies to clarify such issues. Routine tiered testing approaches or standard testing batteries, however, are not recommended for rDNA-derived BMP.

B.4.8.3 Impurities
Safety concerns may arise from the presence of impurities or contaminants. There are potential risks associated with host cell contaminants whether derived from bacteria, yeast, insect, plants, or mammalian cells. The presence of cellular host contaminants can result in allergic reactions and other immunopathological effects. The adverse effects associated with nucleic acid contaminants are theoretical but include potential integration into the host genome (2). For products derived from insect, plant and mammalian cells, or transgenic plants and animals there may be an additional risk of viral infections.
However, it is preferable to rely on manufacturing and quality control processes to deal with these issues (section Part A) rather than to establish a preclinical testing program for their qualification.

B.5 Selection of dose for exploratory clinical trials (first in human use)

When nonclinical data are very limited (e.g. humanized monoclonal antibodies for which there is no relevant species) or when the nonclinical findings demonstrate a potential high risk for the use of the rDNA-derived BMP in humans, a risk mitigating approach is recommended for the first in human use of the rDNA-derived BMP (for example, 34).
Part C. Clinical evaluation

C.1 Good Clinical Practice (GCP)
All clinical trials should be conducted under the principles described in the WHO guidelines for good clinical practice for trials on pharmaceutical product (36) and the WHO guidelines on clinical evaluation of vaccines: regulatory expectations (37).

C.2 Clinical Pharmacology (Phase I)
C.2.1 Initial safety and tolerability studies
Initial safety and tolerability studies start the first-in-human studies of drugs after the completion of essential nonclinical studies (30, 32-34, 38). The safety of participants is the paramount consideration in proceeding to first-in-human studies. Decisions on strategies for the development of a new medicine and the experimental approaches used to assemble information relevant to the safety of first-in-human studies must be science-based and ethically acceptable. Such studies should be closely monitored and conducted with small numbers (20 to 100) of healthy volunteers. However, products with significant potential toxicity and those used for rare diseases are normally studied in patients only. Study protocols should define stopping rules for individual subjects, cohort and the trial itself. Initial safety and tolerability studies are designed to detect common adverse reactions, the tolerated dose range and the potential drug effect. The ultimate goal of the studies is to obtain adequate safety data to permit the design of sufficiently valid phase II studies.

Initial safety and tolerability studies may be non-randomised single-arm studies with no comparator, or open-label, and may range from single dose studies to studies involving multiple doses and lasting several months. Drug doses usually start at low levels, and study participants are monitored very carefully as the dose is escalated. In some settings and depending on the study protocol, individual participants receive only one dose (see also C.2.3 and C.2.4).
From a clinical perspective, rDNA-derived BMPs have particular challenges in comparison to chemically-derived small molecule drugs and present special safety issues to be addressed in the initial safety and tolerability studies:

• The nonclinical data may not be completely predictive of safety in humans. In particular, since rDNA-derived BMPs typically contain non-host proteins and polysaccharides, nonclinical studies are usually not predictive for immunogenicity, i.e. a test species may not react to an rDNA-derived BMP which could cause serious adverse reactions in humans, or a test species may react when humans do not.

• Data from healthy volunteers may not be fully predictive of safety/efficacy in patients, such as target-mediated effect associated with monoclonal antibodies.

• Unlike many small molecule drugs, rDNA-derived BMP have long half-lives and are frequently dosed to the maximum tolerated level to saturate receptors in all patients.

Predicting the potential for severe adverse drug reactions for first-in-human use of an investigational medicinal product, involves the identification of risk factors, which may be related to: 1) the mode of action; 2) the nature of the target; and/or 3) the relevance of animal models. High-risk biologicals (e.g. TGN1412, an anti-CD28 superagonist which caused an acute cytokine storm in humans that was not predicted from animal studies) require extended safety measures, which may include strict sequential inclusion of trial participants with clear stopping rules and extremely careful calculation of the first dose in man (38).

C.2.2 Pharmacogenomics

Pharmacogenomic studies performed early during drug development can provide essential information for the design of robust phase III trials, such as identifying receptor, genetic or phenotypic characteristics and drug response in populations; using biomarkers to identify dose response in individuals; and identifying patients with genetic polymorphisms whose drug dosages should be adjusted for improved safety and/or efficacy or for whom a particular treatment should not be used (39, 40). However, pharmacogenomic effects are not commonly seen with rDNA-derived BMPs. The most
recent guidance documents on this topic from appropriate regulatory agencies should be consulted.

C.2.3 Pharmacokinetics

The pharmacokinetic (PK) profile is a part of the basic description of a medicinal product and should always be investigated. PK studies should be performed for the intended dose range and routes of administration (9). In general, the PKs (absorption, distribution and elimination) of rDNA-derived BMP should be characterized during single-dose and steady-state conditions in relevant populations. However, historically, the PK evaluation of peptide or protein products has suffered from limitations in the assay methodology thus limiting the usefulness of such studies. Immunoassays and bioassays are most frequently used for assaying therapeutic proteins in biological matrices. Special emphasis should, therefore, be given to the analytical method selected and its capability to detect and follow the time course of the protein (the parent molecule and/or degradation and/or metabolic products) in a complex biological matrix that contains many other proteins. The method should be optimized to have satisfactory specificity, sensitivity and a range of quantification with adequate accuracy and precision (9).

If part of the PK information is gathered in healthy volunteers, the validity of extrapolation of that information to the target population needs to be addressed (41). The choice of single-dose studies, steady-state studies, or repeated determination of PK parameters and the study population should be justified (9). A prospective plan for defining the dosing schedule based on observed/calculated PK parameters should be developed and included in the PK study protocol (42). It should be kept in mind that changes in the manufacturing process may alter the PKs of rDNA-derived BMPs.

C.2.3.1 Absorption

The majority of biological products are administered parenterally through intravenous, subcutaneous or intramuscular administration. Alternative routes proposed for delivery of proteins may be considered, e.g. nasal and pulmonary administration, which bypass the
interstitial subcutaneous or intra-muscular environment. Oral delivery of proteins for
systemic effects is still rare due to low bioavailability (41).

Unless the intravenous route is exclusively used, appropriate in vivo studies should be
conducted in healthy volunteers or patients to describe the absorption characteristics of
the rDNA-derived BMP, i.e. the rate and extent of absorption. Single-dose studies are
generally sufficient to characterize absorption and to compare different administration
routes (43). It should be noted that the rate of absorption following intramuscular or
subcutaneous administration may vary according to the site and depth of the injection, the
concentration and volume of the solution injected, and may be influenced by patient
specific factors (41, 43).

Protein therapeutics administered by the subcutaneous route exhibit limited transport into
blood capillaries and enter the systemic circulation indirectly through the lymphatics.
Passage through the lymphatic system usually results in pre-systemic elimination and
consequently a bioavailability of less than 100% is obtained. In addition, small proteins
may undergo proteolytic degradation in tissues as a first-pass mechanism (41). Since
proteases can be affected by disease states and are reported to be upregulated with disease
progression, consideration should be given to patient specific circumstances (43).

C.2.3.2 Distribution

Tissue distribution studies should be undertaken unless otherwise justified. The volume
of distribution of a drug is determined largely by its physicochemical properties (e.g.
charge, lipophilicity), protein binding, and its dependency on active transport processes.
Because most rDNA-derived BMPs are large in size, their volume of distribution is
usually small and limited to the volume of the extracellular space due to their limited
mobility secondary to impaired passage through biomembranes. Site-specific and target-
oriented receptor mediated tissue uptake and binding to intra- and extravascular proteins,
however, can substantially increase the volume of distribution of rDNA-derived BMPs
(44).
Many of the studies regarding biodistribution and/or elimination of rDNA-derived BMP can be elucidated with radiolabeled products. In addition, the binding capacity to plasma proteins (albumin, α-acid glycoprotein) should be studied when considered relevant \((44)\). Pharmacokinetic calculations of steady-state volume of distribution may be problematic for some rDNA-derived BMPs. Noncompartmental determination using statistical moment theory assumes first-order disposition processes with elimination occurring from the rapidly equilibrating or central compartment. This basic assumption, however, is not fulfilled for numerous recombinant peptide and protein products, as proteolysis in peripheral tissues may constitute a substantial fraction of the overall elimination process for such rDNA-derived BMPs \((44)\). There is an inverse correlation between steady-state volume of distribution and molecular weights as well as for permeability and molecular weight. Unlike small molecule chemical drugs, distribution to tissues (i.e. cellular uptake) is often part of the elimination process and not part of the distribution process as such, thus contributing to the small distribution volumes. Thus, a small steady-state volume of distribution 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 \((41)\).

C.2.3.3 Elimination

The main elimination pathway, including the major organs of elimination, should be identified. Radiolabeled proteins can be used for this purpose \((44)\). However, for therapeutic proteins, the main elimination pathway in vivo can be predicted, to a large extent, from the molecular size; thus, specific studies may not be necessary.

Breakdown products may have different PK profiles when compared with the parent rDNA-derived BMP. However, in cases where measurement of separate active peptide fragments is not technically feasible, the PKs of the active moiety could be determined \((41)\).

Metabolism of small proteins and peptides (MW < 50,000 Da) appears to occur mainly in the kidneys. The liver may also play a major role in the metabolism of peptides and proteins, mediated by substance-specific enzymes such as for insulin, glucagon,
epidermal growth factor (EGF), antibodies, and t-Pas (44). If biliary excretion of peptides and proteins occurs, it generally results in subsequent breakdown and metabolism of these compounds in the gastrointestinal tract (44).

Catabolism of proteins usually occurs by proteolysis via the same catabolic pathways as for endogenous or dietary proteins. Proteolytic enzymes such as proteases and peptidases are ubiquitously available throughout the body. Thus, locations of intensive peptide and protein metabolism also include blood and various body tissues (44).

If elimination of the protein is largely dependent on target receptor uptake, differences in receptor density between healthy volunteers and target populations, such as over-expression of receptors in tumors or inflamed tissues can create important pharmacokinetic differences in half-life. These differences should be considered when using healthy volunteer data for predictions to target population (41).

After subcutaneous administration of proteins with relatively rapid elimination, the rate of absorption can be slower than the rate of elimination leading to longer apparent half-lives (flip-flop kinetics) and prolonged exposure when compared to IV administration. As a consequence, dosing frequency may have to be reduced (45).

General tendencies in the in vivo disposition of proteins and peptides may often be predicted from their physiological function. Peptides, for example, frequently have hormone activity and usually have short elimination half-lives. This is desirable for a close regulation of their endogenous levels and thus of their function. In contrast, transport proteins such as albumin or antibodies have elimination half-lives of several days, which ensure the continuous maintenance of necessary concentrations in the bloodstream (44).

C.2.3.4 Subpopulations

The clinical development program should involve studies to support the approval in subpopulations such as patients with organ dysfunction. Whether such studies are necessary depends 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
dedicated studies in the respective population, or from population PK analyses of phase II/III data (4I).

Renal impairment

For proteins with MW lower than 50,000 Da, renal excretion is important for elimination (increasing importance with lower MW) and consequently, for the half-life of the protein. Thus, for these products, PK studies in patients with renal impairment are recommended. It is also conceivable that renal impairment itself may affect functioning of other organs and tissues (e.g. by up- or down-regulation of enzymes or receptors), thereby influencing the PKs and/or PDs of the experimental compound. This should be taken into account in the planning of the clinical pharmacology programme (4I).

Hepatic impairment

Reduced hepatic function may decrease the elimination of a protein for which hepatic degradation is an important elimination pathway. Where relevant, PK studies in patients with different degrees of hepatic impairment are recommended (4I).

C.2.3.5 Interaction studies

The requirements for in vivo drug-drug interaction studies, for example, with respect to cytochrome P450 (CYP) enzymes, are generally lower for therapeutic proteins than for conventional, chemically derived, products. Since elimination of proteins usually involves capacity-limited steps such as receptor binding, the inhibition or induction of receptors might impact pharmacokinetics. However, there is currently lack knowledge about suitable tools to explore such interactions. Development within this area is encouraged (4I).

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 of the product. The dose-proportionality should be evaluated in single- or multiple-dose studies and the clinical consequences discussed. Time-dependent changes in PK parameters may occur
during multiple-dose treatment, e.g. due to down- or up-regulation of receptors responsible for (part of) the elimination of the rDNA-BMP or to formation of anti-drug antibodies. Using appropriate methods, soluble receptors may be measured before treatment and during treatment, differentiating between free and bound receptors. The effect on the PKs should be evaluated and the clinical relevance discussed (30). Apparent time-dependency may also originate from the fact that immunologically active breakdown products may be slowly accumulating and have long half-lives. It is recommended that PKs at several dose levels be determined on several occasions during long-term studies. Population PK analysis of data from long-term trials could be considered (41).

C.2.3.6 Pharmacokinetic data analysis

As for small molecule products the pharmacokinetics may be analyzed using compartment- or noncompartment methods. The choice of the PK model used to derive PK parameters should be justified. Mean (median) and individual results should always be included in a license submission. The inter-subject variability should be estimated and, if possible, the important sources of the variability, e.g. demographic factors such as weight and age, should be identified. Potential additional 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. tumor load), variability in degradation rate (e.g. of de-pegylation) or in degradation pattern. Based on the results, individualized dosing should be considered if necessary from safety and/or efficacy perspectives. The variability within an individual should also 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. Population PK analysis of phase II/III data using a sparse sample approach is recommended for characterizing the pharmacokinetics, the variability of the PK parameters and possible covariate relationships (41). Population analyses may thus support the individualization of doses.
C.2.4 Pharmacodynamics

In many cases, PD parameters are investigated in the context of combined PK/PD studies. Such studies may provide useful information on the relationship between dose/exposure and effect, particularly if performed at different dose levels. PD markers should be selected based on their clinical relevance. Studies in relevant animal models, if available, provide important information on the PD properties of a BMP and may guide PD studies in humans. If no animal model is available, a suitable human population must be chosen. In any case, relevant PD effects should always be confirmed in human subjects with the disease that is being targeted by the BMP.

Human PD studies are usually carried out during phase I or phase II studies. Phase II studies can also be called proof of concept clinical studies and are important for the subsequent development of the product by helping to determine the dose to be used in further confirmatory trials, and providing some level of confidence that the biotherapeutic is pharmacologically active and can do what it is intended to do.

C.2.5 Pharmacokinetic/pharmacodynamic relationship

The relationship between drug concentration and PD response (PK/PD) should always be evaluated as part of drug development. If feasible, markers for both efficacy and safety should be measured, preferably in the same study. It should be noted that PK and PD for a BMP may not necessarily be entirely and fully correlated (e.g. ceiling effect due to saturation of target receptors) and both may be altered by modifications to the molecule, binding to blood components, or formation of anti-drug antibodies. Early pre-clinical and clinical data can be evaluated using appropriate models for a mechanistic understanding of the disease and the PK/PD relationship. PK/PD models may be developed accounting for the time-delay between plasma concentrations and measured effect. The model may also need to take into account the presence or absence of the therapeutic target (e.g. presence of antigen in case of anticancer monoclonal antibodies). PK/PD models may allow extrapolation from volunteers to target population given that suitable assumptions have been made, e.g. regarding pathological factors. These models may provide guidance for dose selection and are helpful when interpreting changes in the PKs in important
subpopulations or when evaluating comparability in the context of a change in manufacturing process. Efforts to explore relevant biomarkers and their link (surrogacy) to safety and efficacy endpoints are encouraged (41).

### C.2.6 Modifications of PK and PD profiles of therapeutic proteins

Many protein drugs display suboptimum therapeutic efficacies due to their inherent poor molecular stability, low systemic bioavailability, and, as a consequence of their innate susceptibility to various clearance mechanisms, short circulatory lifetimes. Higher protein concentrations and increased dosing frequencies, are therefore, often employed to achieve favourable therapeutic responses. Approaches to improve these factors, and thus in vivo efficacy, include targeted mutations, generation of fusion proteins and conjugates, glycosylation engineering, and pegylation (46).

Glycosylation is a co-and post-translational process and refers to the enzymatic covalent attachment of carbohydrate based molecules (glycans) to the surface of proteins or other organic molecules. It is known that glycosylation can influence a variety of physiological processes at both the cellular (e.g. intracellular targeting) and protein levels (e.g. protein-protein binding, protein molecular stability, plasma persistence lifetimes). Since the glycosylation pattern of a BMP may be influenced by even subtle changes in the manufacturing process, the potential effects on PK and PD profiles need to be considered when evaluating comparability of pre- and post-change product in the context of a change in manufacturing process.

### C.3 Efficacy

#### C.3.1 Phase II

In general, phase II studies provide the first test of efficacy in patients with the disease targeted by the rDNA-derived BMP. They aim at determining the correct dosage, identifying common short-term side effects, and defining the best regimen to be used in pivotal clinical trials. Conventionally, the first step (frequently called phase IIa) is focused on an initial proof of concept. This step is to demonstrate that the rDNA-derived BMP interacts correctly with its molecular target and, in turn, alters the disease or its symptoms. Subsequent trials
(frequently called phase IIb trials) are larger and may use placebo and/or active comparator agents and a broader dosage range to obtain a much more robust proof of concept and additional guidance on dose selection.

For proof of concept, single-arm trials may be used with their results interpreted relative to historical control subjects. However, this design could introduce bias since, for example, current study participants may be different from historical control subjects in ways that affect the outcome of interest or because of changes in supportive care that may limit the validity of the conclusions. Therefore, comparative randomized phase II trials are generally preferred.

Phase II trials usually explore a variety of possible endpoints (e.g. time-to-event endpoints, change in a continuous endpoint of tumor size), and provide opportunities for biomarker discovery. A variety of study designs can be used, including the randomized parallel-group design, randomized discontinuation design, single-stage and two-stage designs, delayed-start design, and adaptive (Bayesian) designs. In all cases, clear decision rules should be in place.

Standard study designs for assessing dose-response have been described (47), such as randomized parallel dose response studies. However, the approaches to selecting the optimal dose may differ for rDNA-derived BMP compared to small chemical molecules. For example, biological agents developed in oncology are usually cytostatic and their maximal activities may occur at doses lower than their maximum-tolerated doses.

Combination therapy is an important treatment modality in many disease settings such as cancer. Increased understanding of the pathophysiological processes that underlie complex diseases has provided further impetus for therapeutic approaches using combinations of (new) products directed at multiple therapeutic targets to improve treatment response, minimize development of resistance or improve tolerability. This requires the use of flexible designs and new modeling approaches for the design of clinical trials.

Just like small chemical drugs, rDNA-derived BMPs may affect cardiac electrical activity either directly or indirectly. The amount and type of electrocardiogram (ECG) data considered appropriate should be individualized based on the type of product. A
Thorough QT/QTc Study (TQT) (48) or a study that incorporates many of the key components of a TQT study should be considered (48). However, for large, targeted therapeutic proteins, this may not be necessary if ECG data of good quality are collected during clinical development.

C.3.2 Confirmatory phase III

Phase III clinical trials are designed to evaluate the benefit of the rDNA-derived BMP in a carefully selected patient population with the disease. These trials are to confirm efficacy at the chosen dose(s) and dosing regimen(s), to further evaluate safety and monitor side effects, and sometimes to compare the candidate product to commonly used treatments. For common conditions, phase III studies are usually conducted with large populations consisting of several hundred to several thousand participants who have the disease or the condition of interest.

Confirmatory trials should be prospective randomized trials comparing the test agent against a placebo (in addition to the best supportive care) or an active comparator, usually the best available, evidence-based current standard. If no such comparator is available (e.g. in patients who have failed several lines of therapies), the comparator may be the investigator’s best choice. Ideally, trials should be double blinded, where neither the patient nor the investigator knows the nature of the product received by the patient. Blinding or masking is intended to limit the occurrence of conscious or unconscious bias in the conduct and interpretation of a clinical trial (49).

The design of the trials depends on the hypothesis to be tested: superiority to placebo/active comparator, equivalence or non-inferiority to an active comparator (50). The choice of endpoints depends on the therapeutic indication; there should be sufficient evidence that the primary endpoint can provide a valid and reliable measure of clinically relevant and important treatment benefit in the targeted patient population. If a single primary variable cannot be selected, a composite endpoint integrating or combining multiple measurements into a single variable, using a pre-defined algorithm, can also be used; such validated endpoints are commonly used in inflammatory diseases (e.g. ACR20
in rheumatoid arthritis, ASAS20 in ankylosing spondylitis, CDAI in Crohn’s disease, PASI in psoriasis) or in oncology (disease progression, disease-free survival, or overall survival). Patient reported outcomes and quality of life scales are also important endpoints, which may already be included in some of these composite endpoints.

When direct assessment of the clinical benefit to the patient is not practical, a surrogate endpoint may be considered if it is a reliable predictor of clinical benefit. The strength of the evidence for surrogacy depends upon (i) the biological plausibility of the relationship, (ii) the demonstration of the prognostic value of the surrogate for the clinical outcome in epidemiological studies and (iii) evidence from clinical trials that treatment effects on the surrogate correspond to effects on the clinical outcome. Validated surrogate endpoints are uncommon, but for serious and life-threatening diseases, non-validated markers can be used if they are reasonably likely to predict the desired clinical benefit, e.g. the effect on tumor size, as assessed by imaging, in patients refractory to available treatments.

Specific decisions about the size of the study group will depend on such factors as the magnitude of the effect of interest, characteristics of the study population, and study design (see C.4).

Generally, two confirmatory trials are needed in order to show that the results can be replicated. However, one controlled study with statistically compelling and clinically relevant results may be sufficient, especially in life-threatening conditions or rare disorders. Because most rare diseases have a more homogeneous genetic pattern than common diseases and because they are often characterized by similar or identical genetic or epigenetic defects, patients with these diseases could be expected to have a more uniform therapeutic response. This should reduce the size of phase III studies required to demonstrate efficacy. The use of historical controls (or possibly no controls) may also be justified if the rare disease has a defined course in the absence of treatment that will permit comparisons with the results for the investigational rDNA-derived BMP.

C.3.3 Biomarkers
The identification of laboratory based disease biomarkers has the potential to enhance the benefit-risk profile of rDNA-derived BMP by enabling the selection of patients that are more likely to respond, especially with molecules that target serum or cell markers. In such case, the treatment may only benefit a subset of patients defined by the biomarker, e.g. patients positive for HER-2 or KRAS wild-type tumors. The biomarker evaluation process should consist of the following three steps: (i) analytical validation; (ii) qualification, i.e. assessment of available evidence on associations between the biomarker and disease states, including data showing effects of interventions on both the biomarker and clinical outcomes; and (iii) utilization, i.e. contextual analysis based on the specific use proposed and the applicability of available evidence to this use (50-52). Biomarker qualification should not be part of pivotal phase III trials.

C.3.4 Manufacturing and formulation changes

While manufacturing and formulation changes may be expected during product development, the phase III trials should be conducted with the test rDNA-derived BMP manufactured according to the final manufacturing (commercial) process. If this is not the case, a comparability exercise between the clinical and commercial products is necessary to ensure that the change would not have an adverse impact on the clinical performance of the product (27, 28). This comparability exercise should normally follow a stepwise approach, starting with a comparison of quality attributes of the active substance and relevant intermediates. A comparability exercise should not be limited to release testing but should include more extensive characterization using a range of suitable analytical methods, as appropriate to the product and process changes in question (see A.9) If differences are detected that may have a potential impact on the clinical properties of the product, nonclinical and/or clinical bridging studies may be needed such as PK/PD studies, and possibly immunogenicity studies.

C.3.5 Special populations

As in any clinical development programme, studies in special populations would be expected where relevant to the indications, e.g. in the elderly and in children.
Some rDNA-derived BMPs, such as those developed for cancer, Parkinson’s disease, Alzheimer’s disease, coronary heart disease or diabetes mellitus, may have particular impact on the elderly population (patients aged 65 years or older, and even 75 years and above). The geriatric subpopulation should be represented sufficiently to permit the comparison of treatment effects, dose response and safety between older and younger patients. Where the disease to be treated is characteristically associated with aging, it is expected that geriatric patients will constitute the major portion of the clinical database (53).

The extent of the studies needed in children depends on the possibility of extrapolation from adults and children of other age groups. Some rDNA-derived BMPs may be used in children from the early stages of drug development, especially those targeting genetic diseases where manifestations occur early in life. Evaluation should be made in the appropriate age group and it is usually recommended to begin with older children before extending the trial to younger children and then infants (54). Where justified, extrapolation of efficacy data from adult to paediatric patients may be based on PK and/or PD data. However, safety data usually cannot be extrapolated and need to be generated in children (see C.5).

C.3.6 Post-marketing: Phase IV

Phase IV trials may be required to further evaluate an approved rDNA-derived BMP and obtain more information about safety or effectiveness or both, especially if it has been approved on the basis of a surrogate endpoint.

C.4 Statistical Considerations

C.4.1 General considerations

The application of sound statistical principles to the design, conduct, analysis and interpretation of clinical trials should be considered an important and integral component of the overall development of a rDNA-derived BMP. The success of a trial depends on the appropriateness of the study design, trial conduct and analysis of trial results. Statistical principles are relevant to all three aspects of the clinical trial. In general, details regarding these aspects should be specified in the trial protocol, which should be written
and finalized prior to the start of the trial. Any subsequent amendments to the protocol should be clearly justified and documented in a formal amendment to the protocol, and should include the statistical consequences of the proposed changes.

The scientific integrity of the trial and the credibility of the data from the trial depend substantially on the trial design (55). The study protocol should include a clear description of the specific design selected for a particular trial. Additional details regarding the primary endpoint, which is directly related to the primary objective of the trial, should also be included. If multiple primary endpoints are defined, the criteria for achieving study success should be clearly laid out in order to avoid potential problems with the interpretation of the trial results. The protocol should also clearly define secondary endpoints, and their role in the interpretation of the trial results should be stated. Details regarding measures that have been put in place to avoid or minimize bias in the trial should also be provided, for example, randomization and blinding.

With regards to the type of hypothesis to be tested in a specific trial, it should be clear in the protocol whether the trial is designed to show superiority, non-inferiority, or equivalence. The statistical issues involved in the design, conduct, analysis and interpretation of equivalence and non-inferiority trials are complex and subtle, and require that all aspects of these trials be carefully evaluated. Sample size and power are important for the success of a clinical trial, and should be given careful consideration at the trial design stage. In determining sample size, the specific hypothesis being tested should be taken into consideration.

It is important to ensure that the protocol will provide good quality data that permit an adequate evaluation of the efficacy (and safety) of the product under development. In addition, if formal interim analyses are planned, then the details governing such analyses should be pre-specified in the protocol.

Details regarding the statistical methodology to be applied to the clinical trial should be provided in the protocol, with the more technical details being captured in the Statistical...
Analysis Plan (SAP). The SAP should be prepared and finalized prior to un-blinding the clinical study. Any amendments to the SAP must also be finalized prior to un-blinding.

C.4.2 Specific considerations for rDNA-derived BMPs

Since rDNA-derived BMPs are often indicated to treat severe and/or life threatening diseases and chronic diseases, trials for rDNA-derived BMPs present unique statistical challenges.

Trials in small populations and single arm studies

Some rDNA-derived BMPs are intended for the treatment of rare diseases for which the target population is very small. Consequently, trials that are considered confirmatory for rare disease indications are often based on a limited number of subjects. While such studies must still be designed with the rigor of traditional trials, and should be conducted with high quality in order to provide reliable and valid data for assessing efficacy and safety, some flexibility is needed with regards to the statistical methods that will be utilized in such trials. Single arm studies with comparisons being made to an external control can sometimes be justified.

Tumor-based endpoints in oncology trials and composite endpoints

In confirmatory oncology trials for rDNA-derived BMPs, the use of tumor-based endpoints such as disease-free survival and progression-free survival as the primary endpoint is not uncommon (56). The use of tumor-based endpoints as the primary endpoint creates several statistical challenges, and considerations for the collection and analysis of such endpoints have been discussed (e.g. 57). Clinical trials may involve the use of a composite primary endpoint, arising from the combination of multiple clinical measurements or outcomes (e.g. major adverse cardiac events, MACE, the most commonly used composite endpoint in cardiovascular studies). For such a composite endpoint, it is important that the individual components are analyzed separately (usually as secondary endpoints) in order to ensure that the treatment effect is shown across all components, and is of similar magnitude.
Missing data is a common problem in long-term trials of rDNA-derived BMPs targeting chronic diseases, for example, diabetes and rheumatoid arthritis, while it is usually not a problem in short-term trials. The impact of missing data on the validity of trial results should be carefully assessed using sensitivity analyses with appropriate underlying assumptions.

**Adaptive clinical trial designs**

In an era when it is recognized that improvements in the drug development process are needed in order to increase the likelihood of trial success, decrease costs, and increase the efficiency with which efficacious and safe medicines are brought to market, adaptive clinical trial designs are increasingly considered as one tool through which these improvements can be achieved. Adaptive design refers to a clinical study design that uses accumulating data to decide how to modify aspects of the study as it continues, without undermining the validity and integrity of the trial (58, 59). A key statistical issue for adaptive designs is the preservation of the Type I error rate. The methods used to properly control the Type I error rate should be described in the study protocol, with additional details provided in the SAP.

**C.5 Safety**

Pre-licensing safety data should be obtained in a sufficient number of patients to characterize and quantify the safety profile of the rDNA-derived BMP including type, frequency and severity of adverse drug reactions (ADRs). The safety evaluation should cover a reasonable duration of time, taking into account the intended duration of use of the drug, to assess potential changes in the ADR profile over time and to capture delayed ADRs.

For drugs intended for long-term treatment of non-life-threatening conditions, a 12-month exposure of at least 100 patients to the investigational medicinal product at the intended clinical dosage should be considered (60). When no serious ADR is observed in a one-year exposure period, this number of patients can provide reasonable assurance that the true cumulative one year incidence is no greater than 3%. This estimate is based on the
statistical “rule of three”, which states that if no major ADR occurred in a group of \( n \) people, there can be 95% confidence that the chance of a major ADR is less than one in \( n/3 \) (or equivalently, less than 3 in \( n \)). This estimate is considered a good approximation for \( n > 30 \).

The safety database may need to be larger or may require longer patient observation if a safety signal is identified, if the drug is expected to cause late developing ADRs, or if ADRs increase in severity or frequency over time. Concerns requiring a larger safety database may arise from non-clinical or early clinical data or from experience with other products of the same or related pharmacologic class. A smaller safety database may be acceptable if the intended treatment population is small.

Safety data should be obtained from prospective and preferably controlled studies including a placebo or active comparator arm since comparison with an external control group (e.g. with published data) is usually hampered by differences in the investigated patient population, concomitant therapy, observation period and/or reporting. Causality assessment, i.e. whether the observed adverse event is causally related to the investigational drug, is usually easiest in placebo-controlled studies.

Generally accepted definitions and terminology as well as procedures to harmonize the way to gather and, if necessary, to take action on important clinical safety information arising during clinical development are important \(^{61} \). The term “adverse event” (AE) describes any untoward medical occurrence developing with administration of a pharmaceutical product irrespective of a causal relationship. The term “adverse drug reaction” (ADR), on the other hand, should only be used for adverse events that have at least a reasonable possible causal relationship to the pharmaceutical agent.

Standardized reporting is important for transmission of pre- or post-marketing safety information, for example, between reporting source or pharmaceutical industry and regulatory authorities or between regulatory authorities and WHO Collaborating Center for International Drug Monitoring \(^{62} \). Data elements to be included in individual case safety reports should comprise all important information on the primary source, date, sender and receiver of the information, the type, seriousness, duration and outcome of the AE or ADR, detailed patient characteristics and drug information, actions taken with the
drug (e.g. dose reduction, discontinuation), and an assessment of the degree of suspected relatedness of the drug to the AE (62).

To facilitate sharing of regulatory safety information internationally for medical products used by humans, specific MedDRA terminology has been developed, a rich and highly specific standardized medical terminology for accurate and consistent safety information allowing for aggregation of reported terms in medically meaningful groupings (63). Products covered by the scope of MedDRA include pharmaceuticals, vaccines and drug-device combination products.

Since safety data obtained from pre-marketing clinical trials can be expected to detect mainly common and shorter-term ADRs, further monitoring of clinical safety of the biological product to detect rare but sometimes serious adverse effects and an ongoing benefit-risk evaluation are necessary in the post-marketing phase (see C.7).

C.5.1 Special populations

Elderly population

The safety of rDNA-derived BMPs should be investigated in geriatric patients during clinical drug development (64, 65), except where there is no intention to use the rDNA-derived BMP in this age group. Geriatric patients are more prone to adverse effects since they often have co-morbidities and are taking concomitant medication that could interact with the investigational drug. The adverse effects can be more severe, or less tolerated, and may have more serious consequences than in the non-geriatric population. Depending on the mechanism of action of the drug and/or the characteristics of the disease, specific effects on cognitive function, balance and falls, urinary incontinence or retention, weight loss and sarcopenia should be investigated.

Elderly patients may be included in the main Phase III or Phase II/III studies or in separate studies. Inclusion of younger and elderly patients in the same studies has the advantage of allowing direct comparisons using data collected in similar ways. Certain assessments, however, such as studies of cognitive function, require special planning and can be best accomplished in separate studies.
Where enrolment of geriatric patients has been insufficient despite the efforts of the applicant, a specific plan to collect data post-marketing should be presented in the marketing application.

**Paediatric population**

Data on the safety of medicinal products in the pediatric population should be generated unless their use is clearly inappropriate (54). During clinical development, the timing of pediatric studies will depend on the medicinal product, the type of disease being treated, safety considerations, and the efficacy and safety of alternative treatments. Justification for the timing and the approach to the clinical program needs to be clearly addressed with regulatory authorities.

Medicinal products may affect physical and cognitive growth and development, and the adverse event profile may differ in pediatric compared to adult patients. In addition, adverse effects may not be apparent immediately, but only at a later stage of development. Long-term studies or surveillance data while patients are on chronic therapy and/or during the posttherapy period, may be needed to determine possible effects on skeletal, behavioral, cognitive, sexual, and immune maturation and development.

**C.6 Immunogenicity**

rDNA-derived BMPs may induce unwanted humoral and/or cellular immune responses in recipients. Immunogenicity of rDNA-derived BMP should therefore always be investigated pre-authorization (66). Since animal data are usually not predictive of the immune response in humans, immunogenicity needs to be investigated in the target population. Although in-silico modeling may help to identify T-cell epitopes related to immunogenicity (i.e. T-helper epitopes) this does not predict whether immunogenicity will occur. The frequency and type of anti-drug antibodies induced as well as possible clinical consequences of the immune response should be thoroughly assessed.

The immune response against a biotherapeutic is influenced by many factors such as the nature of the drug substance, product- and process-related impurities (e.g. host-cell-proteins, aggregates), excipients and stability of the product, route of administration (s.c.
administration usually more immunogenic than i.v. administration), dosing regimen (intermittent use usually more immunogenic than continuous use), and patient-, disease- and/or therapy-related factors (e.g. antibody development more likely in immunocompetent than immunosuppressed state and potentially enhanced in the presence of autoimmune disease). The consequences of unwanted immunogenicity on safety may vary considerably, ranging from clinically irrelevant to serious and life-threatening (e.g. serious infusion/anaphylactic) reactions. Neutralizing antibodies directly alter the PD effect of a product (i.e. by blocking the active site of the protein) leading to reduced or loss of efficacy. Binding antibodies often affect pharmacokinetics and, thereby, may indirectly influence pharmacodynamics. Thus, an altered effect of the product over time due to anti-product antibody formation might be a composite of pharmacokinetic, PD and safety effects.

The proposed antibody testing strategy should be appropriately justified including the selection, assessment, and characterization of assays, identification of appropriate sampling time points (including baseline samples), sample storage and processing as well as selection of statistical methods for analysis of data (66). Antibody assays should be validated for their intended purpose. Validation studies need to establish appropriately linear responses to relevant analytes as well as appropriate accuracy, precision, sensitivity, specificity and robustness of the assay(s). A highly sensitive screening assay should be used for antibody detection and a complementary assay, usually with a different assay format, to confirm the presence of antibodies and eliminate false positive results. A neutralization assay should be available for further characterization of antibodies, if present, unless the development of neutralizing antibodies can be excluded with a high probability (e.g. based on experience with the substance class). Possible interference of the circulating antigen with the antibody assay(s) should be taken into account.

If the rDNA-derived BMP is a monoclonal antibody (mAb), the development of assays to detect antibodies against this mAb can be technically challenging (67). Many standard assay formats involve the use of anti-immunoglobulin reagents such as antibodies against immunoglobulins, protein A or protein G, but these are inappropriate for use in detecting
antibodies against mAbs as they very often bind to the product itself. Different assay approaches have been developed to overcome this problem such as the ‘bridging’ ELISA format or Surface Plasmon Resonance (SPR) procedures which do not require anti-immunoglobulin reagents but may be less sensitive than other immunoassay methods (68).

Detected antibodies should be further characterized with regard to antibody content (concentration/titre) and, depending on case-by-case considerations, possibly other criteria such as antibody class and subclass (isotype), affinity and specificity. For example, the isotype of the antibodies could be determined if this may be predictive of safety (such as development of IgE antibodies causing allergic and anaphylactic responses). Potential clinical implications of detected antibodies regarding safety, efficacy and pharmacokinetics should always be evaluated. Special attention should be paid to the possibility that the immune response seriously affects the endogenous protein and its unique biological function (e.g. neutralizing anti-epoetin antibodies cross-reacting with endogenous erythropoietin and causing pure red cell aplasia).

The required observation period for immunogenicity testing will depend on the intended duration of therapy and the expected time of antibody development, if known, and should be justified. In the case of chronic administration, one-year data will usually be appropriate pre-licensing to assess antibody incidence and possible clinical implications. If considered clinically relevant, development of antibody titers, their persistence over time, potential changes in the character of the antibody response and the possible clinical implications should be assessed pre- and post-marketing.

Since pre-licensing immunogenicity data are often limited, further characterization of the immunogenicity profile may be necessary post-marketing, particularly, if rare but clinically meaningful or even serious antibody-related ADRs have been encountered with biological agents of the same or related substance class that are not likely to be detected in the pre-marketing phase.

C.7 Pharmacovigilance and risk management planning

NRAs should be vigilant that the health of the public is protected. The aim is to ensure that the risks associated with rDNA-derived BMPs are actively minimized. Patient safety
is a key concern for all medicinal products that are on the market: rDNA-derived BMPs are no exception. Due to the specific characteristics of rDNA-derived BMPs already discussed in this Guideline, PhV activities required for rDNA-derived BMPs may differ in some respects from those required for small molecule drugs. For example, BMP use may lead to antibody formation with consequences for clinical efficacy and/or safety.

A risk management plan (RMP) should be submitted and agreed to by the NRA. The key components of a RMP should include:

1. Safety Specifications, which summarize the known and potential safety issues and missing information about the rDNA-derived product;
2. A PhV plan to further evaluate important known or potential safety concerns and, provide postmarketing data where relevant information is missing; and
3. A risk minimization plan (RMinP), which provides proposals on how to minimize any identified or potential safety risk.

In the RMP, the known or potential risks should be described and PhV and risk minimization activities be proposed to identify, characterize, prevent, or minimize risks related to the use of the rDNA-derived BMP; to assess the effectiveness of those interventions; and to communicate those risks to both patients and health care providers.

PhV and risk minimization activities that might be included in an RMP fall into two categories: routine activities, which would generally be conducted for any medicine where no special safety concerns have arisen, and additional activities designed to address identified and potential safety concerns that could have an impact on the benefit-risk balance of a product. Routine PhV activities would include the monitoring and reporting of spontaneous adverse events post-approval (e.g. ADR; Periodic Safety Update Report (PSUR) or a Period Benefit/Risk Evaluation Report (PBRER)) and, any safety evaluations incorporated in clinical trials that may be initiated by the marketing authorization holder following marketing authorization for a wide variety of reasons. In case of relevant safety issues, NRAs may request additional PhV activities in the form of active surveillance (e.g. registries), epidemiology studies, further clinical studies, and drug utilization studies. Routine RMin activities would ensure that suitable
contraindications and warnings are included in the product information and that this information is updated on an ongoing basis. A RMinP can further specify other risk minimization activities, as appropriate, which could include: specific educational material about the product and its use, patient- or physician-oriented training programs, restricted use of the product and registration programs for patients, doctors and/or pharmacists.

Once on the market, manufacturers should monitor the effectiveness of their RMinPs, and revise them if new safety and effectiveness concerns are identified. Changes in the manufacturing processes introduced post-marketing could also influence the safety profile (e.g. by enhancing immunogenicity) of rDNA-derived BMPs and may necessitate enhanced safety monitoring.

In case a relevant or even serious potentially drug-related adverse event occurs, it is important to be able to identify the specific biological causing this event. Therefore, all ADR reports should carry the proprietary (brand) name, the INN, identification code (if there is one), and lot information of the respective biological to quickly trace an ADR to a specific product and ascertain any relation to causality.

An RMP will not reduce the scientific and clinical standards or the data requirements for the market authorization of rDNA-derived BMPs; nor will it replace the precautionary approach that is taken to managing the risks associated with those products. To the contrary, implementation of an RMP will further strengthen the rigor of post-market surveillance, allowing for earlier identification of risks associated with rDNA-derived BMPs and earlier interventions to minimize those risks.

C.8 Additional guidance

Further guidance on various aspects of clinical trials is available from several other bodies such as the International Conference on Harmonization (ICH), the European Medicines Agency (EMA), the United States Food and Drug Administration (FDA) as well as from several other NRAs. The WHO guidelines are not intended to conflict with, but rather to complement these other documents with respects to medicinal products prepared by rDNA technology.
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Appendix 1. Manufacturing process validation

Process validation is the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce a drug product, drug substance or intermediate meeting its predetermined specifications and quality attributes.

Process validation should include the collection and evaluation of data, from the process design stage throughout production, to establish scientific evidence that a process is capable of consistently delivering a quality drug substance. The manufacturing process should be validated before commercial distribution of drug product. It generally includes collection of data on an appropriate number of production batches. The number of batches can depend on several factors including but not limited to: (1) the complexity of the process being validated; (2) the level of process variability; and (3) the amount of experimental data and/or process knowledge available on the specific process.

Process validation studies should include appropriate evaluation of the commercial process and process steps (e.g. cell culture, harvest, purification, mixing, sterilisation, filling), providing evidence that they are capable of consistently delivering quality product and intermediates (i.e. meeting their predetermined specifications and quality attributes).

The capacity of the purification procedures to remove product and process-related impurities (e.g. unwanted variants, host cell proteins, nucleic acids, resin leachates) should be investigated thoroughly. Process conditions (e.g. column loading capacity, column regeneration and sanitisation, height) should be appropriately evaluated. Columns should also be evaluated throughout their expected life span of the column regarding their purification ability (e.g. impurity clearance, collection of intended variants), leaching of ligands (e.g. dye, affinity ligand) and/or chromatographic material (e.g. resin).
Process validation activities should normally include the evaluation of resin lifetime, including maximum cycles and/or maximum time duration, using small scale studies to ensure proper performance and integrity of the columns. In addition, the results should normally be verified at full scale through the lifecycle of the product. These studies should also confirm the suitability of the column cleaning, storage and regeneration procedures.

Where hold times are applied to intermediates (e.g. harvest, column eluate), the impact of hold times and hold conditions on the product quality should be appropriately evaluated (e.g. degradation).

Evaluation of selected step(s) (e.g. steps for which high impurity or viral clearance are claimed) operating in worst case and/or challenging conditions (e.g. maximum hold times, spiking challenge) could be performed to demonstrate the robustness of the process. Depending on the relevance of experimental model with regards to the final process (e.g. scale, materials, equipment, operating conditions), these studies could be leveraged in support of process validation and/or quality control data requirements.

The information provided in the dossier in support of process validation usually contains both commercial-scale process validation studies and small-scale studies. Process validation batches should be representative of the commercial process, taking into account the batch definition as detailed in the process description.

Extensive process changes at the level of fermentation and/or purification during progression to full scale commercial production may have considerable consequences for the quality of the product, yield and/or in quantitative and qualitative differences in impurities. Therefore, the contribution of data from small-scale studies to the overall validation package will depend upon demonstration that the small-scale model is an appropriate representation of the proposed commercial scale. Data demonstrating that the model is scalable and representative of the proposed commercial process should be provided. Successful demonstration of the suitability of the small-scale model can enable manufacturers to propose process validation with reduced dependence on testing of commercial-scale batches. Data derived from commercial-scale batches should confirm
results obtained from small scale studies used to generate data in support of process validation. Scientific rationale, or reference to guidelines can be an appropriate justification to conduct certain studies only at small scale (e.g. viral removal).
Appendix 2. Characterization of rDNA-derived BMP

This Appendix provides details of approaches expected to be applied to the characterization of a rDNA-derived BMP.

1. Physicochemical characterization

The amino acid sequence should be deduced from the DNA sequence and confirmed experimentally by appropriate methods (e.g. peptide mapping, amino acid sequencing, mass spectrometry analysis). Attention should be paid to the possible presence of N-terminal methionine (e.g. in Escherichia coli derived products), signal or leader sequences and other possible N- and C-terminal modifications (such as acetylation, amidation or partial degradation by exopetidases). The variability of N- and C-terminal amino-acid sequences should be analysed (e.g. C-terminal lysine(s)).

Free sulphhydril groups and disulfide bridges should be determined. Disulfide bridge integrity and mismatch should be analysed. Experimentally determined disulfide bonding patterns should be compared to the predicted structure based on the class of the molecule. Post-translational modifications, such as glycosylation should be identified and adequately characterized. The carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined if linked to clearance or activity. In addition, the structure of the carbohydrate chains should be analysed. This includes the overall profiles of sialo- and asialoglycans as well as analysis of site specific glycan profile and analysis of site occupancy. Particular attention should be paid to glycan structures that may be associated with adverse effects, such as non-human structures or residues. Further tests to be conducted include analysis of charge heterogeneity. Consideration should be given to the possibility that such modifications are likely to be different from those found in the natural counterpart and may influence the biological, pharmacological and immunological properties of the rDNA-derived BMP.

Higher-order structure should be characterised by appropriate physicochemical methodologies and confirmed by biological function. The analysis of pegylated proteins
should include, but not be limited to the average rate of modification, the location of modification, and analysis of site occupancy.

2. Biological activity

Assessment of the biological properties of a product constitutes an essential step in establishing a complete characterization profile.

The biological activity should be assessed by in vitro, in vivo, biochemical (including immunochemical assays) and/or physicochemical assays as appropriate.

For antibody products, where effector function may play a role in the mechanism of action, and/or have an impact on the product safety and efficacy, a detailed analysis of biological activity demonstrating the mechanism of action (e.g. antibody dependent cellular cytotoxicity, complement dependent cytotoxicity, apoptosis), ability for complement binding and activation and other effector functions, including Fc gamma receptor binding activity, and FcRn binding activity should be provided, as appropriate.

The mechanism of action should be discussed, and where relevant, the importance (or consequences) of other functions (e.g. effector functions) with regards to the safety and efficacy of the product should be included.

Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties, whereas, quantity (expressed in mass) is a physicochemical measure of protein content. For assessing potency, use of bioassays that reflect the biological activity in the clinical situation is preferable, but not always possible or necessary for lot release. For example, bioassays which assess some functional aspect of the protein or mechanism of action (rather than the intended clinical effect) can also be used as the basis for a potency assay.

3. Immunochemical properties

Where relevant (e.g. for monoclonal antibody products), the immunological properties should be fully characterised. Binding assays using purified antigens and defined regions
of antigens should be performed, where feasible, to determine affinity, avidity and
immunoreactivity (including cross-reactivity with other structurally homologous proteins).

Unintentional reactivity/cytotoxicity for human tissues distinct from the intended target
should be documented. Cross-reactivity with a range of human tissues should be
determined using immunohistochemical procedures. The CDR regions should be
identified and justified.

The part of the target molecule bearing the relevant epitope should be defined. This
should include biochemical identification of these structures (e.g. protein, oligosaccharide,
glycoprotein, glycolipid), and relevant characterisation studies (amino acid sequence,
carbohydrate structure) to the extent possible.

Unless otherwise justified, the ability for complement binding and activation, and/or
other effector functions should be evaluated, even if the intended biological activity does
not require such functions.

4. Purity, impurity and contaminant

Biotechnological products commonly display several sources of heterogeneity (e.g. C-
terminal processing, N-terminal pyroglutamation, deamidation, oxidation, isomerisation,
fragmentation, disulfide bond mismatch, N-linked and O-linked oligosaccharide,
glycation, aggregation), which lead to a complex purity/impurity profile comprising
several molecular entities or variants. This purity/impurity profile should be assessed by a
combination of methods, and individual and/or collective acceptance criteria should be
established for relevant product-related variants.

These methods generally include the determination of physicochemical properties such as
molecular weight or size, isoform pattern, determination of hydrophobicity,
electrophoretic profiles, chromatographic data including peptide mapping and
spectroscopic profiles including mass spectroscopy.

Multimers and aggregates should also be appropriately characterised using a combination
of methods. Unless otherwise justified, the formation of aggregates, sub-visible and
visible particulates in the drug product is important and should be investigated and
closely monitored at the time of release and during stability studies.

Impurities may be either process or product-related. They can be ones of known structure,
partially characterized, or unidentified. When adequate quantities of impurities can be
generated, these materials should be thoroughly characterized and, where possible, their
biological activities should be evaluated.

Potential process-related impurities (e.g. HCP, host cell DNA, cell culture residues,
downstream processing residues) should be identified, and evaluated qualitatively and/or
quantitatively, as appropriate.

Contaminants, which include all adventitiously introduced materials not intended to be
part of the manufacturing process (e.g. microbial species, endotoxins) should be strictly
avoided and/or suitably controlled. Where non-endotoxin pro-inflammatory contaminants,
such as peptidoglycan, are suspected, the use of additional testing, such as the monocyte
activation test, should be considered.

5. Quantity
Quantity should be determined using an appropriate physicochemical and/or
immunochemical assay.
Appendix 3. Technical approaches to analytical characterization

This appendix provides examples of technical approaches which might be considered for structural characterization and confirmation, and evaluation of physicochemical and biological properties of the desired product, drug substance and/or drug product. These methods should provide an understanding of the product with sufficient level of detail, e.g. complete primary structure, properties for the higher order structure, qualitative and quantitative analysis of product related substances and product and process related impurities, assessment of biological functions. The specific technical approach employed will vary from product to product and alternative approaches, other than those included in this appendix, will be appropriate in many cases. New analytical technologies and modifications to existing technologies are continuously being developed and should be utilized when appropriate.

A subset of the methods described below can be used for routine batch release testing. Others are subject to extended characterization of the desired product during product and process development, and are also often used to support process evaluation/validation and/or comparability studies, e.g. after making significant process changes. The selection of release testing methods depends on the overall design of quality control for which the release testing is only one element among others. For example, if a certain quality attribute can be controlled by in-process tests and/or demonstrated manufacturing process capability (e.g. high impurity clearance), such attribute may not need to be tested routinely on every batch.

1. Primary structure

The primary structure (i.e. amino acid sequence including the disulfide linkages) of the desired product can be determined to the extent possible using combined approaches such as those described in items a) through c) and then compared with the sequence of the amino acids deduced from the gene sequence of the desired product.

a) Peptide map
Selective fragmentation of the product into discrete peptides is performed using suitable enzymes or chemicals and the resulting peptide fragments are analysed by HPLC or other appropriate analytical procedures. The peptide fragments should be identified to the extent possible using appropriate techniques such as mass spectrometry methods (e.g. electrospray ionization mass spectrometry (ESI-MS), matrix-assisted laser-desorption ionization time-of flight mass spectrometry (MALDI TOF MS)). The use of MS/MS coupling should also be considered, as it could reveal more detailed sequence information of the analysed peptide fragment. If one fragmentation method does not deliver the complete amino acid sequence, the use of an orthogonal enzyme or chemical cleavage method can increase the sequence coverage. The correct formation of the disulfide bridges may be characterized by the use of peptide mapping under reducing and non-reducing conditions.

b) Molecular weight determination by mass spectrometry
The molecular weight of the intact molecule as determined by mass spectrometry serves as an additional confirmation of the primary structure. For smaller peptides, MS/MS sequencing can provide the complete amino acid sequence. Mass spectrometry can be performed under reduced and non-reduced conditions and deglycosylated and intact conditions for multi-subunit and glycosylated protein molecules such as monoclonal antibodies.

c) Other methods
Methods such as amino acid analysis or Edman sequencing can also be used although their importance has decreased due to the technical advances in mass spectrometry.

2. Higher-order structure
The complete assessment of the three dimensional chemical structure in the context of product characterization is rarely achieved, because absolute methods such as X-ray crystallography or NMR with isotope labeled amino acids deliver only an approximation to the structure of the product of interest. They measure the product either in a non-relevant state or require a separate production of the isotope labeled sample. However,
the use of applicable but relative orthogonal methods as described below enable the
determination and characterization of discrete folding and also the assessment of changes
in the higher order structure, e.g. in the case of comparability studies.

a) Spectroscopic methods
The higher-order structure of the product should be examined using appropriate
procedures such as circular dichroism (CD), Fourier transform infrared spectroscopy (FT-
IR), fluorescence, differential scanning calorimetry, proton nuclear magnetic resonance
(1H-NMR) and/or other suitable techniques, for example hydrogen-deuterium exchange
MS. When using these methods, their capabilities and limitations need to be considered
(e.g. impact of protein concentration). For instance, FT-IR and CD in the far UV range
deliver information on the secondary structure, whereas CD in the near UV reflects to
some extent the tertiary and quaternary structure.

b) Bioassays
Bioassays can serve as an additional confirmation of the higher order structure in addition
to demonstrating biological function.

3. Glycan structure
For glycoproteins, the glycan content (neutral sugars, amino sugars, and sialic acids) is
determined. In addition, the structure of the glycan chains, the glycan pattern (antennary
profile native glycan profile and site specific glycan analysis) and the glycosylation site(s)
of the polypeptide chain is analysed, as far as possible. This task can be achieved by the
combination of enzymatic or chemical hydrolytic cleavage with a variety of separation
methods (HPLC, electrophoresis) and detection/identification methods (mass
spectrometry including MS/MS, UV, fluorescence detection, electrochemical detection).
The quantitative oligosaccharide analysis (chemical or enzymatic cleavage followed by
HPLC) provides additional useful qualitative and quantitative information on the glycan
structure.
Measurement of the quantitative charge patterns of the intact glycoprotein, e.g. by
measuring the charge based isoforms using appropriate method (e.g. capillary
electrophoresis, isoelectric focusing) may be useful as an overall measure of the degree of sialylation and antennary profile.

4. Process-related impurities and contaminants

This section lists potential impurities, their sources and examples of relevant analytical approaches for their detection. The process-related impurities are derived from the manufacturing process itself and could be classified into three major categories: cell substrate-derived, cell culture-derived and downstream-derived. Contaminants, on the other hand represent unwanted material which are introduced by unintentional means into the manufacturing process such as adventitious viruses.

a) Cell substrate-derived impurities include, but are not limited to, proteins derived from the host organism, nucleic acid (host cell genomic, vector, or total DNA). For host cell proteins, a sensitive assay e.g. immunoassay, capable of detecting a wide range of protein impurities is generally utilized. In the case of an immunoassay, polyclonal antibodies used in the test are typically generated by immunization of animals with an appropriate preparation derived from the production cell minus the product-coding gene, which have been cultured in conditions representative of the intended culture and appropriately collected (e.g. filtered harvest, partial purification).

The level of DNA from the host cells can be detected by direct analysis on the product (e.g. qPCR, immunoenzymatic techniques). Clearance studies, which could include spiking experiments conducted at the small scale, to demonstrate the removal of cell substrate-derived impurities such as nucleic acids and host cell proteins may sometimes be used to eliminate the need for establishing acceptance criteria for these impurities.

b) Cell culture-derived impurities include, but are not limited to, inducers antibiotics, serum, and other media components. These impurities need to be tested and evaluated on a case-by-case basis using a risk-assessment and management approach. In the case of a potential impact on the safety of the product, the removal of such impurities to acceptably low levels during downstream purification may need to be validated, or end product testing and specification limits established.
c) Downstream-derived impurities include, but are not limited to, enzymes, chemical and biochemical processing reagents (e.g. guanidine, dyes, oxidizing and reducing agents), inorganic salts (e.g. heavy metals, non-metallic ions), solvents, carriers, ligands (e.g. protein A), and other leachables. As for cell-culture-derived impurities, these impurities should be evaluated on a case-by-case basis using a risk-assessment and management approach. Where appropriate, development of analytical methods for these impurities and validation of their removal could be considered.

5. Product-related substances and impurities including degradation products

The following represents the most frequently encountered molecular variants of the desired product and lists relevant technology for their assessment. Such variants may need considerable effort in isolation and characterization in order to identify the type of modification(s). Degradation products arising during manufacture and/or storage in significant amounts should be tested for and monitored against appropriately established acceptance criteria. When these variants of the desired product have properties comparable to those of the desired product with respect to activity, efficacy and safety, they are considered product-related substances and not impurities.

a) Truncated forms

Hydrolytic enzymes or chemicals may catalyse the cleavage of peptide bonds. This may lead to terminal heterogeneity; e.g. for C-terminal Lys in monoclonal antibodies. These may be detected by HPLC and/or electrophoretic methods and verified by mass spectrometry. Peptide mapping may also be useful, depending on the property of the variant.

b) Amino acid modifications

Individual amino acid modification may include deamidation (Asp/Gln to Asp, Glu), oxidation (e.g. Met to Met-sulfoxide), spontaneous formation of pyroglutamate out of N-terminal Glu or Gln residues, glycation of Lys residues and others. These forms may be
detected and characterized by relevant analytical methods (e.g., HPLC, capillary electrophoresis, mass spectrometry). In some cases peptide mapping is important to clearly identify and localize the site and nature of the amino acid modification.

c) High molecular weight species (HMWS) and particles
HMWS includes dimers and higher oligomers of the desired product. Particles include intrinsic visible particles of the desired product. These are generally resolved from the desired product and product-related substances, and quantitated by appropriate separation procedures (e.g. size exclusion chromatography, field flow fractionation, analytical ultracentrifugation) coupled with sensitive detection methods (e.g. UV, fluorescence, light scattering). Using orthogonal methods and/or procedures with overlapping analytical windows (e.g. light obscuration testing, micro-flow imaging (MFI) for testing of sub visible particles) can greatly enhance the characterization of aggregates and particles. Foreign particles are not intended to be part of the product and should strictly be avoided.

6. Biological activity
The assessment of the biological properties constitutes an essential step in establishing a complete characterization profile. The biological activity describes the specific ability or capacity of a product to achieve a defined biological effect. Description of a valid biological assay to measure the biological activity should be provided by the manufacturer. Examples of procedures used to measure biological activity include:

- Animal-based biological assays, which measure an organism's biological response to the product;
- Cell culture-based biological assays, which measure biochemical or physiological response at the cellular level;
- Biochemical assays, which measure biological activities such as receptor or ligand binding, enzymatic reaction rates or biological responses induced by immunological interactions.

7. Content
The protein content (expressed in mass units) can be determined by measuring the sample against an appropriate reference standard using a suitable method (e.g. HPLC).

The protein content can also be measured in an absolute way, e.g. by UV photometry using an extinction coefficient e.g. at 280 nm. Although the calculated extinction coefficient delivers a satisfactory accuracy for many cases, it is advisable to use a second absolute method (e.g. amino acid analysis, Kjeldahl) for verification. If the deviation is too large, re-determination by another method could be considered.
Appendix 4. Routine control of rDNA-derived BMP

This appendix discusses approaches which should be taken to routine control of a rDNA-derived BMP.

1. Specification

A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance and drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. “Conformance to specification” means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria. The justification of specification should take into account relevant development data and data from nonclinical, clinical and stability studies. The setting of acceptance ranges should also take into account the sensitivity of the analytical method used.

The selection of tests to be included in the specifications is product specific, and should take into account the quality attributes (e.g. potential influence on safety, efficacy or stability), the process performance (e.g. clearance capability, content), the controls in place through the manufacturing process (e.g. multiple testing points), the material used in relevant nonclinical and clinical studies. These tests could include criteria such as potency, the nature and quantity of product-related substances, product-related impurities, process-related impurities, and absence of contaminants. Such attributes can be assessed by multiple analytical procedures, each yielding different results. Since specifications are chosen to confirm the quality rather than to characterize the product, the rationale and justification for including and/or excluding testing for specific quality attributes should be provided.

The rationale used to establish the acceptable range of acceptance criteria should be described. Acceptance criteria should be established and justified based on data obtained
from lots used in nonclinical and/or clinical studies. Nevertheless, where appropriately justified, data from lots used for stability studies, or relevant development data could support limits beyond ranges used in clinical studies.

2. Identity
The identity test(s) should be highly specific and should be based on unique aspects of the product’s molecular structure and/or other specific properties (e.g. peptide map, anti-idiotypic immunoassay, or other appropriate method). Depending on the product, more than one test (physicochemical, biological and/or immunochemical) may be necessary to establish identity, and such test(s) should possess sufficient specificity that they can discriminate other products that may be manufactured in the same facility.

3. Purity and impurities
As noted in the characterisation section, recombinant proteins may display a complex purity/impurity profile that should be assessed by a combination of orthogonal methods, and for which individual and/or collective acceptance criteria should be established for relevant product-related variants. Chromatographic and/or electrophoretic methods capable of detecting product truncation, dissociation and aggregation should be included, and quantitative limits should be proposed for these, as appropriate. Considering that glycosylation and pegylation may have an impact on the pharmacokinetic of the product, and may modulate its immunogenic properties, appropriate acceptance criteria should be considered for this attribute. In addition, as appropriate, such control will further confirm the consistency of the product. The control of relevant process-related impurities should be included in the plan for quality control. Control of process related impurities (e.g. protein A, HCP, DNA and other potential culture or purification residues) are typically part of the drug substance specification, as appropriate. In some situations, and where appropriately demonstrated, their control may be performed on an intermediate product, at an appropriate process step. Routine testing may not be necessary for some impurities for which the process has been demonstrated to achieve high reduction levels.
4. Potency

Potency is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties. A relevant potency assay should be part of the specifications for drug substance and/or drug product, should reflect the biological activity whenever possible. Specific activity (biological activity per unit mass) is of considerable value to demonstrate consistency of production.

The potency of each batch of the drug substance and the final dosage form should be established using, wherever possible, an appropriate national or international reference material (e.g. A.1.3) which is normally calibrated in units of biological activity, for example International Units (IU). In the absence of such preparations, an approved in-house reference preparation may be used for assay standardization.

For biologicals with antagonist activity, it may be appropriate to calibrate the potency assay using the standard/reference preparation for the agonist and express activity of the antagonist in terms of inhibition of biological activity i.e. units of the agonist. For example for TNF antagonists, bioassays can be calibrated using the IS for TNF-alpha and activity expressed as number of IU of TNF neutralized by the amount of the antagonist.

5. Quantity

The quantity of the drug substance and drug product, usually based on protein content, should be determined using an appropriate assay.

6. General tests

General tests should be performed in accordance to relevant monographs, which could include appearance, solubility, pH, osmolality, extractable volume, sterility, bacterial endotoxins, metal ions, stabiliser and water, visible and sub-visible particulate, as appropriate.
Appendix 5. Product specific guidance in nonclinical evaluation (examples)

1. Anticancer pharmaceuticals
For anticancer pharmaceuticals, nonclinical evaluations are intended to identify the pharmacologic properties, establish a safe initial dose level for the first human exposure, and understand the toxicological profile, e.g. identification of the target organ, estimation of the safety margin, and reversibility. In the development of anticancer drugs, most often the clinical studies involve cancer patients whose disease condition is often progressive and fatal. In addition, the clinical dose levels often are close to or at the adverse effect dose levels. For these reasons, the type and timing and flexibility called for in designing of nonclinical studies of anticancer pharmaceuticals can have a different pattern from those for other pharmaceuticals (1, 2).

2. Monoclonal antibodies
For monoclonal antibodies, the immunological properties of the antibody should be described in detail, including its antigenic specificity, complement binding, and any unintentional reactivity and/or cytotoxicity towards human tissues distinct from the intended target. Such cross-reactivity studies should be carried out by appropriate IHC procedures using a range of human tissues (see also B.3.3).

For monoclonal antibodies and other related antibody products directed at foreign targets (i.e. bacterial, viral targets etc.), a short-term safety study in one species (choice of species to be justified by the sponsor) can be considered; no additional toxicity studies, including reproductive toxicity studies, are needed. When animal models of disease are used to obtain proof of principle, a safety assessment can be included to provide information on potential target-associated safety aspects. Where this is not feasible, appropriate risk mitigation strategies should be adopted for clinical trials.

Antibody-drug/toxin conjugates
Species selection for an antibody-drug/toxin conjugate (ADC) incorporating a novel toxin/toxicant should follow the same general principles as an unconjugated antibody. If
two species have been used to assess the safety of the ADC, an additional short-term
study or arm in a short-term study should be conducted in at least one species with the
unconjugated toxin. In these cases a rodent is preferred unless the toxin is not active in
the rodent. If only one pharmacologically relevant species is available, then the ADC
should be tested in this species. A novel toxicant calls for an approach to species
selection similar to that used for a new chemical entity on a case-by case approach (e.g.
for anticancer products as described in ICH S9 Guideline) (2). For toxins or toxicants
which are not novel and for which there is a sufficient body of scientific information
available, separate evaluation of the unconjugated toxin is not warranted. Data should be
provided to compare the metabolic stability of the ADC in animals with human.

References
1. ICH S6(R1) guideline. Preclinical safety evaluation of biotechnology-derived
   pharmaceuticals, 2011.
Appendix 6. Animal species/model selection

1. Species selection

The biological activity together with species and/or tissue specificity of many rDNA-derived BMP often preclude standard pharmacological/toxicity testing designs in commonly used species (e.g. rats and dogs). Pharmacological and safety evaluation programs should include the use of relevant species. A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies). In addition to receptor expression, the cellular/tissue distribution of receptors is an important consideration in selection of appropriate species. A variety of techniques (e.g. immunochemical or functional tests) can be used to identify a relevant species. Knowledge of receptor/epitope distribution can provide greater understanding of potential in vivo toxicity.

A number of factors should be taken into account when determining species relevancy. Comparisons of target sequence homology between species can be an appropriate starting point, followed by in vitro assays to make qualitative and quantitative cross-species comparisons of relative target binding affinities and receptor/ligand occupancy and kinetics. Assessments of functional activity are also recommended. Functional activity can be demonstrated in species-specific cell-based systems and/or in vivo pharmacology or toxicology studies. Modulation of a known biologic response or of a PD marker can provide evidence for functional activity to support species relevance. Consideration of species differences in target binding and functional activity in the context of the intended dosing regimens should provide confidence that a model is capable of demonstrating potentially adverse consequences of target modulation. When the target is expressed at very low levels in typical healthy preclinical species (e.g. inflammatory cytokines or tumor antigens), binding affinity and activity in cell-based systems can be sufficient to guide species selection. Tissue cross reactivity in animal tissues is of limited value for species selection. However, in specific cases (i.e. where the approaches described above cannot be used to demonstrate a pharmacologically relevant species) TCR studies can be used to guide
selection of species to be used in toxicology studies by comparison of tissue binding profiles in human and those animal tissues where target binding is expected (see also B.3.3). An animal species which does not express the desired epitope may still be of some relevance for assessing toxicity if comparable unintentional tissue cross-reactivity to humans is demonstrated.

When no relevant species exists, the use of relevant transgenic animals expressing the human receptor or the use of homologous proteins should be considered.

2. Number of species

Safety evaluation programs should normally include two relevant species. However, in certain justified cases one relevant species may suffice (e.g. when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood).

In addition, even where two species may be necessary to characterize toxicity in short term studies, it may be possible to justify the use of only one species for subsequent long term toxicity studies. If there are two pharmacologically relevant species for the clinical candidate (one rodent and one non-rodent), then both species should be used for short-term (up to 1 month duration) general toxicology studies. If the toxicological findings from these studies are similar or the findings are understood from the mechanism of action of the product, then longer-term general toxicity studies in one species are usually considered sufficient. The rodent species should be considered unless there is a scientific rationale for using non-rodents. Studies in two non-rodent species are not appropriate.

The use of one species for all general toxicity studies is justified when the clinical candidate is pharmacologically active in only one species. Studies in a second species with a homologous product (see below) are not considered to add further value for risk assessment and are not recommended.

Transgenic animals
The information gained from use of a transgenic animal model expressing the human receptor is optimized when the interaction of the product and the humanized receptor has similar physiological consequences to those expected in humans.

**Homologous proteins**

While useful information may also be gained from the use of homologous proteins, it should be noted that the production process, range of impurities/contaminants, pharmacokinetics, and exact pharmacological mechanism(s) may differ between the homologous form and the product intended for clinical use.

Studies with homologous proteins can be used for hazard detection and understanding the potential for adverse effects due to exaggerated pharmacology, but are generally not useful for quantitative risk assessment. Therefore, for the purposes of hazard identification it can be possible to conduct safety evaluation studies using a control group and one treatment group, provided there is a scientific justification for the study design and the dose(s) selected (e.g. maximum pharmacological dose).

**Nonclinical testing in a non-relevant species**

Pharmacological/toxicity studies in non-relevant species may be misleading and are generally discouraged. However, where it is not possible to identify a relevant species or to use transgenic animal models or if it is not possible to use a homologous protein for testing purposes, it may still be prudent to assess some aspects of potential toxicity in a limited toxicity evaluation in a single species, e.g. a repeated dose toxicity study of ≤14 days duration that includes an evaluation of important functional endpoints (e.g. cardiovascular and respiratory).

**3. Animal models of disease**

In recent years, there has been much progress in the development of animal models that are thought to be similar to the human disease. These animal models include induced and spontaneous models of disease, gene knockou(t)s or knockin(s), and transgenic animals. These models may provide further insight, not only in determining the pharmacological action of the product, pharmacokinetics, and dosimetry, but may also be useful in the
determination of safety (e.g. evaluation of undesirable promotion of disease progression).

In certain cases, studies performed in animal models of disease may be used as an acceptable alternative to toxicity studies in normal animals. Animal models of disease may be useful in defining toxicity endpoints, selection of clinical indications, and determination of appropriate formulations, route of administration, and treatment regimen. It should be noted that with these models of disease there is often a paucity of historical data for use as a reference when evaluating study results. Therefore, the collection of concurrent control and baseline data is critical to optimize study design.

The scientific justification for the use of these animal models of disease to support safety should be provided.
Appendix 7. Explanatory notes

Note 1: The species-specific profile of embryo-fetal exposure during gestation should be considered in interpreting studies. High molecular weight proteins (>5,000 D) do not cross the placenta by simple diffusion. For monoclonal antibodies with molecular weight as high as 150,000 D, there exists a specific transport mechanism, the neonatal Fc receptor (FcRn) which determines fetal exposure and varies across species. In the NHPs and humans, IgG placental transfer is low in the period of organogenesis and begins to increase in early second trimester, reaching highest levels late in the third trimester. Therefore, standard embryo-fetal studies in NHPs, which are dosed from early pregnancy up to gestation day 50, might not be of value to assess direct embryo-fetal effects in the period of organogenesis, although effects on embryo-fetal development as an indirect result of maternal effects can be evaluated. Furthermore, maternal dosing in NHPs after delivery is generally without relevance as IgG is only excreted in the milk initially (i.e. in the colostrum), and not later during the lactation and nursing phase. Rodents differ from the NHPs and humans, as IgG crosses the yolk sac in rodents by FcRn transport mechanisms and exposure can occur relatively earlier in gestation than with NHPs and humans. In addition, delivery of rodents occurs at a stage of development when the pups are not as mature as the NHP or the human neonate. Therefore, rat/mouse dams should be dosed during lactation in order to expose pups via the milk up to at least day 9 of lactation when the offspring are at an equivalent stage of development as human neonates.

Note 2: The minimum duration of post-natal follow-up should be one month to cover early functional testing (e.g. growth and behaviour). In general, if there is evidence for adverse effects on the immune system (or immune function) in the general toxicology studies, immune function testing in the offspring during the post-partum phase of the enhanced pre/post-natal development (ePPND) study is warranted. When appropriate, immunophenotyping can be obtained as early as post-natal day 28. The duration of post-natal follow-up for assessment of immune function can be 3-6 months depending on the functional tests used.
Neurobehavioural assessment can be limited to clinical behavioural observations.

Instrumental learning calls for a training period, which would result in a post-natal duration of at least 9 months and is not recommended.

**Note 3:** A detailed discussion of the approach to determine group sizes in cynomolgus monkey ePPND studies can be found in Jarvis P et al.: “The Cynomolgous Monkey as a model for Developmental Toxicity Studies: Variability of Pregnancy losses, Statistical power estimates, and Group Size considerations.” Birth Defects Research (Part B) 2010, 89: 175-187. Group sizes in ePPND studies should yield a sufficient number of infants (6-8 per group at post-natal day 7) in order to assess post-natal development and provide the opportunity for specialist evaluation if necessary (e.g. immune system).

Most ePPND studies accrue pregnant animals over weeks and months. Consideration should be given to terminating further accrual of pregnant animals into the study, and adapting the study design (e.g. by Caesarian section) when pre-natal losses in a test item group indicate a treatment-related effect.

Reuse of vehicle-control treated maternal animals is encouraged.

If there is some cause for concern that the mechanism of action might lead to an effect on EFD or pregnancy loss, studies can be conducted in a limited number of animals in order to confirm the hazard.

**Note 4:** An example of an appropriate scientific justification would be a monoclonal antibody which binds a soluble target with a clinical dosing regimen intended to saturate target binding. If such a saturation of target binding can be demonstrated in the animal species selected and there is an up to 10-fold exposure multiple over therapeutic drug levels, a single dose level and control group would provide adequate evidence of hazard to embryo-fetal development.

**Note 5:** Endpoints to be included in an interim report of an ePPND study in NHPs:
- Dam data: survival, clinical observations, bodyweight, gestational exposure data (if available), any specific PD endpoints;
- Pregnancy data: number of pregnant animals started on study, pregnancy status at both the end of organogenesis (gestation day (GD) 50) and at GD100, occurrence of abortions and timing of abortions. There is no need for ultrasound determinations of fetal size in the interim report; these are not considered essential since actual birth weight will be available;

- Pregnancy outcome data: number of live births/still births, infant birth weight, infant survival and bodyweight at day 7 post-partum, qualitative external morphological assessment (i.e. confirming appearance is within normal limits), infant exposure data (if available), any specific PD endpoints in the infant if appropriate.