Monoclonal Antibodies Developed as Anticancer Drugs: EU Clinical Trial Application With Focus on IMPD Requirements

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List of Abbreviations

ADCC	Antibody dependent cell mediated cytotoxicity
ADME	Absorption, Distribution, Metabolism, Excretion
ADR	Adverse drug reaction
AUC	Area under the plasma or serum concentration vs. time curve
BDS	Bulk drug substance
BSC	Best supportive care
BWP	Biologics working party
CA	Competent authority
CR	Complete response
CDC	Complement dependent cytotoxicity
Cmax	Maximum serum concentration
CHMP	Committee for human medicinal products
CMC	Chemistry, manufacturing, control
CP	Centralised procedure
CR	Colorectal cancer
CTA	Clinical trial application
CTD	Common technical document
Da	Dalton
DFS	Disease-free survival (time from randomization until objective tumor recurrence or death from any cause)
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DP	Drug product
DS	Drug substance
EEG	Electroencephalogram
EC	Ethics committee
ECG	Electrocardiogram
EFS	Event-free survival
EFPIA	European Federation of Pharmaceutical Industry and Associations
EGFR	Epidermal growth factor receptor
ELISA	Enzyme linked immunosorbent assay
EMEA	European medicines agency
EOPC	End of production cells
EOPCB	End of production cell bank
EP	European Pharmacopeia
EPAR	European public assessment report
EU	European Union
EudraCT	European clinical trials database
EWP	Efficacy working party
Fc	Constant region (complement binding fragment of antibody)

GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practices
HCP	Host cell protein
HERG	Human ether-a-go-go related gene
HRQoL	Health-related quality of life
IHC	Immunohistochemistry
IB	Investigator´s brochure
ICH	International conference on harmonisation
IEF	Isoelectric focussing
IEC	Ion exchange chromatography
I.V.	Intravenous
IMP	Investigational medicinal product
IMPD	Investigational medicinal product dossier
IDSM	Independent drug safety monitoring board
IPC	In-process control
LoD	Level of detection
LoQ	Level of quantification
mAb	Monoclonal antibody
MW	Molecular weight
PCR	Polymerase chain reaction
MAA	Marketing authorization application
MAH	Marketing authorization holder
MABEL	Minimum anticipated biological effect level
mBC	Metastatic breast cancer
MTD	Maximum tolerated dose
NBE	New biological entity
NCE	New chemical entity
NIMP	Non investigational medicinal product
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NtA	Notice to applicants
OOS	Out of specifications
ORR	Objective response rate (proportion of patients where a CR or PR was observed)
OS	Overall survival (time from randomization to death from any cause)
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PDCO	Paediatric committee
PFS	Progression-free survival (time from randomization until objective tumor progression or death from any cause)
PIP	Paediatric investigation plan
PK	Pharmacokinetics

POC	Proof of concept
PR	Partial response (i.e. tumor parameter reduction >50%)
PSA	Prostate specific antigen
QA	Quality assurance
QOL	Quality of life
QP	Qualified person
QT interval	Time between start of the Q wave and the end of the T wave
SCID	Severe combined immunodeficiency
SDS-PAGE	Sodiumdodecylsulfate polyacrylamide gelelectrophoresis
SEC	Size exclusion chromatography
SmPC	Summary of product characteristics
SUSAR	Suspected unexpected serious adverse reaction
SWP	Safety working party
t1/2	Elimination half life
tg	transgenic
Tmax	Time to reach the maximal concentration Cmax
TSE	Transmissible spongiform encephalopathy
TTF	Time to treatment failure
TTP	Time to progression (time from randomization to discontinuation of treatment for any reason – incl. progression, treatment toxicity and death)
UF/DF	Ultrafiltration/Diafiltration
UK	United Kingdom
VLP	Virus-like particles
Vss	Apparent volume of distribution at steady state
WCB	Working cell bank

1. Introduction

The Directive 2001/20/EC (1) of the European Parliament and of the Council of April 2001 (the so called “Clinical Trials Directive”) set the scene for the harmonisation of requirements for the conduct of clinical trials in the EU. As foreseen by the EU legal system, the member states were to implement the Directive into their national laws by May 1, 2004. According to the current EFPIA status report, the Directive has now been enforced in all EU member states with the exception of Poland (60). GCP requirements were further defined in the follow-up Directive 2005/28/EC (51).

As an important principle, a clinical trial application (CTA) must be submitted to both the concerned national competent authorities (CA) and to the involved ethic committees (EC). Both applications can be submitted in parallel. The positive opinion of the EC and approval of the national CA is required before a sponsor can initiate a clinical trial in an EU member state. The Directive 2001/20/EC foresees after submission of a valid application 60 days for the EC opinion, and up to 90 days for the assessment of the CTA by the national CA. After implementation into their national laws, different CTA review periods with regards to different types of medicinal products exist in some of the member states. Furthermore, there are different lengths of validation periods and time periods for the sponsor to answer to authority objections (52, as an example).

Essential components of the clinical trial application package for both national CA and EC are covering letter, application form, receipt of confirmation of EudraCT number, clinical study protocol, informed consent and investigator´s brochure (IB). There are other documents which are required either only by national CA or by EC. Further details are specified in the European Commission guidelines ENTR/CT1 (3), ENTR/CT2 (50), and in national guidance issued by some member states (6, 52, as an example). Whereas guideline ENTR/CT2 is dealing with the CTA procedure at the ethic committees, ENTR/CT1 is the central guidance document giving advice on procedures, documentation requirements and application forms relevant for the competent authorities.

One of the key elements for clinical trial application is the need to submit to the national CA an “Investigational Medicinal Product Dossier” (IMPD) (3). It is not required to submit an IMPD to the EC. The IMPD should contain relevant information on quality, safety, and if available, previous human experience with the investigational medicinal product (IMP) to be used in the clinical trial. The definition of an IMP is already given in Directive 2001/20/EC, and is further specified in “Guidance on Investigational Medicinal Products (IMPs) and other medicinal products used in Clinical Trials” (2), which is part of Notice to Applicants, Volume

10. As a general principle, an IMPD is required for all investigational medicinal products to be tested in a clinical study, including placebos and reference products. For registered products to be used in the trial, the submission of a simplified IMPD is sufficient (3). A simplified IMPD usually consists of the current approved SmPC, and may be supplemented with additional data. Conditions and requirements for a simplified IMPD are described in ENTR/CT1 (3). Noteworthy, although the IMPD should be regarded as the central core document for the IMP to be used in a clinical trial, as an exception the national authorities of The Netherlands, Greece, Lithuania and Poland do not require an IMPD for clinical trial application.

Monoclonal Antibodies (mAbs) belong to the class of biological/biotechnological medicinal products. These molecules are also called immunoglobulins, and bind to a corresponding antigen in a highly specific manner. Antibodies recognize their antigen with their variable regions. Different modes of action have been reported for mAb therapeutics. Some types of mAbs mediate cytotoxicity through their Fc domain which activates the complement system, or they interact with receptors on antigen-presenting cells, mediating effector functions such as antibody dependent cell mediated cytotoxicity (ADCC), and complement dependent cytotoxicity (CDC). Furthermore, by interaction with their specific cellular target, antibodies have the potential to modulate signalling pathways thereby inducing desired pharmacological effects (11, 12).

However, the use of antibodies to target molecular structures, and the development of antibodies as medicines for human use, have for a long time been hampered by the lack of suitable production methods. This situation changed in the year 1975 by the breakthrough of the invention of the hybridoma technology by Köhler and Milstein (13). This method employs the fusion of B cells derived from the spleen of immunized mice with plasmacytoma tumour cells. The resulting hybrydoma cell clones produce only one specific monoclonal antibody, and have the intrinsic property for indefinite growth in cell culture. Today, by means of modern DNA technology and polymerase chain reaction (PCR) methods, recombinant antibody molecules can be engineered which are produced in large-scale bioreactors.

Initially the first licensed monoclonal antibody products were fully murine, and thus were recognized by the human immune system as foreign antigens. In addition to immunogenicity concerns, murine antibodies are short-lived in humans and may have inefficient effector functions. Consequently, chimeric antibodies were constructed where only the variable regions consisted of murine sequences. As next step in the innovation chain, antibodies were “humanized” by the insertion of human sequences. Today, fully human antibodies can be regarded as state-of-the art approach (12), and advanced phage display and library techniques are employed for generation of those molecules.

At present, information on 17 different licensed mAb therapeutics is available from European Public Assessment Reports (EPARs) on the EMEA website (14). Several mechanisms of action are described for anticancer monoclonal antibodies in the literature (11), including

i) binding to cancer antigens thereby mediating antibody dependent cell-mediated cytotoxicity (ADCC),
ii) targeting of tumour vascularization by interference with growth factors, or growth factor receptors,
iii) modulation of the immune system by neutralization of cytokines, or
iv) antagonistic, or agonistic interaction with membrane-bound receptors to inhibit, or to induce downstream signalling

From a biochemical perspective, mAbs are high molecular weight proteins characterised by complex secondary and tertiary structures. They are subject to post-translational modifications, and show a high degree of inherent microheterogeneity. The manufacturing process is usually very complex, and is divided in upstream processing (production of the mAb in cell culture) and downstream processing (purification of the mAb). Thus, as with other biotechnological-derived products, mAbs usually contain impurities originating from the production cell line (i.e. host cell DNA and host cell proteins), and also other impurities originating from the upstream- or downstream process. Furthermore, product-related impurities such as mAb aggregates or degradation products are normally observed. The upstream manufacturing process usually utilizes complex starting materials that cannot be chemically defined, and often also employs animal-derived materials like e.g. bovine sera.

As a consequence, only minor fluctuations in the manufacturing process can affect critical quality attributes, with potential impact on pharmacokinetics, pharmacodynamics, and toxicity profile of the mAb. Accordingly, the major sections of an IMPD (quality data, non-clinical safety data, efficacy data) characterizing a mAb product are interlinked, and should be looked at as a whole. As proposed by Schneider et al., the development of antibodies as medicines for humans should thus be regarded as an integrated, interdependent “threesome” process (11).

In summary, monoclonal antibodies are typical biological/biotechnological products in terms of manufacturing and product attributes. Antibodies can target cancer antigens in a highly specific way, and thus can possibly be more effective than conventional drugs in treating oncology diseases. Based on their specific properties, mAbs have also a potential for less side effects compared with conventional cytotoxic drugs. Consequently, mAb therapeutics will become increasingly important for the treatment of malignant diseases.

2. Chemistry, Manufacturing and Control

From a regulatory perspective, mAbs belong to the class of biological medicinal products, and can as such be distinguished from conventional medicinal products. A definition for a biological medicinal product is initially given in Annex I of Directive 2001/83/EC, 3.2.1.1.b (15). According to the Directive, “a biological medicinal product is a product, which active substance is a biological substance”. The Directive further clarifies that “a biological substance is produced or extracted from a biological source”. Biological medicinal products are thus complex therapeutics, and cannot fully be characterised by analytical methods alone. For complete product characterisation and determination of its quality, “a combination of physicochemical and biological tests together with the production process and its control is required” (15). Thus, the quality part of the IMPD is pivotal for determination of product performance, and builds the basis for assuring safety and efficacy of the drug.

General IMPD quality data requirements are covered by the EU quality guideline CHMP/QWP/185401/2004, which however does not specifically address biopharmaceuticals (4). Furthermore, essential virus safety requirements are described the new EU draft guideline EMEA/CHMP/BWP/398498/2005 (5). There are only few national guidelines existing which address data requirements for biotechnological investigational medicinal products (6-8). These national guidelines provide important insight in authority expectations, and are very useful for the development of mAb therapeutics. Obviously, the quality requirements for anticancer mAbs in comparison to other biological medicinal products are essentially the same.

For marketing authorisation for biological/biotechnological medicinal products, relevant ICH guidelines Q5A, Q5B, Q5C, Q5D, Q5E, and Q6B (26, 17, 23, 18, 24, 21) need to be followed. However, due to a lack of specific EU or ICH guidance for development of biopharmaceuticals, it is difficult to assess which data should be presented at which stage of development in the IMPD for clinical trial application. Substantial information has already to be available for the first-in-human clinical trial application, while other results might be presented in CTAs for clinical phase III. Particular data might be required only for marketing authorisation, or might even be provided after approval. As a consequence, developmental strategies are often based on previous experience with similar products.

Recently a new EMEA draft guideline “Production and quality control of monoclonal antibodies” (53) has been issued replacing the former outdated guideline “Production and quality control of antibodies” (3AB4A) of 1995 (54). Noteworthy, the draft version of this new guideline currently contains some expectations exceeding even current ICH data

requirements for marketing authorisation (e.g. new specification requirements). Furthermore, two EP monographs (16, 19) are pivotal for marketing authorisation of mAbs in the EU. Manufacturing and quality control of mAbs is addressed in the first monograph (19), and the second monograph is deals with the generation and control of cell substrates derived from recombinant DNA technology.

According to the CTD format, and as proposed in ENTR/CT1 (3) and in several national guidelines (6-10), the quality part of an IMPD should be divided in “Drug substance” (DS) and “Drug product” (DP) parts, and should be further sub-structured in sections S.1-S.7 and P.1-P.8. The following chapters describe pivotal quality data requirements concerning manufacturing, chemistry and control, which have to be provided in an IMPD. Main topics include cell banking, up- and downstream processing, product characterisation and stability, virus safety, and comparability. General data requirements for the IMPD at the different developmental stages are outlined where possible.

2.1 Generation of Cell Banks

In general, mAbs are produced with cell lines generated by incorporation of appropriate recombinant expression constructs. Description of all steps for production of the cell banks has to be provided in the IMPD in section S.2.3 “Control of materials”. For the generation and control of such cell substrates derived from recombinant DNA technology, the above-mentioned monograph of the European Pharmacopeia (16) has to be followed. Furthermore, also ICH guideline Q5B (17) addresses this topic. Both guidance documents are in principle applicable for marketing authorisation, but most of the requirements apply also for development of biopharmaceuticals such as mAbs.

The generation of the Master Cell Bank (MCB) starts with the construction of the expression vector by utilizing PCR and recombinant DNA technology. The final expression vector provides the coding sequence for the recombinant mAb protein, and is transfected into appropriate host cells to generate a production cell line. The steps in the assembly of the expression construct must be described in detail in the IMPD, and with this respect there is no difference to marketing authorisation requirements. The purpose of analysing the expression construct is to verify that the correct coding sequence of the product has been incorporated into the host cell and is maintained during the culture process until the end of production. Source and function of components of the expression construct (e.g. origins of replication, antibiotic resistance genes, promoters, and enhancers) have to be described. Furthermore, a detailed map of the final expression vector has to be shown in the IMPD, and

the nucleotide sequence of the final expression vector used for transfection of parental cells should be verified already for phase I clinical trial application.

The production of mAb products should be based on well-defined Master Cell Banks and Working Cell Banks as described in ICH guideline Q5D (18). The Master Cell Bank (MCB) is usually derived from a cell clone that shows sufficient stability and a high expression level of the desired mAb. Generally, the MCB is generated first in development. The MCB can be prepared directly from the initially selected cell clone, or from a preliminary cell bank derived from the initial clone.

A Working Cell Bank (WCB) can be derived from one or more vials of the MCB, and is typically expanded to several hundred vials of cells. The WCB is normally intended to provide cells for the manufacturing process. Additional WCBs may be generated from the MCB as needed. However, a newly prepared WCB should be appropriately qualified by characterisation and testing before its use in the production process (18). Establishing a WCB is not mandatory at early stages of development. Alternatively, manufacturing runs may be inoculated directly with cells of the MCB, and a WCB might be established later in development (18).

Importantly, cell line history and generation of all cell banks should be described in detail in the IMPD already for the initial phase I clinical trial application. This includes the origin and characterisation of the parental cell line, the steps used for transfection and for clone selection, and expansion of the final cell clone to cell banks. In addition, authenticity testing of the cell lines is usually demonstrated by isoenzyme analysis via PCR analysis.

ICH Q5B requires that the “limit for in vitro cell age for production” (i.e. proposed maximum of generation times) should be based on data derived from production cells expanded to the proposed in vitro cell age or beyond (17). To determine this limit, the phenotypic stability of production cells investigated, which is usually done by measuring the expression rate of the antibody product in cell culture over time.

At the latest for marketing authorisation, a detailed genetic characterisation of the MCB and WCB is required. For genetic characterisation the expression construct is isolated from the cells, and the sequence of the coding region and other important components is verified. Genetic characterisation further includes restriction enzyme mapping or hybridisation techniques to investigate potential deletions, and the determination of copy number and integration sites of the expression construct. It is usually accepted by the authorities that those data will be provided later in development (i.e. between clinical phase II and phase III, according to Merck KGaA experience). However, some information on the integrity of the

desired product should to be provided in the IMPD already at earlier stages of development. This can either be demonstrated by confirming the DNA sequence of the coding region in production cells, or by verification of the amino acid sequence of the purified mAb. Furthermore, end-of-production cells (EOPC) have to be collected after a representative production run, and should be used to generate EOP cell banks (EOPCB). For marketing authorisation, also the EOPCB must be fully characterised. According to Merck KGaA experience, a lack of such data seems to be accepted by the competent authorities at earlier phases in development, but should be available at the latest for clinical phase III.

Already at early stages of development it is essential to evaluate the viral safety of MCB, WCB, and possibly also EOPCB. As most host cell lines contain endogenous viruses, the number of virus particles contained in the cell banks needs to be determined. IMPD requirements are described more in detail in chapter 2.6 “Virus safety testing” in this thesis. In addition to the assessment of potential viral contaminants, the cell banks have to be carefully analysed for sterility (i.e. tests for bacteria, fungi and mycoplasma).

2.2 Upstream- and Downstream Processing

In principle, manufacturing of monoclonal antibodies can be divided in upstream- and downstream processing. The upstream process starts with the inoculation of growth media with cells of the MCB or WCB. The cells are grown in a bioreactor to a definite passage (i.e. number of generation times). During the fermentation process, the mAb protein is expressed the host cells and secreted into the cell culture media. In a “fed-batch” production run, the upstream process ends with the harvest of the cell culture (“bulk harvest”). After filtration or centrifugation of the bulk harvest, the resulting clarified harvest is used as starting material for the downstream purification process.

During downstream purification, impurities like DNA and proteins originating from the host cells and media components are removed. The aim of the purification process is to achieve maximum purity without affecting the biological activity and integrity of the mAb, and with minimal product losses. Protein A affinity chromatography selective for the Fc-region of mAbs is normally used to remove the majority of the host proteins. Subsequent purification steps can e.g. include ion exchange, gel filtration or hydrophobic interaction chromatography. In addition, steps to inactivate/remove viruses are part of the purification process. Purification usually ends with a concentration step, combined with a buffer exchange into a suitable storage buffer, which is followed by sterile filtration. The final purified mAb protein represents “(bulk) drug substance”. The annex of this thesis provides an example for a medium-scale manufacturing process applied by Merck KGaA.

Upstream and downstream manufacturing processes have to be described in detail in the IMPD in chapter S.2 “Manufacture” (6). It is very important to give a batch size definition for the fermentation and a scale for the purification process. Preferably a range for the batch size should be indicated in order to keep some flexibility. Furthermore, a flow chart should be provided illustrating the up- and downstream process and also the respective in process controls (IPCs). IPCs are usually conducted during fermentation, on the bulk harvest, during purification, and on the purified mAb (i.e. drug substance). These can include cell density and viability, bioburden, protein concentration, pH, and virus and mycoplasma testing. IPCs should be described in the IMPD briefly already for phase I clinical trial application. At least for steps critical for product performance, preliminary IPC limits should be set already at early stages of development, but at latest during phase II (Merck KGaA experience). Limits can initially be broad, but are expected by the authorities to be tightened during development.

A formal process validation to demonstrate consistency of up- and downstream processes is not yet required during clinical development. An important exception is the assurance of the purification process for its capacity to remove and inactivate viruses. “Virus validation” of the purification process is required by national authorities for clinical trial authorisation (6), and represents a basic principle of the new EU draft guideline “Virus safety evaluation of biotechnological investigational medicinal products” (5). Thus, a formal virus clearance validation must be presented already for initial phase I clinical trial application. For clinical phase I/II however, the number of investigated model viruses, as compared with ICH Q5A marketing authorisation requirements, can be reduced (see also section 2.6 of this thesis).

It is essential to list in the IMPD in section S.2.3 “Control of materials” all starting materials, with indication of the grade (i.e. compendial or non-compendial), or at least of the purity of the material. Such materials can be growth media, sera and media components used for cell culture, or columns, filters, buffers used in the downstream purification process. It must be clearly indicated in the IMPD if materials are derived of animal or human origin. Importantly, the viral safety of the materials must be assured, and their compliance with the EU guidance for TSE risk minimization (20) has to be demonstrated already for the initial CTA. If possible, raw materials of animal or human origin should be avoided, as it is today considered as state-of-the-art not to use such products for manufacture of mAb therapeutics.

2.3 Characterisation

MAb products are different from small molecules because of their size, complexity and derivation, and are characterised by highly specific secondary and tertiary structures necessary to maintain their functional capacities. Due to translational modifications (e.g.

glycosylation) the final product can be very heterogeneous. Noteworthy, product variants showing a different glycosylation pattern are considered as product-related substances (i.e. isoforms) and not as impurities, provided those have the same biological activity as the desired product. Additional heterogeneity is introduced by product-related impurities caused by de-amidation or by oxidation of amino acids. Further product-related impurities include antibody multimers and aggregates, or cleaved/truncated antibodies.

Thus, thorough characterisation of mAb products is required in order to assure their identity, purity, potency and safety. As a consequence, comprehensive characterisation data (to be provided in section S.3 “Characterisation”) is required in the IMPD already for first-in-human clinical trial application (6, 34). In general, characterisation occurs through the whole lifecycle of a mAb product. While a broad range of assays is used for initial product characterisation, for batch release only parameters relevant for product safety and efficacy are monitored in a reduced number of release assays. Examples for acceptable analytical characterisation methods are given in the appendix of ICH Q6B (21). In general, the characterisation of a mAb should include the determination of physico-chemical properties, biological activity, immunochemical properties, purity, impurities, and contaminants:

- Physico-chemical properties

  A physicochemical characterisation programme generally includes elucidation and conformation of the structure (amino acid sequence), determination of the composition, and assessment of the physical properties of the desired product. In addition, the heterogeneity of the product has to be analysed, which includes the investigation of post-translational modifications (e.g. glycosylation).

- Biological activity (potency)

  The biological activity describes the specific ability of a mAb product to exert its desired biological effect. The bioactivity of a mAbs is measured by utilising a bioassay (other name potency assay). Prerequisite for bioassay development is in-depth knowledge on the mode of action of the mAb and on the biology of the target. Furthermore, robust knowledge on response pathways and downstream signalling should be available. The most common bioassays used for mAb anticancer therapies are cell-based assays that measure cell survival, cell proliferation, or downstream signalling events such as receptor phosphorylation or cytokine release (12). A bioassay represents also a critical tool for batch release, since it monitors the biological activity where changes might not be reflected by physico-chemical characterisation alone. ICH Q6B requires that a correlation between expected clinical efficacy and the bioassay should be established (21). Thus, the bioassay should provide a quantitative measure of biological activity based on the

product attribute(s) that are linked to the relevant biological properties, and should provide a correlate to the expected clinical efficacy (21).

A sponsor should to establish a bioassay already at early stages of development. Since an immunoassay can measure only binding of the antibody to its antigen, it is unable to determine its full functionality and can usually not replace a bioassay (21). Thus, to substitute a bioassay by a binding assay (immunoassay) is a major challenge, and should be thoroughly justified in the IMPD (34).

- Immunochemical properties

Binding assays (immunoassays) to purified antigens and defined regions of antigens should be performed in order to characterize affinity and immunoreactivity of the mAb (21). As a prerequisite, the specific antigen should be biochemically well defined. Specific binding of the antibody to its antigen/epitope is usually demonstrated by employing immunoassays such as Western-Blot, ELISA, or Biacore assay. Binding assays can also be performed to analyse identity, purity, or for quantification purpose. Immunoassays are thus also performed as part of the testing for batch release. A binding assay does not allow the assessment of the biological activity of an antibody, and does normally not substitute for a potency assay. Noteworthy, in situations where no effector functions are involved in the mode of action (i.e. for binding/neutralizing antibodies), the new draft guideline EMEA/CHMP/BWP/157653/2007 accepts the replacement of a potency assay by a binding assay (53).

- Purity, impurities and contaminants

The purity of a mAb product is usually assessed by a combination of analytical methods. Purity testing should assess, at a minimum, the structural integrity, isoform distribution, and biochemical purity. Commonly used methods for assessment of purity (antibody aggregates, antibody degradation products) are SDS-PAGE and SEC (size exclusion chromatography). For the detection and quantification of isoforms, isoelectric focussing (IEF) or ion exchange chromatography (IEC) can be applied. For process-related impurities such as host cell DNA, host cell proteins, and Protein A (which may bleed off protein A columns used for purification), often commercially available ELISA kits are employed.

Validation of analytical methods used for characterisation is linked to the clinical development phase. For initial phase I clinical trial application, only parameters and acceptance limits have to be evaluated, and the suitability of the respective methods should be confirmed. For phase II studies, tabulated summaries showing validation parameters, acceptance criteria and results have to be provided. Analytical methods should be fully

validated for phase III clinical trial application (6). Complete validation reports are usually not attached to the CTA, but have to be submitted to the competent authorities on request (6).

2.4 Specifications

According ICH guidance Q6B (21) specifications are defined as a list of test methods, analytical procedures used, and appropriate acceptance criteria (which can be numerical limits, ranges, or other criteria). Specifications establish the set of criteria to which the mAb product should conform, in order to be considered as acceptable for its intended use as medicinal product. Thus, specifications are part of a total control strategy designed to ensure product quality and consistency. Specifications are critical quality standards that have to be proposed and justified in the IMPD, and product specifications must be approved by the national CA. Standard specifications for parenteral mAb therapeutics include appearance (colour/clarity), identity, purity and impurities, potency, quantity, pH, sterility, endotoxin content, and particulate matters. Depending on the formulation and on the pharmaceutical form of the drug product, additional specifications on residual moisture, preservatives, or excipients might be applicable.

As a general principle, a thorough characterisation of the product during development is a prerequisite for establishing relevant specifications. Specifications should be based on analytical data obtained from several production lots to demonstrate manufacturing consistency. Furthermore, linking specifications to a specific manufacturing process is particular important, especially with regards to product-related and process-related impurities (21). In general, specifications are usually broader at early stages of development and in situations when there is only limited manufacturing experience. Regulatory authorities expect the sponsor to tighten specifications during development, which also needs to be reflected in the IMPD. Specifications are established for drug substance (i.e. the purified mAb), and also for the formulated drug product (usually a solution or a lyophilisate). Specifications for drug substance and drug product are provided in the IMPD in sections S.4 “Control of drug substance”, and P.5 “Control of medicinal product”, respectively.

In principle, a sponsor can differentiate between release and shelf specifications. The concept of release limits vs. shelf-life limits allows the establishment of limits for certain specifications that are tighter for the release than for the shelf-life. According to ICH Q6B (21), acceptable examples may include potency and degradation products, but such approaches should be justified on a case-by-case basis. However, mainly due to a different drug regulation history, agencies in some regions have a diverging view on this approach. In the US as an example, the concept of release vs. shelf life specifications is not accepted for

regulatory purpose. In case of a “global” product development, it might thus be more feasible to apply identical specifications for release/shelf-life.

At early stages of development, it is often difficult to define limits/acceptance criteria, since there is only limited knowledge on manufacturing and product performance. Thus, there is risk that a production lot can not be released since specifications are not met, or that a batch runs out of specifications (OOS) after long-term storage. Based on experience with previous Merck KGaA IMPDs, it may be acceptable during clinical phase I to specify particular impurities such as host cell protein/DNA or protein A as “report results”. During clinical phase II however, limits/acceptance criteria for such typical process-related impurities should be established.

2.5 Stability Testing

ICH guideline Q1A rev “Stability testing of new drug substances and products” (22) covers all types of medicinal products and in principle also biopharmaceuticals. Guideline ICH Q5C applies to “well-characterised proteins or polypeptides”, which also includes mAbs, and gives guidance to applicants regarding the type of stability studies that should be provided for biotechnology products in support of marketing applications (23). In addition, EU guideline CHMP/QWP/185401/2004 (4) and also the German national guidance document (6) provide useful information regarding stability data requirements for IMPs.

The unique characteristics of proteins as compared to small molecules should be reflected in the design of a testing programme to confirm their stability during the intended storage period. Protein products might be particularly sensitive to environmental factors such as temperature, oxidation, light, osmolality, and shear stress. Thus, long-term storage conditions have usually to be very stringent to preserve the integrity of the product. Physicochemical changes that occur over time can alter potency, pharmacokinetics and bioavailability, which can also impact safety and efficacy of the product proposed for clinical trials. Examples include fragmented antibodies, which can show altered clearing rates, or antibody aggregates which can increase the immunogenicity and/or change the PK profile of the product (12).

As outlined in ICH guideline Q5C, the development of mAb therapeutics should include a well-defined testing programme for the drug substance and drug product. Testing should cover physicochemical, biological and microbiological attributes. Stability tests should not be restricted to release assays, but also contain a subset of stability-indicating characterisation methods. Stability protocols must investigate long-term storage at the intended storage conditions (i.e. real-time stability data) as well as accelerated stability conditions. Accelerated

storage conditions increase the rate of degradation and of physicochemical changes, which helps to select appropriate stability indicating tests. Importantly, potential degradation pathways should be identified, but this is usually not expected at early developmental stages. Mechanisms involved in antibody degradation may include deamidation, oxidation, hydrolysis, proteolysis, or disulfide bond breakage.

Drug substance stability data

According to CHMP/QWP/185401/2004, stability data available at the respective stage of development should be summarised in the IMPD in tabular format in section S.7 “Stability”. The parameters known to be critical for stability of the drug substance should be presented, e.g. chemical and physical sensitivity, photosensitivity, or hygroscopicity. Usually, results from studies under long-term conditions and under accelerated storage conditions should be provided. As an important principle, the drug substance stability data presented in the IMPD should cover at a minimum the time period until to further processing to the final drug product.

Drug product stability data

Drug product stability data has to be provided in the IMPD in section P.8 “Stability”. The EU guideline CHMP/QWP/185401/2004 states that the shelf-life should be defined based on the stability profile of drug substance and drug product batches of the IMP. An extrapolation of the shelf-life may be used, provided that stability studies with the clinical trial material are conducted in parallel to the clinical studies and throughout their entire duration. A proposal and justification for a re-test date and for shelf-life extension should be given In the IMPD. As a general principle, stability of the mAb drug product over the entire duration of the clinical trial has to be assured. There are different data requirements for phase I and phase II/III clinical trial application, as outlined in CHMP/QWP/185401/2004 (4) and in the German national guidance document (6):

- For phase I clinical trials, it should be confirmed that an ongoing stability programme is carried out with the relevant batch(es) used for the clinical trial. Before initiating a trial, at least studies under accelerated and long-term storage conditions have to be started. However, accelerated testing may be omitted for biotechnological products if justified (6). Where available, results from these long-term and accelerated studies should be summarised in a tabulated form in the IMPD. In addition, available data of supportive batches that are representative for the clinical trial material should be provided. Finally, an evaluation of available stability data and justification of the proposed shelf-life to be assigned to the IMP in the clinical study is required (6, 4).

- For phase II and phase III trials however, stability data generated with the material proposed for the clinical trial to be presented in the IMPD. All available stability data should be summarised in a tabulated form. An evaluation of the stability data and justification of the proposed shelf-life to be assigned to the IMP in the clinical study should be provided. Data should include results from studies performed under accelerated and long-term storage conditions (6, 4). If justified, accelerated storage conditions might be omitted for biotechnological products (6).

Stability during administration

Pharmaceutical forms for mAb therapeutics can include frozen/liquid solutions, but also lyophilisates that have to be reconstituted prior to use in humans. A dilution step is often involved when preparing the final solution for infusion/injection. Importantly, the stability of the solution administered to clinical trial subjects must be addressed already for the initial clinical trial application. Data demonstrating stability of the mAb during administration should be provided in the "Pharmaceutical development" section P.2.6 of the IMPD. In the same section, also compatibility data investigating the interaction of the product with its container components has to be presented.

Critical product performance criteria to be monitored in administration tests are e.g. protein concentration, potency, binding activity, and particulate matters. Antibody molecules might interact with the plastic material of the administration set-up (i.e. infusion bag, filters, tubing), which can lead to product losses. Adsorption processes and potential product losses might be particular critical if the mAb is highly diluted to prepare small doses or low concentrations. This is especially important to be considered for first-in-human trials, where in general very low doses are used (34). Shear stress induced by reconstitution, dilution, and infusion processes can trigger the formation of particulates. Formulation development should aim to avoid such particulate matters, which are in general not accepted for parenteral drugs. As a last option, during clinical development the use of in-line filters might be acceptable.

2.6 Virus Safety Testing

Assuring the viral safety of biological/biotechnological medicinal products is a complex process, and in-depth assessment of the viral safety is particular important for approval of a clinical trial application. The majority of the studies must be conducted at early stages of development, in order to be able provide comprehensive virus safety data for initial phase I clinical trial application. Thus, state-of-the–art virus safety testing of mAb therapeutics is pivotal for a successful development. Summarized virus safety data should be presented in

IMPD appendix A.2 “Adventitious viruses”. Noteworthy, some national authorities (e.g. France, Germany) require submission of complete virus safety study reports for clinical trial application.

Available guidance documents are ICH Q5A (26), CPMP/BWP/268/95 (27), and the new EU draft “Guideline on virus safety evaluation of biotechnological investigational medicinal products” (5), which also covers mAb drugs. As a general principle, the aim of virus safety studies is to demonstrate an acceptable level of safety for clinical trial subjects. Virus safety is assured by three complementary approaches, involving

(i) testing of all raw materials, and of cell banks (MCB, WCB, EOPCB) for viral contaminants,
(ii) assessment of the capacity of the purification process to remove or inactivate viruses, and
(iii) testing the product at appropriate steps for contaminating viruses (including assessment of unprocessed bulk harvest, purified drug substance, and final drug product

In summary, the principle of assuring viral safety combines direct testing of cell banks, raw materials, and product for viruses, together with validating the manufacturing process for its ability to inactivate/remove adventitious viruses.

Importantly, regarding virus safety testing of cell lines, raw materials and the product, the new EU draft guideline (5) refers to ICH Q5A. With this respect, there is no difference between authorized products and investigational medicinal products used in initial phase I clinical trials. If this requirement would be found also in the final version of the guideline, this would impose a new hurdle for sponsors, as some types of studies (e.g. virus safety testing of EOPC banks) are usually not yet performed for phase I clinical trial application.

The new draft guideline also describes virus validation requirements for the purification process at different stages of development. As an important principle, before initiation of phase III trials, the full programme of virus validation studies according ICH Q5A has to be completed (5). For phase I/II clinical trials however, taking into account the developmental nature of the manufacturing process and of the product, reduced validation studies on virus inactivation/removal are appropriate. More in detail, the new EU draft guideline recommends that, prior to phase I studies, the downstream process should evaluated for its capacity to inactivate/remove at least two model viruses (5). The guideline suggests the investigation of an enveloped virus (e.g. a retrovirus) and of a small non-enveloped virus. Further examples for acceptable model viruses can be found in appendix 2 of ICH Q5A (26). In principle, two

orthogonal inactivation steps should be assessed (e.g. low pH and filtration). Importantly, a reduction of the virus validation programme for phase I/II development must be discussed and justified in the IMPD, and might be based on (5)

i) use/non-use of material of animal origin,
ii) previous manufacturing experience employing the same process, or
iii) published literature results.

As an exception from the rule that study reports are usually not submitted for clinical trial application, the new EU draft guideline states that for virus validation testing of the manufacturing process, “raw data or complete reports may be required” (5). As a new requirement, the new draft guideline expects sponsors to provide an integrated “virus safety risk assessment”, which should cover all raw materials, cell banks, up- and downstream processes and also the final drug product (5). This overall virus safety risk assessment can be included in IMPD appendix A.2 “Adventitious viruses”, and should be based on the calculation of the estimated number of virus particles per administered dose (5).

In general, it is expected that not more than one virus particle is present in one million clinical doses (see ICH Q5A, appendix 5), but there seems to be some flexibility taking into account the nature of the particles. As an example, in case of non-infectious murine virus-like particles (VLPs), a slightly higher particle load might be accepted at early stages of development (Merck KGaA experience). In such a scenario, virus removal/inactivation steps should be optimized when approaching clinical phase III trials at the latest.

2.7 Manufacturing Changes and Comparability

Manufacturing of biotechnological products such as mAbs is a complex process. It is common that manufacturing changes are introduced during development, which can have a potential impact on quality attributes and performance of the product. Reasons for changes include improvement of the manufacturing process, up-scaling, improvement of product stability, or complying with changes in regulatory requirements.

Changes in the expression system, cell culture conditions, or purification can influence the level of product-related substances (i.e. isoforms), and the degree of heterogeneity of the product. Also the profile of product-related impurities (e.g. aggregates, degradation products), or process-related impurities (e.g. HCP, protein A, DNA) can be altered as a result of manufacturing changes. Furthermore, formulation changes in the final drug product can influence product quality attributes and performance. Manufacturing changes for drug

substance should be described in the IMPD in section S.2.6 “Manufacturing process development”, and for drug product in section P.2 “Pharmaceutical development” respectively.

Basically, the concept of comparability requires that physicochemical properties, biological activity and immunochemical properties should be highly similar between pre-change and post-change product. Principles for assessing comparability are addressed in ICH and EU guidelines (24, 25). In case different product quality attributes should be observed, the differences should not alter the safety or efficacy profile of the mAb. In general, a determination of comparability is based on in-depth analytical testing including investigation of biological and immunochemical properties. Importantly, conformity to specifications alone is not considered to be sufficient to establish product comparability. Thus, the analytical comparability exercise should comprise further characterisation assays, which are not part of the standard release testing (24). If a sponsor can provide assurance of comparability through analytical testing alone, further non-clinical or clinical studies are not warranted (25). However, if analytical differences have been detected, and an effect of those differences on safety/efficacy cannot be ruled out, additional non-clinical and/or clinical studies are required to demonstrate comparability (24, 25). As a general principle, all studies should be of comparative nature, directly comparing pre-change with post-change material (25).

As an example, the amount and nature of glycosylation can influence the pharmacokinetic profile of mAbs by decreased or increased clearance rates. As an example, differences in the glycosylation pattern after a manufacturing change may warrant additional bridging PK studies in animals or humans, in order to verify the pharmacokinetic comparability of the post-change product (12). Furthermore, differences in process- or product related impurities or changes in product formulation could alter the safety/efficacy profile of a mAb. As a consequence, the potential risk for unwanted immunogenicity or anaphylactic reactions may be increased, which might require additional preclinical or clinical testing (see also chapters 3.3 and 4.2 of this thesis).

In general, the comparability exercise is determined by the nature and degree of the manufacturing changes, and by the stage of clinical development. Thus, changes introduced after confirmatory clinical trials will require in-depth comparability testing not different to the approach for authorized products. Less comparability testing may be acceptable for manufacturing changes at earlier stages of development, i.e. before the confirmatory trials (25). Importantly, manufacturing changes during confirmatory trials are discouraged, and the EU comparability guideline suggests that the sponsor should seek scientific advice in such a developmental scenario (25).

As a general rule, the material used for non-clinical testing should be representative to the material proposed for human clinical trials. This aspect of product comparability and has to be addressed already in initial phase I clinical trial applications (6, 34), and the potential impact of any differences for extrapolation of the animal findings to humans should be carefully considered in the IMPD.

To create a reviewer-friendly document for submission to the competent authorities, all results of comparability studies should be compiled in a summarising section in the IMPD. From previous Merck KGaA experience, section S.2.6 “Manufacturing process development” might be an appropriate location to provide such comparability data.

2.8 GMP Requirements

Compliance with current GMP principles is an important prerequisite for the manufacture of IMPs. In principle, manufacturing comprises all steps in the production, purification, and formulation of the mAb, which also includes packaging, labelling and distribution of the final drug product. Relevant regulatory documents include Volume 4 “Good Manufacturing Practices” of the rules governing medicinal products in the European Union, in particular Annex 13 (62), and EMEA/410/01 Rev.2 (20).

Importantly, all manufacturing sites and facilities have to be listed in the IMPD, and their compliance to GMP has to be clearly indicated. Thus, it should be assured by the QA system of the sponsor that also external CROs involved in manufacturing of the IMP work in accordance with current GMP principles. Manufacturing authorisation(s) and/or GMP-certificates for all sites and facilities have to be included in the clinical trial application package.

For biotechnological medicinal products, a separate IMPD appendix “A.1 Facilities and equipment”, describing all production facilities and equipment needs to be attached to IMPD, although the level of details provided can be much lower as compared to marketing authorisation application (3, 6). It should be noted that appendix A.1 is not required for chemical pharmaceuticals.

In case the active biological substance is manufactured outside the EU, a statement of the EU qualified person is required declaring that the respective manufacturing site works in accordance with current GMP principles at least equivalent to EU GMP. In such a situation, also a certification of the GMP status of the active substance and a copy of the importers manufacturing authorisation needs to be provided for clinical trial application (3, 6).

3. Preclinical Requirements

The preclinical development programme for mAb therapeutics is different from a testing programme for conventional small molecules. ICH guideline M3 (R1) addresses general principles for the development of non-clinical strategies and on the timing of toxicity studies in relation to the conduct of clinical trials (28). Several general requirements as outlined in ICH M3 are however not warranted, whereas other preclinical testing strategies are unique for biopharmaceutical products such as mAbs.

For anticancer therapies some general non-clinical development approaches are not applicable and different testing strategies have to be followed. The preclinical evaluation of anticancer medicinal products is specifically addressed in EU guideline CPMP/SWP/997/96 (30). However, this guideline primarily concerns small molecules that are presumed to have a direct toxic effect on tumour cells, and is thus not directly applicable to biopharmaceuticals. Important principles for preclinical safety requirements for biotechnological products are described in ICH guideline S6 (29). In addition, general recommendations for early preclinical development of IMPs (including biopharmaceuticals) can be found in the recent EMEA document “Guideline on strategies to identify and mitigate risks for first-in-man human clinical trials with investigational medicinal products” (34).

Noteworthy, the ICH is currently preparing a new tripartite guideline S9 “Preclinical guideline on oncology therapeutic development”, which is planned to cover all therapeutic classes of anticancer products, including biopharmaceuticals. The final S9 concept paper has recently been endorsed by the steering committee (55). A step 4 document is planned to be completed by early 2010 and should provide useful harmonized guidance for non-clinical development of all types of anticancer drugs, which is currently missing.

In general, preclinical studies for anticancer mAbs comprise investigations on mode of action, in vitro and in vivo efficacy, cross-reactivity and safety pharmacology. Furthermore, toxicological and pharmacokinetic properties of the product characterised. The primary goals of preclinical studies for the development of anticancer mAb therapeutics are

i) assessment of mechanism of action,
ii) proof of activity and efficacy using in vitro models, and in vivo animal models,
iii) identification of a safe starting dose in humans,
iv) establishment of appropriate dose escalation schemes and limits for clinical trials,
v) identification of potential target organs to predict toxicity in humans, and
vi) determination of an acceptable risk-benefit ratio for human use

In line with the ENTR-CT1 guidance, the preclinical part of the IMPD 2.2 “Non-clinical pharmacology and toxicology data” comprises the sections 2.2.1 “Pharmacodynamics”, 2.2.2 “Pharmacokinetics”, and 2.2.3 “Toxicology”. (3). As a general principle, only summarizing results of the preclinical studies should be presented in the IMPD, preferably in tabular format. The GLP status of the respective studies should be clearly indicated. Importantly, pivotal toxicology studies (including evaluation of toxicokinetics) and safety pharmacology studies should be conducted in accordance with GLP. There is no requirement to attach preclinical study reports to the IMPD. However, reports should be available at the time of submission of the clinical trial application, since those may have to be provided to the competent authorities on request during the review process (6).

3.1 Pharmacodynamics

Pharmacodynamics of mAb anticancer drugs is addressed by different types of experiments such as mode of action studies, in vitro/in vivo efficacy tests and tissue cross-reactivity studies. Furthermore, results of safety pharmacology studies are presented in the pharmacodynamics section of the IMPD. Investigations can in principle be separated in primary and in secondary pharmacodynamic studies. Primary pharmacodynamics effects are caused by the intended mechanism of action, i.e. by interaction of the mAb with its defined target. Secondary pharmacodynamic effects however can be induced by interaction of the mAb with structurally related epitopes different from the intended target.

Mode of action studies

In general, the degree and the nature of mode of action studies depend on the specific mAb product and needs to be considered on a case-by-case basis. As a prerequisite for mode of action studies, the target (which is usually a cell surface receptor) needs to be clearly defined, and should biochemically and genetically be characterised in detail. This includes knowledge on target sequence, sequence homology to humans, tissue distribution, receptor occupation, and binding affinity and avidity (11, 53). Mode of action studies should also provide insight in the involved cellular signalling events. A comparison of the downstream signalling cascade to the humans is also important for the proof of relevance of the preclinical animal model (see chapter 3.2 of this thesis).

In addition, mAb effector functions such as antibody dependent cell mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) should be elucidated. This includes also potential interaction of the mAb with Fc-receptors, taking into account available information on differences between the animal species and humans (11).

The use of in vitro cell lines and/or primary cell cultures can be useful to examine the direct effects on cellular phenotype and proliferation, a type of study that is often performed for conventional anticancer products. However, due to their unique mode of action, such direct unspecific effects of mAbs are normally not expected. As a prerequisite for development of the mAb in specific cancer indications, also the expression level of the target epitope in human tumours needs to be determined.

In vitro and in vivo efficacy testing

Non-clinical pharmacology studies for antineoplastic agents usually include in vitro and in vivo efficacy testing. To determine anticancer activity mediated by the mAb molecule, the growth inhibition of appropriate tumour cell lines in vitro is investigated. Such experiments can also be part of the screening process to select the most suitable production cell clone, or can serve as basis for bioassay development to measure the biological activity of the mAb. Complementary to in vitro studies, specific in vivo mouse tumour models (e.g. xenograft models) can be investigated to determine the antineoplastic activity under physiological conditions. In xenograft experiments, human malignant cells are transplanted either in nude mice or in immunodeficient (SCID) mice to generate solid tumours. Depending on the experimental set-up, the impact of mAb treatment on tumour growth, eradication of established tumours, or overall survival can be analysed.

Tissue cross-reactivity studies

Based on their physiological function, antibodies might cross-react with other epitopes that have a similar structure as the defined target. The immunological properties of a mAb should thus be described in detail in the IMPD, which includes the antigenic specificity, complement binding activity, and any unintentional reactivity towards human tissues distinct from the intended target (53). It has to be considered that cancer-associated antigens might also be expressed to some extent in normal human tissues. Because binding to non-target tissues might have undesirable consequences, testing of potential tissue cross-reactivity is an important requirement for mAb products. Human cryosections from three individual donors are usually investigated, and various different tissues are screened for potential cross-reactivity with the mAb (53). Results of tissue cross-reactivity studies can help to predict the clinical safety profile, and should be thoroughly considered for monitoring of adverse reactions in initial clinical trials.

Tissue cross-reactivity studies are also performed to identify an appropriate animal species for toxicity testing. In such studies, tissue panels from a variety of animal species like mouse, rat, dog, or non-human primates are investigated, and the results are compared against the human cross-reactivity pattern. The challenges in the approach to select a relevant preclinical animal model are described in this thesis chapter 3.2 “Toxicological testing”.

Safety pharmacology

The aim of safety pharmacology is to reveal undesired pharmacodynamic effects of the mAb on vital organ functions. Safety pharmacology measures functional indices of potential toxicity in relation to exposure within and above the planned therapeutic range. The core battery of studies, as described by ICH guidance, includes the assessment of cardiovascular system, central nervous system and respiratory system. Usually, in-life parameters such as EEG, ECG and respiratory flow are measured. In contrast to toxicity studies, the experimental animals are not sacrificed, since histophathological analysis is not required for assessment of safety pharmacology. Importantly, all studies should be conducted in accordance with GLP principles (6).

Safety pharmacology may also include dedicated in vitro studies such as investigation of cardiotoxicity by utilizing HERG-channel assays as a measure of QT-interval prolongation. Additional studies are only warranted if cardiotoxic signs have been observed in initial standard battery experiments, an outcome which is normally not expected for mAbs. In principle, safety pharmacology studies can either be integrated in the design of repeated dose toxicology studies, or can be performed as separate single studies (28, 29).

Importantly, adequate safety pharmacology information has to be available prior to first human exposure (29). Thus, it is pivotal to perform such studies at early stages in development, and respective results have to be presented in the IMPD for the first-in-human clinical trial application.

3.2 Toxicological Testing

Toxicological testing of mAbs is covered by ICH guideline S6 (29) describing the preclinical safety requirements for biotechnological products. The guideline addresses the use of animal disease models, determines if genotoxicity assays and carcinogenicity studies should be performed, and discusses the impact of antibody formation. Furthermore, ICH guideline M3 recommends standards for non-clinical safety studies which are needed to support human clinical trials of a given scope and duration. However, standard approaches to toxicity testing of pharmaceuticals are usually not appropriate for protein products due to their unique structural and biological properties (29), whereas other requirements are usually not applicable for anticancer drugs. As a consequence, the toxicological testing programme for anticancer mAbs needs to be specifically tailored and must be designed on a case-by-case basis.

General principles of toxicological studies

There are important general principles, which have to be applied for the strategy and design of a toxicological testing program for mAb therapeutics:

- The material used for pharmacology and toxicology testing should be comparable and representative to the product proposed for clinical studies. Importantly, the comparability of the test material should be justified when changes in the manufacturing process or changes in product formulation have been introduced (see also section 2.7 of this thesis). The potential impact of such changes for extrapolation of the animal findings to humans should be carefully considered in the IMPD.

- Safety concerns may arise from insufficient purity of the product, or from the presence of contaminants. Examples are product-related impurities like antibody aggregates, process-related impurities (HCP or host-cell DNA), and microbial/viral contaminants. In principle, it is recommended to rely on a robust and scientifically sound purification process to remove such impurities and contaminants, rather than to establish a preclinical testing programme for their qualification (29). With regards to microbial contaminants/sterility, as for marketed products, the requirements of the European Pharmacopeia are applicable.

- The route and frequency of administration of the mAb product should be identical, or at least as close as possible, to that used in the clinical trial. Consideration should be given to pharmacokinetics and bioavailability of the product in the tested animal species. In situations where clearance is observed, the dose and frequency of administration might be adjusted in the test species. Furthermore, the volume and concentration of the product, which can be safely and ethically administered to test animals needs to, considered (29).

- Dose levels should be selected to provide information on a dose-response relationship, including a toxic dose, a no observed adverse effect level (NOAEL), and possibly a no observed effect level (NOEL). In general, the calculation of the first human dose is based on the NOAEL determined in non-clinical safety studies in the most sensitive and relevant animal species, adjusted with allometric factors, or on the basis of pharmacokinetics. The relevant dose is usually adjusted by appropriate safety factors according to the particular aspects of the molecule and design of the clinical trial. In case factors of risk for potential severe adverse reactions in first-in-human trials are identified, the MABEL (minimal anticipated biological effect level) concept may be more appropriate to calculate an initial human dose. When the

NOAEL and MABEL methods give different estimations of the first human dose, in general the lowest obtained value should be used (34) (see also chapter 4.1).

- Toxicity studies should be performed in compliance with Good Laboratory Practices (GLP). However, some studies employing specialised test systems, which may be used for biotechnological products might not be able to comply fully with GLP. This is acknowledged by competent authorities, but needs to be justified in the IMPD (6, 29).

Selection of relevant animal model

Reflecting their unique mechanism of action, mAbs can be highly species-specific. The identification of an appropriate species for toxicological testing ("relevant species") is thus pivotal to assess preclinical safety of a mAb. A relevant species is an animal species in which the mAb is pharmacologically active due to the expression of the defined target epitope. The relevant species should show a similar tissue cross-reactivity profile as human tissues, since this allows extrapolation of toxicity arising from the binding to the epitope, and also the prediction of any unintentional tissue cross-reactivity (29). ICH guideline S6 further states that toxicity studies in non-relevant species may be misleading and are discouraged. Animal species which do not express the desired target epitope may still have some limited relevance for assessing toxicity, in case comparable unintentional tissue cross-reactivity compared with humans can be demonstrated. However, toxicity caused by the intended pharmacological action of the mAb cannot be investigated in such species that do not express the homologue of the human target. Steps employed in the identification of a relevant animal model are usually as follows:

1. Comparison of DNA and protein sequences of the human target epitope with the animal homologue expressed in the candidate species. Ideally, a very high degree of homology between the animal and human sequences is desired.
2. Expression of the target protein is investigated by IHC in the candidate species, and compared to the expression pattern in humans. Also binding affinity and avidity of the mAb to the human epitope should be comparable with its binding properties to the homologous animal protein.
3. Downstream signalling components and signalling events should also be compared (11), since binding to the target alone doesn´t necessarily imply that there is also the same pharmacological effect in the selected animal species (34).

For some products however, it might not possible to identify an appropriate relevant species. In this scenario, alternative approaches should be considered (29, 34). For example, transgenic mice expressing the desired human epitope might be used for preclinical testing.

However, there is the possibility that the tissue expression pattern of the target protein and/or the downstream signalling events might be different to humans.

Alternatively, a homologous animal model can be employed, which however requires the availability of a homologous surrogate mAb recognizing the corresponding animal epitope. In order to be able to extrapolate the obtained data, the quality attributes of the surrogate mAb should be comparable to the IMP (53). Also differences in downstream signalling pathways might limit the extrapolation of data derived from the surrogate animal model.

Where it is not possible to use transgenic animal models or homologous animal models, it is suggested to assess some aspects of potential toxicity in a limited toxicity study in a single species, e.g. a repeated dose study of < 14 days duration that includes an evaluation of important functional endpoints (e.g. on cardiovascular and respiratory system). In such scenario, it might be acceptable to establish in vitro approaches using human cell lines, ex-vivo experiments using primary human cells, or to use animal models of disease (29, 34).

Single dose and repeated dose toxicity studies

Toxicological programmes should normally include two species, of which one is a non-rodent species. However, according ICH S6 in certain cases only one relevant species may be sufficient (e.g. when only one relevant species exists, or where the biological activity is well understood). In case of mAbs recognizing human epitopes, a single non-human primate species might be sufficient (Merck KGaA experience). Even where two species are used in single dose studies, it may be possible to use only one species for subsequent long-term toxicity studies (e.g. in case the toxicity profile in the two species is comparable in single dose studies) (29). Potential limitations in sample size, as it is often the case for non-human primate studies, may be in part overcome by increasing the frequency and duration of monitoring. Importantly, both genders should be used in the pivotal toxicology studies unless otherwise justified (29).

Single dose studies may provide useful data to describe the relationship of dose to systemic and/or local toxicity. Data from single dose toxicity studies are helpful to select appropriate doses for the repeated dose toxicity studies. Safety pharmacology or local tolerance parameters might also be integrated in the design of the single dose studies (29). However, in case of mAb products, it might be acceptable not to perform single dose testing, and instead to proceed directly with repeated dose studies (Merck KGaA experience). In general, the route and dosing regimen should reflect the intended clinical use and exposure as close as possible (29). Studies should also include toxicokinetic investigations to assure exposure of the animals to the mAb (see also chapter 3.3 of this thesis). The duration of the pivotal repeated dose studies should be based on the intended duration of the clinical trial and the

indication. General guidance on duration of the pivotal repeated dose toxicity studies is given in ICH M3 (28). According to ICH S6, and different from requirements for conventional small molecules, the duration of animal dosing is normally 1-3 months for biotechnological products. For biopharmaceuticals intended for short-term use (< to 7 days) and also for life-threatening diseases, repeated dose studies up to two weeks are adequate to support clinical studies. For biotechnological products intended to treat chronic indications, studies of 6 months duration are suggested (29). However, pivotal toxicological studies for mAb products should be considered on a case-by-case basis, and there might be situations where even longer durations (e.g. 9 months) might be appropriate to support confirmatory trials. The duration of the long-term toxicity studies needs to be justified in the IMPD (6).

Local tolerance studies

In addition to single dose and/or repeated dose studies, other dedicated toxicity studies should be performed as part of a toxicological programme for a mAb. Before the first human exposure, local tolerance studies have to be conducted using the intended route of clinical administration (28, 29). Importantly, the formulation intended for clinical testing should be tested in such studies (29). The assessment of local tolerance might also be included in other studies, i.e. in single or repeated dose toxicity studies. The results of local tolerance studies have to be presented in the IMPD for initial phase I clinical trial application.

Genotoxcity/Carcinogenicity

For chemical pharmaceuticals, in vitro tests for the evaluation of mutations and chromosomal damage are generally needed before first human exposure, and the standard battery of genotoxicity testing should be completed prior to phase II clinical trials (28). Genotoxicity testing usually does not apply for biotechnological products, as it is not expected that proteins directly interact with chromatin (6, 28). Likewise, carcinogenicity studies are also not adequate for protein products. Dependent on the on the mechanism of action and quality attributes of mAbs, a reduced testing programme might still be required. However, applying ICH standard battery approaches may often not be feasible, and product-specific methods have to be developed. Examples for potential genotoxic concerns with regards to mAb therapeutics include

- potential indirect mutagenic properties (e.g. an interference with cellular DNA-repair mechanisms),
- mechanisms involving the stimulation or induction of cell proliferation, or
- pegylation or organic linker molecules present in the product (organic molecules usually require a standard battery testing approach).

Reproductive/Developmental toxicity

According to ICH guideline S6, the need for reproductive/developmental toxicological studies is dependent on the product, the clinical indication, the phase of clinical development, and the intended patient population. In general, assessment of embryo-fetal development should be completed prior to phase I trials in women of childbearing potential (28). However, in case of life-threatening diseases, it might be acceptable to include women of childbearing potential in the trial without prior assessment of embryo-fetal toxicity, provided that highly effective contraceptive measures are applied during the study. Furthermore, a first assessment of male fertility needs to be provided in the IMPD prior to first exposure in humans (6). Comparable to local toxicity testing approaches, the evaluation of male fertility might be integrated repeat-dose toxicology studies.

Immunotoxicity

An important aspect of immunotoxicity concerns the assessment of potential immunogenicity (i.e. formation of anti-drug antibodies), which is addressed in chapter 3.3 in this thesis. In addition to antibody responses, also cell-mediated immunity might play a role for some types of mAb products. For antibodies intended to stimulate or to suppress the immune system, it might be required to perform dedicated immunotoxicity studies to investigate potential effects on cell-mediated immunity. Inflammatory reactions at the injection site might be indicative of an unintended stimulatory response or reaction, but could also be caused by direct toxic effects or by formulation components. However, routine testing approaches or standard batteries as described in the guidelines are not suitable for biotechnological products such as mAbs, and usually product-specific methods need to be developed.

3.3 Pharmacokinetics, Toxicokinetics, and Immunogenicity

The pharmacokinetic (PK) investigation of mAb products is different from that for chemical pharmaceuticals, since classical ADME (absorption, distribution, metabolism, excretion) studies are usually not warranted for proteins. Pharmacokinetic evaluation of mAb therapeutics usually comprises single dose and multiple dose pharmacokinetic studies, toxicokinetic studies investigating the exposure of the animals during toxicological testing, and. At later stages of development, tissue distribution studies and elucidation of main elimination pathways are performed, which is also part of clinical PK. As an important principle, the pharmacokinetic investigations have to be performed in the relevant animal species (29). In addition, potential immunogenicity of the mAb should be tested in the relevant animal, although it is difficult if not impossible to translate observed immunogenic effects to clinical trial subjects.

Pharmacokinetics

PK studies should use preparations representative for toxicological testing and subsequent clinical trials, and should follow the same route of administration as in the clinical studies (29). The bioanalytical assays employed for PK studies have to be fully validated to support a clinical trial application. For proteins such as mAbs a standard ADME testing programme is usually not warranted. In case the mAb is administered via the intravenous route, bioavailability and absorption studies usually not necessary. Tissue distribution studies in the relevant species might be useful, but are normally not required for phase I clinical trial application. The typical metabolic pathway of proteins is proteolysis leading to degradation into peptides and amino acids, and the involved steps are in general well understood. Standard biotransformation studies as performed for chemical pharmaceuticals are thus not required. Mass-balance studies are usually not warranted, as proteins are not necessarily recovered in urine or faces as intact molecules, but are instead reabsorbed, metabolised and used for protein synthesis (29). Main elimination pathways for the mAb should be identified at later stages of clinical development. Importantly, elimination pathways should also be addressed in the clinical PK studies (see chapter 4.2 of this thesis). In the PK studies, the dose-concentration exposure relationship has to be demonstrated. In general, single dose and multiple dose PK studies are conducted to determine the systemic exposure, measuring standard PK parameters such as Tmax, Cmax, AUC, t1/2, Vss and clearance.

Toxicokinetics

As an important principle, at the pivotal toxicological studies that support a clinical trial should be accompanied by toxicokinetic PK studies. Also pilot toxicology studies might require toxicokinetics to design appropriate pivotal toxicity studies. Toxicokinetics investigates the systemic exposure during repeated-dose toxicity testing, which is prerequisite for evaluation of the obtained results. Obviously, only where sufficient systemic exposure to the mAb can be demonstrated, toxicology studies can provide meaningful results. Toxicokinetic PK studies should be performed in accordance with GLP principles, considering “Note for guidance on toxicokinetics: The assessment of systemic exposure in toxicity studies” (31).

Immunogenicity

MAb therapeutics containing human protein sequences can be highly immunogenic in animals, and the detection of anti-drug antibodies during preclinical testing is a frequent phenomenon. Unwanted immune responses are likely to occur during toxicological testing, in particular when mAbs are administered repeatedly to animals. Antibody responses might either lead to increased or decreased clearance rates, and thus can alter AUC and exposure. Due to increased clearance rates the pharmacokinetic profile might be changed, which can affect also toxicological outcomes. Furthermore, immune reactions directed against the

variable regions of the mAb (so called “neutralizing antibodies”) may prevent binding to the target epitope and might neutralize the intended pharmacological activity. In such situations, toxicological studies would not be expected to provide meaningful results.

Thus, comprehensive immunogenicity data in the relevant animal species is essential for the interpretation of results derived from toxicity studies. Antibody responses should thoroughly be characterised, and their appearance has to be correlated with any observed pharmacological or toxicological changes. (29). However, detected antibody responses might also be transient or without any consequence on pharmacological activity or pharmacokinetic profile. Such antibody responses are not expected to affect the outcome of the toxicological studies. In conclusion, observed immunogenicity should not result in premature termination of toxicology studies, unless the antibody response neutralizes the activity of the product, or leads to an increased elimination of the mAb from the circulation.

Immunogenicity should also be considered with regards to potential differences in product attributes after manufacturing changes (see section 2.7 of this thesis). Noteworthy, altered quality attributes might also influence immunogenic properties and PK profile of the mAb. Also formulation, pharmaceutical form and route and frequency of administration can have an impact on immunogenic properties of the product. The reason for this phenomenon is at present not very well understood. It is assumed that e.g. changes in glycosylation or impurity profiles (i.e. degradation products, aggregates, host-cell derived impurities) can influence immunogenicity. Consequently, when changes in critical quality attributes are observed after a manufacturing change, immunogenic and pharmacokinetic properties should be re-assessed in side-by-side comparison with the pre-change material (25).

Due to the inevitable immunogenicity of human proteins in animals, the predictive value of animal models is rather low. The consequences of immune reactions in humans range from transient appearance of antibodies without any clinical significance to severe life-threatening conditions, although the latter is unlikely for mAb products. In order to identify suitable strategies for clinical phase III immunogenicity testing, it is important to implement already in phase I/II trials approaches to determine potential immune responses (32, 33). At later stages of development, the bioanalytical assays employed in clinical trials should be able to distinguish between neutralizing and non-neutralizing antibodies. However, it should be noted that there is no clear regulatory guidance at which stage of development such neutralizing mAb assays have to be implemented. In general, the strategy will be determined by the expected clinical outcomes of neutralizing antibodies, and should be decided on a case-by-case basis (see also chapter 4.2 of this thesis).

4. Clinical Particulars

In general, the clinical development of mAbs as anticancer medicines follows the approach applied for conventional antineoplastic drugs. All clinical trials studies have to be conducted in accordance with GCP principles, and applicable ICH and EU guidance has to be followed. Differences to development of small molecules can be found primarily at early clinical stages, and concern e.g. investigation of unwanted immunogenicity, dose escalation strategies, and approaches to establish the therapeutic dose range for phase II. There are fewer differences in the approach to confirmatory trials, although the design of phase II studies might be special for mAb anticancer therapeutics. Also investigation of pharmacokinetics and immunogenic properties is different from strategies applied for chemical pharmaceuticals. However, general criteria for establishing clinical benefit in confirmatory trials do not differ.

The pivotal document for clinical development of anticancer mAbs is the recently revised EU guideline “Evaluation of anticancer medicinal products in man” CPMP/EWP/205/95/Rev.3 (35). This guideline covers cytotoxic and non-cytotoxic compounds, and is thus also applicable for mAb anticancer drugs. Additional information with regards to paediatric drug development and childhood malignancies are provided in “Addendum on paediatric oncology” (36) as appendix to this guideline. Importantly, as for all investigational medicinal products, the development of mAb pharmaceuticals must follow the new EU paediatric regulation, which came into force in January 2007 (37).

In the approach to first-in human trials, the new document “Guideline on strategies to identify and mitigate risks for first-in-man human clinical trials with investigational medicinal products” (EMEA/CHMP/SWP/294648/2007) (34) needs to be considered. This guideline assists sponsors in the transition phase from preclinical testing to the first administration in humans, and is applicable for all types of investigational medicinal products. Importantly, the sponsor should identify potential risk factors involved with first human administration of the IMP, and implement appropriate risk mitigation strategies if appropriate. Risk criteria as outlined in the new guideline should be discussed in the initial clinical trial application (34).

In accordance with the ENTR/CT1 guidance, relevant clinical information is provided in the IMPD in section 2.3 “Clinical data” (3). Obviously, an IMPD for a first-in-human clinical trial does include data on previous clinical use, and the clinical information provided is limited to section 2.3.4 “Risks benefit assessment”. At later stages of clinical development, also IMPD sections 2.3.1 “Clinical pharmacology”, 2.3.2 “Clinical pharmacokinetics”, and 2.3.3 “Human exposure” are required to support a clinical trial application.

4.1 First-in-Human Trials

The transition from preclinical testing to first human subjects is a particular critical step in development. A severe incident during a first-in-human trial in March 2006 in the UK with TeGenero´s antibody TGN1412 showed that there are molecules with higher risk potential than others for first human exposure. The sponsor followed apparently all relevant regulatory guidelines, and responsible EC and national CA on the grounds of a positive risk-benefit assessment approved the clinical trial. However, clinical trial subjects exposed to TGN1412 developed life-threatening adverse reactions due to cytokine release syndrome and acute shock (11, 38). This sad accident raised a lot of questions, which have not sufficiently been answered until today. Apparently, the claimed relevant animal model used for toxicological testing was not appropriate to predict a safe starting dose, and thus the first human dose calculated with the NOAEL approach was too high. Furthermore, the design of the clinical trial was criticised since the initial dose of TGN1412 was administered nearly simultaneously to all eight healthy volunteers in the same cohort.

As a direct consequence of the TGN1412 incident, an expert group established in the UK issued a report in order to increase the safety of future first-in-man trials of novel compounds (so called “Duff-Report”). The expert group made 22 recommendations covering non-clinical and early clinical development, determination and administration of the initial starting dose in man, clinical environment for first-in-man studies, and for development of specific expertise (39).

Furthermore, Schneider et al. of the German national competent authority PEI suggested three criteria for identifying mAb products that would warrant “more stringent regulatory oversight” (38), and indicated that data requirements could in future be substantially higher for those products. A subsequent publication of other PEI assessors suggests concrete safety measures, such as introduction of criteria for high-risk antibodies and sequential inclusion of trial participants (57). The principles for potential “high-risk antibodies” as proposed by Schneider et al. (38) were:

i) the antibody employs a new mechanism of action,
ii) the antibody addresses a target that lacks appropriate animal models, and
iii) the antibody comprises a new type of engineered structural format (e.g. changes in amino acid sequence, or recombinant fusion proteins).

As a final consequence of the TGN1412 disaster, a new EU guideline “Strategies to identify and mitigate risks for first-in-man human clinical trials with investigational medicinal products” was issued (34). This new guideline is applicable for all types of IMPs and covers both small

molecules and biopharmaceuticals. According to this document, predicting potential severe adverse reactions for the first-in-human use of an investigational medicinal product involves identification of the factors of risk. Particular concerns might be derived from:

(i) the mode of action of the IMP,
(ii) the nature of the target, and/or
(iii) the relevance of animal models.

Importantly, when planning a first-in-human clinical trial, sponsors should identify potential factors of risk and apply risk mitigation strategies as laid down in the guideline (34). The new guidance document further outlines quality, non-clinical and clinical aspects to be considered in the approach to initial clinical testing, and suggests potential risk-mitigation strategies for the first-in-human trial.

Quality aspects

Key issues to be considered for a potential high-risk medicinal product include determination of strength and potency, characterisation, comparability with the material used in preclinical studies, and reliability of very small doses (34). Noteworthy, these are clearly general requirements essential for development of all types of biopharmaceuticals (described in this thesis in chapters 2.3, 2.5, 2.7, and 3.2). However, it can be expected that in case of a potential “high-risk” antibody, the lack of essential data in the IMPD (e.g. the absence of a bioassay) and might lead to authority objections or even to a rejection of a clinical trial application. In case substantial data would be missing in the IMPD for a proposed “low-risk” antibody product, there might be more possibilities for negotiations with the competent authorities during the CTA review process.

Non-clinical aspects

A state-of-the-art preclinical testing approach should be followed for all IMPs, which includes extensive primary and secondary pharmacodynamic investigations such as receptor binding and occupancy, duration of effect, and dose-response curves (34). In particular, for medicinal products targeting the immune system, potential unintended effects should be investigated, e.g. by performing in vitro studies using human material. In the new guideline there is a special focus on the identification of appropriate animal models for toxicological testing. The demonstration of relevance of the preclinical animal model should include a comparison of pharmacodynamics (receptor homology, receptor binding and downstream signalling, including additional functional domains like Fc receptor binding), and cross-reactivity studies using human and animal tissues (34).

For the calculation of a safe first human dose, the concept of the minimum anticipated biological effect level (MABEL) is introduced by the new guideline (34). The MABEL should be determined on the basis of a PK/PD relationship by utilising all relevant in vitro and in vivo information from pharmacodynamic and pharmacokinetic data, such as:

i) target binding and receptor occupancy studies in vitro in target cells from human and in the relevant animal species/model,
ii) concentration-response curves in vitro in target cells from human and in the relevant animal species/model, and dose/exposure-response in vivo in the relevant animal species/model, and
iii) exposures at pharmacological doses in the relevant animal species/model.

Safety factors are usually applied for the calculation of the first human dose from MABEL. The calculation of safety factors should take into account criteria of risks such as the novelty of the active substance, its biological potency and mode of action, the degree of species specificity, and the shape of the dose-response curve (PK/PD relationship). However, particular for anticancer medicinal products a, PK/PD relationship may be often difficult to establish due to the lack of appropriate PD parameters as surrogates for expected clinical efficacy. Biomarkers which might fulfil this role are difficult to identify, and are often not yet available at early stages of development.

Clinical risk mitigation strategies

As outlined in the new guideline, key aspects to be considered for planning and conduct of first-in-human trials with potential high-risk products include

i) the risks associated with the type of medicinal product,
ii) the molecular target,
iii) immediate and long term toxicity,
iv) the expression of the target (in healthy volunteers or in patients only), and
v) the possibly higher variability in patients.

In general, subjects participating in a first-in-man trial are not expected to derive any clinical benefit from their participation. The selection of the study population (i.e. healthy subjects vs. patients) should thus be fully justified on a case-by-case basis (34). Anticancer mAbs might be administered to healthy subjects due to their favourable toxic profiles, which is different from development of conventional cytotoxic drugs. However, PK profiles and safety outcomes might not be clinically meaningful in healthy volunteers that show a reduced expression of the target antigen, and may thus difficult to be translated to patients. In

general, for mAbs targeting cancer antigens often patients are selected as population for the initial clinical trial.

The route and the frequency of administration need carefully to be considered. In the case of a planned I.V. administration of the product, a slow infusion over several hours could be more appropriate than a slow bolus over several minutes. A slow infusion would allow continuous monitoring for any adverse reactions during administration, and thus allow a discontinuation of the infusion in order to prevent a serious clinical outcome.

The new EU guideline recommends a sequential dose administration design within each cohort in order to minimise any unpredictable risks. The sponsor must justify non-sequential dose administration within each cohort. Furthermore, all results from all subjects of the preceding cohorts need to be carefully analysed before initiation of the next dose cohort. Importantly, the suggested dose escalation scheme should reflect dose/toxicity or dose/effect relations observed in the non-clinical studies (34).

Stopping rules must be defined for an individual subject, for cohorts and for the clinical trial. The trial design should provide a specific plan to monitor for adverse events or adverse reactions, and the clinical trial staff should be trained appropriately how to identify and to respond to these reactions. Special consideration should be given to potential long-term consequences on organ systems, and to potential long-term safety problems. Thus, long-term monitoring might be required for potential high-risk medicinal products in the follow-up period of the trial. The sponsor should justify the length of the monitoring period as part of the risks managing strategy applied for the trial (34).

The guideline further recommends that the first human administration of potential high-risk products should take place in suitable clinical facilities. Trials should be conducted by medical staff with appropriate level of training and expertise and sufficient understanding of the investigational medicinal product, its target and mechanism of action. There should be an immediate access to facilities for the treatment of medical emergencies that might occur during the trial. Dose escalation trials with potential high-risk medicinal products should preferably be conducted as single protocol/single site studies, since this helps to minimise the overall risks and aids to the safety of all trial participants (34).

The monitoring and reporting of suspected unexpected serious adverse reactions (SUSARs) are particularly important. It has to be ensured that appropriate processes for expedited reporting of SUSARs are in place before the start of the first-in-human study. As with all IMPs, the SUSARs have to be reported to the EudraVigilance clinical trial module in accordance with Directive 2001/20/EC (1) and chapter 2 of volume 10 of NtA.

4.2 Clinical Pharmacokinetics of Therapeutic Proteins

The main objective of pharmacokinetic investigation of medicinal products is to contribute to assurance of efficacy and safety of subjects participating in clinical trials. Generally, the pharmacokinetics of biopharmaceuticals such as mAbs should be evaluated based on essentially the same scientific grounds as for small molecules. However, the unique features of therapeutic proteins as compared to conventional NCEs have to be reflected in the design of the PK studies. The pharmacokinetic evaluation of proteins such as mAbs has long been hampered from limitations in assay methodology and derived pharmacokinetic parameters. Today, substantial progress in bioanalytical methods allows appropriate investigation of those products, which is also outlined in the new EU guideline “Clinical investigation of the pharmacokinetics of therapeutic proteins” (40). For clinical PK studies, also available NtA guidance (41) and ICH guidelines need to be followed, although these documents do not specifically address biotechnological products. With respect to analysis of potential immunogenicity of protein therapeutics, a recent EU guideline (32) and two ”white paper” publications (33, 56) can be considered as essential core documents.

General aspects of clinical PK studies

In general, the same principles as for small molecules apply for PK studies with mAbs. The pharmacokinetic profile should be characterised during single-dose and steady-state conditions (i.e. multiple dosing) in relevant populations. A justification of investigating healthy volunteers vs. patients should be given, since over-expression of the target in patients (or its absence in healthy volunteers) might impact the pharmacokinetic profile of the mAb product. Absorption studies are usually not warranted if the route of administration is exclusively I.V. Changes in route of administration during development may alter the PK profile, and need to be addressed in appropriate studies.

Main elimination pathways have to be defined at later stages of clinical development. With regards to elimination of protein therapeutics, smaller polypeptides of MW < 50,000 Da are usually eliminated through renal filtration, followed by tubular re-absorption and subsequent catabolism. For larger proteins like antibodies, elimination in other tissues and/or in target cells (e.g. by receptor-mediated endocytosis) followed by metabolic catabolism plays a more important role. Dedicated mass-balance studies are not useful due to metabolism and re-use of amino acids for protein synthesis.

Smaller Vss (steady state volumes of distribution) of therapeutic proteins do not necessarily correspond to low tissue penetration, since there is an inverse correlation between observed Vss and the MW of the protein. Unlike conventional molecules, distribution to tissues is often part of elimination and not part of the distribution process, which contributes to small

distribution volumes of mAbs (40). Although plasma levels might often be rather low, adequate therapeutic concentrations of the mAb is achieved by specific localisation to its target antigen on malignant cells.

The dose-concentration relationship of therapeutic proteins may be non-proportional. In case of targeted mAb products, a saturable elimination pathway may dominate at lower doses, which is determined by expression rate and density of the target epitope. Time-dependent changes in pharmacokinetic parameters may occur during multiple-dose treatment, e.g. due to down- or up-regulation of receptors responsible for cellular uptake, or by formation of anti-drug antibodies which influence clearance rates. Standard interaction studies as for small molecules addressing e.g. the cytochrome P450 system are not warranted for mAb products, since antibodies are not metabolized via these pathways (40).

Special attention should be given to potential inter-subject variability. Sources of variability might be formation of anti-drug antibodies, absorption variability, variability in target expression and target density, and variability in degradation rate or in degradation pattern. Clinical pharmacokinetics should include studies to support the approval in special sub-populations as in patients with organ dysfunction. E.g. for patients suffering from renal and hepatic impairment, specific guidance on the conduct of pharmacokinetic studies is available (42, 43). Data might also be derived from conventional pharmacokinetic studies in a specific population, or from phase II/III studies conducting population pharmacokinetic analysis.

Bioanalytical assays

The bioanalytical methods employed are key components in the investigation of clinical PK. The new EU guideline (40) recommends combination of a bioassay with a binding assay in pharmacokinetic studies. Since a binding assay detects not only intact mAb molecules, but also structurally related compounds (i.e. antibody aggregates or degradants) regardless whether these are active or not, a complementary bioassay or other appropriate assay should be used to ensure the activity of the detected antibody molecules (40).

Bioanalytical methods for the investigation of clinical PK are often derived from immunoassays and bioassays developed for product characterisation and batch release (see chapter 2.3 of this thesis). However, potential issues need to be considered when adapting those assays for pharmacokinetic testing. With regards to the immunoassay, there might be interference by other product-related immunoactive molecules, or interaction with anti-drug antibodies that are binding to the mAb. Matrix effects and interference with endogenous proteins and plasma components may also be observed. Compared to immunoassays, a typical bioassay may exhibit low precision and sensitivity, and might not be specific for the

mAb analyte. Furthermore, the presence of plasma proteins can change the bioactivity of the mAb, or influence the overall performance of the bioassay.

Importantly, suitable and pure reference material serving as calibration standard has to be available for the development of bioassays. Care should be taken to ensure that this reference material is representative to the material used in clinical trials (40), which however can be a challenge at early stages of development.

Bioanalytical assays should be fully validated before their use in clinical trials. One must distinguish between pre-study validation of assays, which includes investigation of the stability of the analyte in matrix, specificity, accuracy, precision, LoD/LoQ, and dose-response relationship; and with-in study validation of assays with control samples to confirm correct method performance (40).

Unwanted immunogenicity

Antibody responses elicited by mAb therapeutics occur frequently during repeated exposure in humans, and can alter PD and PK profiles of the product. The immunogenic properties of protein therapeutics may be influenced by many factors, such as expression system, purification process, final formulation and route of administration. Potential immunogenicity in humans cannot be predicted from animal studies (see also chapter 3.3 of this thesis). Thus, data should be collected in clinical trials from a sufficient number of subjects to investigate a potential immune response against the mAb.

Immunogenicity testing is especially important for multi-dose or long-term treatment. Data should be collected early during phase I/II, in order to guide planning of pivotal Phase III studies. As general principle, due to variability of individuals, samples should be collected from the same subject pre- and post-dosing. At a minimum, sampling during phase III studies should take place after the first and last dose to compare plasma levels, and to compare accumulation in antibody-positive with antibody-negative patients. Samples should be drawn after 6-7 half-lives of the mAb, in order to avoid potential interference of the mAb with the bioanalytical assay (40).

One can distinguish between neutralising and non-neutralising immune responses. Neutralising antibodies bind to the variable, epitope-recognising region of a mAb. As a consequence, binding to its specific target can be inhibited, and the pharmacological activity of a mAb can be neutralised. The PK profile however can be affected independent of the neutralising capacity of the antibodies responses, e.g. by altering the clearance rates of the mAb. Thus, potential effects of immune reactions to mAbs in clinical trials may be a combination of both PD and PK changes. The degree and nature of the immune responses

(i.e. neutralising vs. non-neutralising antibodies) should be fully characterised during clinical development (40, 32).

Consequences of immune responses in humans can range from transient appearance of antibodies without any clinical significance to severe life-threatening conditions. A well-known example is the neutralisation of human endogenous erythropoietin by anti-drug antibodies after repeated administration of recombinant erythropoietin (e.g. epoetin alpha). Thus, if there is risk for severe clinical adverse reactions due to neutralisation of the endogenous counterpart of the therapeutic protein, an assay for detection of neutralising antibodies should be in place already in the first in-man trial (56). However, this is usually not warranted for standard mAb products, since there are in general no human counterparts of mAbs existing. In conclusion, the testing strategy should be guided by the expected clinical outcomes of neutralising antibodies, and should take into account relevant product-specific properties (56).

4.3 Phase I/II Exploratory Trials

As outlined in chapter 4.1, planning and conduct of first-in-human trials needs special caution if potential factors of risk might be involved with first human administration (34). During clinical phase I studies, determination of an optimal therapeutic dose for phase II, dose escalation, and design/endpoints for phase II trials are particular important issues. Unlike for conventional cytotoxic drugs, a strict delineation between phase I and phase II trials may not be appropriate for anticancer mAb medicines. Importantly, measures of anti-tumour activity might be needed already early in development in order to define an optimal dose and schedule for phase II. Key aspects for clinical evaluation of anticancer medicinal products are addressed in a recently revised EU guideline covering both NCEs and NBEs (35).

Dose escalation

Typically, cytotoxic anticancer drugs are studied by a modified Fibonacci scheme. The modification of the Fibonacci sequence used in phase I trials is usually 2n, 3.3n, 5n, 7n, 9n, 12n, 16n as multiples of the initial dose, corresponding to 100%, 65%, 52%, 40%, 29%, 33%, 33% increases over the previous dose (58). Alternatively, a linear dose escalation not exceeding 100% increments is applied for conventional small molecules.

However, due to more favourable safety profiles and wider safety margins, a typical dose-escalation for anticancer mAbs is based on a logarithmic scale with increments in half-logs (e.g. 0.1 mg/m^2, 0.3 mg/m^2, 1.0 mg/m^2, 3.0 mg/m^2). Dose escalation schemes applied in phase I trials for anticancer mAbs are variable, and should take into account all available

preclinical safety data. Thus, in first-in-human clinical trials with potential “high-risk” mAb products a more cautious dose-escalation design might be appropriate (34). In general, administered doses are calculated per m^2 body surface on individual patient basis (35).

Determination of therapeutic dose

A key aspect of phase I trials is to determine an optimal active dose for phase II trials. The phase II dose for small anticancer molecules is commonly defined as the MTD (maximum tolerated dose), which is determined by the incidence of DLTs (dose-limiting toxicities) observed during phase I trials. Thus, toxicity endpoints are used to establish an optimal therapeutic dose for conventional cytotoxic drugs. However, this approach is normally not feasible for mAb therapeutics, since their toxicity profile is very different from that of small molecules. Noteworthy, direct toxicological effects caused by many anticancer mAb drugs are mild and transient (as an example, mAbs targeting EGFR induce skin toxicity as most common adverse reaction).

The phase II dose for anticancer mAbs is often based on the concept of the “optimal biological dose”, which is determined by the assessment of relevant pharmacodynamic effects. In principle, any PD measures predictive for anti-tumour activity (e.g. binding of target epitope, receptor occupation, or downstream signalling) might be employed to establish an optimal therapeutic dose for phase II. As prerequisite, the predictive value of those parameters should have been assured in preceding preclinical PD studies. Other surrogate markers include functional imaging, biomarkers, tumour markers, genomics, proteomics, or immunological outcomes (e.g. changes in lymphocyte populations).

In situations where there is no other possibility to obtain information on the drug exposure-drug activity relationship, biopsies from tumours or in some cases normal tissues might be analysed to obtain data on target saturation or downstream events (35). Even in case the saturation of the target for drug activity can be demonstrated without significant toxicity, it might still be appropriate to investigate higher doses of the mAb to gain more knowledge on the overall safety profile. Before approaching phase II trials and dosing larger patient populations, a suitable target population should be selected with regards to tumour type and expression level of the target. Thus, tumour cells should be screened for expression of the target epitope, and the relationship between target expression and anti-tumour activity should be determined already at early clinical stages (35).

Phase II study designs and endpoints

It may often impossible to predict whether an anticancer mAb product predominantly acts through tumour growth inhibition or by eliciting early tumour shrinkage. Thus, it might be

difficult to determine whether TTP (time to progression) or ORR (objective response rates) will be more appropriate to measure the anti-tumour activity. In situations where only limited knowledge is available, it might be reasonable to assume that TTP more appropriately reflects the anti-tumour activity, and the study should be designed accordingly to investigate TTP as primary endpoint (35). In such studies, documented progressive disease should be an inclusion criterion in the study protocol, and the sponsor should apply short time intervals for tumour assessments. The revised version of the guideline “Evaluation of anticancer medicinal products in man” (35) favours investigation of TTP over PFS (progression-free survival) at early phases in small patient populations. The main argument might be that early deaths that are included in calculation of PFS might reduce the sensitivity of the study to define anti-tumour activity. TTP as primary endpoint has the clear disadvantage that unwanted toxicity of the drug leading to death is not reflected in the analysis, which however should not be an issue for the majority of mAb products. According to the revised EU guideline (35), the following designs using TTP as primary endpoint might be acceptable:

i) randomised dose comparative trial (i.e. comparing different pharmacologically active doses and the effect on TPP),
ii) randomised withdrawal (defined period of experimental therapy with non-progressive disease),
iii) within-patient comparison (TTP after previous therapy compared to TTP with experimental therapy), or
iv) randomised study vs. compound known to be active (or placebo/BSC if justified).

TTP is considered as a function of the underlying tumour growth rate and the activity of the anti-tumour compound. Underlying growth rates however are difficult to define in most patients with progressive disease, and available historical data might not be sufficient to allow robust interpretation of the results. Accordingly, the EU guideline suggests the use of a randomised reference in phase II trials that address TTP as primary endpoint (35). Thus, the sponsor has to justify a TTP based design without internal control. In such situations, the EU guideline recommends a systematic literature review. For example, fixed-time related endpoints such as percentage of patients without progression after a predefined period of experimental therapy may be used, in order to assess the observed anti-tumour activity of the compound (35).

Alternatively, ORR as measure of tumour shrinkage might be used as primary endpoint in phase II clinical trials. Since spontaneous tumour regression fulfilling at least the definition of a partial response (> 50% reduction in tumour parameters) is uncommon, there is usually no randomised control required for interpretation of such studies (35).

Randomized controlled trials as suggested by the EU anticancer guideline clearly show some advantages, such as a broader estimate of benefit and an increased power to detect adverse reactions. However, it should be noted that these studies are longer, larger and more expensive as compared with single arm clinical trials. Furthermore, there is risk that group sizes in randomized controlled studies might not be large enough to prevent selection biases. If a randomised study design is employed and if blinding of the trial is possible, the use of generally accepted tools to estimate health-related quality of life (HRQoL) or symptom control is recommended, since this may provide valuable additional information (35).

4.4 Confirmatory Clinical Trials

In principle, confirmatory trials for anticancer mAb products follow the same approach as for conventional small molecules. Prerequisite for initiation of confirmatory trials is sufficient clinical experience in terms of safety and anti-tumour activity in relation to dose and schedule in the defined population. Studies are designed in order to establish the risk-benefit profile in a well-defined target population, and the results should clearly demonstrate clinical benefit. Target population and reference regimen (or BSC) should be defined by disease, disease stage, and prior lines of therapy. Confirmatory phase III trials usually serve as pivotal studies for marketing authorisation, and should thus be designed as randomised controlled trials (35). However, also phase II or phase II/III designs might be acceptable in the EU as pivotal registration trials (see discussion)

Eligibility criteria, randomisation and blinding

In general, any exclusion criteria have to be justified from the perspective of patients participating in the study and to be treated in clinical practice. Thus, patients representative for those likely to be treated in clinical practice should be included in confirmatory trials. Patients are expected to be characterised e.g. by relevant tumour parameters, previous lines of therapy, performance status, co-morbidity, and organ dysfunction (35). If exploratory studies provide information for including/excluding patients based on the characterisation of the tumour-type (e.g. polymorphism or expression-level of the target epitope), this is acceptable for the phase III trial design. Furthermore, a stratification strategy based on established prognostic covariates is suggested (35). Randomisation and stratification should follow the general principles as described in current guidelines (46, 47).

The EU guideline recommends the blinding of confirmatory trials, if applicable. However, a double-blind design is often not feasible due to obvious differences in toxicity between regimens, or due to safety concerns. A double-blind design might be applicable in case study regimens with comparable toxicity are used in both treatment and control arm. Due to the

known limitations of open-label study designs (e.g. potential bias, choice of study endpoints), an open-label approach in confirmatory trials needs to be justified by the sponsor (35).

Regulatory endpoints for confirmatory trials

Primary endpoints recommended by the EU guideline are mainly overall survival (OS) and progression free survival or disease free interval (PFS/DFS) (35). In general, PFS is more frequently employed as primary endpoint. DFS is an accepted endpoint in particular adjuvant settings, where patients are disease-free and can hope for cure until recurrence occurs. In general, if one of both parameters (OS or PFS/DFS) is selected as primary endpoint, the other parameter should be reported as secondary endpoint. Furthermore, consistency of results obtained for both OS and PFS/DFS estimates should be demonstrated (35).

OS, as a direct measure of patient benefit, is a well-accepted primary endpoint for clinical confirmatory trials. Survival rates can easily be measured, and there are usually no methodological issues involved in this approach. However, the disadvantages of survival endpoints are the long study duration and a potential effect of next line treatments, which are usually administered after tumour progression. Next line therapies might influence OS and may interfere with the detection of a relevant treatment effect of the investigational drug in the trial. The EU guideline regards OS as a preferred endpoint if the toxicity profile of the control regimen is favourable, and if there are no further next-line evidence-based therapies existing. Also, in situations where the time period from disease progression to death is expected to be short, OS is regarded as suitable primary study endpoint (35).

PFS may be acceptable as primary endpoint for confirmatory trials in situations where further lines of treatment exist, and may also be used for non-inferiority designs. However, the quality of historical evidence-based data must be sufficiently high to allow an adequate definition of the non-inferiority margin (35, 49). If there is either a large effect of the investigational drug on PFS, a clearly favourable safety profile compared with the control treatment, or a long survival time after tumour progression, precise estimates of OS may not be required for marketing authorisation (35). However, when PFS is reported as primary endpoint, it has to be assured that there is no negative effect of the investigational drug on the secondary survival endpoint.

In summary, the acceptance of PFS/DFS as primary endpoint for pivotal oncology trials has clearly increased during the last years. Thus, PFS has been accepted as primary endpoint for confirmatory studies e.g. in first-line CRC and mBC. This is also underlined by appendix 1 to the EU anticancer guideline, which provides general guidance on PFS methodology with regards to definitions, frequency and methods of assessment, ascertainment bias, handling of deviations/missing data, and radiological review (44).

The EU guideline further states that under certain circumstances primary endpoints other than OS and PFS/DFS might be suitable for confirmatory trials, but it is highly recommended to seek scientific advice in such situations (35). Alternative primary endpoints for pivotal oncology trials include TTP, TTF, EFS, ORR, symptom control (in late stage indications), or tumour response-related activities (e.g. limb-saving surgery). Also tumour markers that have been convincingly demonstrated to adequately reflect tumour burden, together with other measures of tumour load, might be employed as surrogates for tumour growth and progression (35). However, the clinical validation of tumour markers used as surrogate endpoints is a challenge. Until today, no biomarker assayed from blood or body fluids has been accepted by regulatory agencies as a surrogate endpoint for registration purpose. Presently, PSA (prostate specific antigen) seems to be the most advanced biomarker likely to predict clinical benefit, e.g. by measuring the PSA doubling time as surrogate endpoint in prostate cancer trials.

Reference therapy

A crucial aspect of phase III confirmatory trials in oncology is the choice of appropriate reference therapies. The reference regimen should be selected from the best available, evidence-based therapeutic options and should be justified in the study protocol. The reference should be a widely used regimen with a clearly favourable benefit-risk ratio, which has been demonstrated through randomised controlled trials (35). The control regimen has not necessarily to be licensed, but should be at least as good as other alternative evidence-based treatment options. Among the best available references, regimens with similar cycle lengths should be preferred as this facilitates the identical scheduling of tumour assessments during the trial. For superiority studies (reference vs. test), any regional-preferred reference can be used, provided it is evidence-based. For add-on studies (reference + test vs. reference), a few, different region-preferred references might be used in the trial. However, this approach needs to be justified by the sponsor, and scientific advice is recommended in the planning phase of such studies (35).

In situations where no regimen with a favourable, evidence-based risk-benefit profile exists, an accepted regimen with a well-documented safety profile used in clinical practice might be used. Alternatively, so called “investigator’s best choice” among a few selected regimens is acceptable. In such cases, however, superiority of the investigational regimen has to be shown versus the combined results of the reference arm. The absence of evidence-based therapies often applies to last-line indications, and to patients who have failed several lines of therapy. In such situations however, it may be more appropriate to conduct randomised trials in less advanced patients, supported by “salvage” last-line studies (35).

Special populations

Special populations to be considered in the design of confirmatory trials are e.g. the elderly, patients with impaired organ function, and children. Obviously, in many cancer indications elderly patients represent the majority of the population. The sponsor has to ensure that this distribution is adequately reflected in the clinical trial database. For certain tumours and/or therapies, a difference in anti-tumour activity in relation to gender has been demonstrated. Where there is likelihood for a treatment by gender interaction, this has to be taken into account in the design of the study, and subgroup analyses differentiated by gender should be conducted.

For the design of trials for patients suffering from renal or hepatic dysfunction, guidelines on investigation of clinical PK are available which should be considered if applicable for the product and the disease (42, 43). If justified by the target indication and patient population, additional PK investigations in these populations may also be conducted after marketing authorisation in phase IV studies.

In general ICH guideline E11 gives guidance for clinical trials in the paediatric population (48). Furthermore, the revised EU anticancer guideline (35) with its addendum on paediatric oncology should be considered for the conduct of trials in children (36). Importantly, the new EU paediatric regulation (37) requires for new active substances mandatory studies in all paediatric age groups. Noteworthy, these studies have to be conducted in compliance with a so-called PIP (paediatric investigation plan), which can be regarded as an overall paediatric development plan for the product. The PIP addresses not only clinical development of a compound. In addition, the document may include pre-clinical studies in juvenile animals, and also development of special formulations for children. As an important principle, the PIP has to be agreed by the new PDCO (paediatric committee) established at the EMEA. Paediatric studies might be waived or deferred by the sponsor, but those waivers/deferrals have also to be agreed by the PDCO (37).

For many anticancer drugs however, paediatric development seems not be reasonable because the indication in which the product is developed occurs only in adults. Thus, waivers on the grounds of prevalence/ incidence of the disease in the paediatric population might be a standard approach for those products. Importantly, either results of studies conducted in accordance with an agreed PIP, or an agreed waiver/deferral will be required for future marketing authorisation of all new active substances. In case the applicant will not be able to submit such information, the marketing authorisation application will be invalidated and rejected (37).

5. Summary

Monoclonal antibodies developed as anticancer drugs have revolutionized modern medicine, and an increasing number of therapeutics is in clinical use or in the pipeline. As with small molecules, successful drug development is based on chemical-pharmaceutical quality, non-clinical pharmacology/toxicology, and well-designed clinical trials. However, the unique features of protein products such as monoclonal antibodies require a specific development programme.

- Key quality aspects for monoclonal antibody therapeutics include derivation of from biological sources, complex manufacturing process, and inherent structural complexity and heterogeneity. Thus, for complete product characterisation and quality determination, a combination of physicochemical and biological tests together with the production process and its control is required. This also includes comprehensive assessment of microbial, viral and TSE safety. Since also minor differences in product quality attributes can impact pre-clinical and clinical outcomes, manufacturing changes can be critical, and require the demonstration of comparability between pre- and post-change product.

- In-depth understanding of the pharmacodynamic, pharmacokinetic and toxcicological properties derived from appropriate preclinical studies is essential for successful drug development. In comparison to conventional small molecules however, the non-clinical testing programme needs to be specifically tailored for monoclonal antibodies. As antibodies can be highly species-specific, the establishment of relevant animal models to assess pre-clinical safety and to establish clinical dosing can be a significant challenge. Robust knowledge on the mode of action is prerequisite for development of bioassays, which are important tools to monitor batch release to assure safety and efficacy.

- In general, clinical development of monoclonal antibodies as anticancer therapeutics follows the approach as for conventional cytotoxic drugs. If there are potential factors of risk involved with first human administration, risk mitigation strategies for the first-in-man trial should be applied. Differences to clinical development of small molecules include bioanalytical analysis, dose escalation strategies, and approaches to establish therapeutic doses. The phenomenon of unwanted immunogenicity can affect dose exposure, safety profile and efficacy. Since animal models are not predictive for humans, a potential immune response should be carefully monitored during clinical trials. Study designs for confirmatory trials are usual comparable to those applied for cytotoxic drugs.

In summary, development of anticancer monoclonal antibodies should follow an integrated, interdependent “threesome” process, and usually involves product-specific approaches. Quality data, non-clinical safety data and efficacy data are interlinked, and should be looked at as a whole. This thesis identifies cornerstones for a successful development of those molecules, with particular focus on IMPD requirements for clinical trial application in the EU.

6. Discussion

Monoclonal antibodies are typical biological medicinal products defined by complex manufacturing processes and product attributes. Consequently, these product-specific characteristics are also reflected in the IMPD that needs to be submitted for clinical trial application. Compared with other NBEs, the fundamental requirements for antibody products with regards to the IMPD quality documentation are essentially the same. However, the preclinical programme for monoclonal antibodies is different from a development programme applied for other NBEs or chemical pharmaceuticals. In particular the pharmacodynamic investigation of antibody products is special, and includes in-depth characterisation of mode-of-action and of immunological properties. With regards to toxicological testing, the duration of repeat-dose toxicity studies can vary between small molecules and biopharmaceuticals, and e.g. genotoxicity testing is usually not required for protein products such as antibodies. In general, clinical investigation of mAb anticancer therapeutics follows the same approach as for conventional cytotoxic drugs. Differences in clinical development include investigation of unwanted immunogenicity, dose escalation strategies and approaches to establish the therapeutic dose range.

Directives 2001/20/EC and 2005/28/EC and also available explanatory texts give rather high-level information on IMP data requirements for clinical trial application. Thus, EU guideline CHMP/QWP/185401/2004 provides general information on quality requirements for IMPs, but has the limitation that it does not specifically address investigational biotechnological products (4). There are numerous ICH and EU guidelines, which describe marketing authorization requirements with regards to quality, safety and efficacy of biopharmaceuticals. However, concerning data requirements for clinical trial application at the different stages of development, no clear guidance is available. Also current FDA guidance to industry that might serve as additional supportive information has its focus on marketing authorization. To date, the only EU guidance specific for investigational biotechnological products is the new draft guideline on virus safety (5). As a consequence, development strategies are often based on previous experience, and there is a tendency to seek for scientific advice at the regulatory agencies already at early stages of development.

Noteworthy, in the EU some national guidelines exist which provide information on IMPD requirements for biopharmaceuticals. Respective guidance has been issued by the national competent authorities of Germany, Ireland, and UK (6-8). Furthermore, the French regulatory agency has provided specific guidelines for first-in-human clinical trials and for phase I clinical trial application (9, 10). However, these documents have the clear disadvantage that they reflect merely the view of single national authorities, and the level of details is usually

rather low. As the sponsor prepares one IMPD used throughout the EU, it might be a suitable strategy to follow the very comprehensive national German guideline “3. Bekanntmachung zur klinischen Prüfung von Arzneimitteln am Menschen” (6). Although the IMPD is the core document for the investigational product proposed for the clinical trial, as an exception the competent authorities of The Netherlands, Greece, Lithuania and Poland do not require an IMPD. Also regarding duration of the CTA process and documentation requirements, a complete harmonization within the EU has not yet been achieved. Interestingly, the EFPIA has recently issued a position paper on five major issues that impact the performance of clinical trials in Europe. The five main issues identified were national differences in CTA core documentation, ADR reporting, GMP requirements, definition/requirements on IMPs vs. NIMPs, and substantial amendments to CTAs (61).

Where there is an obvious lack of general guidelines for the developmental requirements for biopharmaceuticals and mAb products, the sponsor is confronted in few specific areas with very explicit and detailed regulatory expectations (5, 32). Thus, regarding assurance of virus safety of investigational biotechnological products, standards in the EU are very high. Requirements might even increase if the new EU virus safety guideline (5) would come into effect in its current form. With regards to testing of cell lines, raw materials and product, the new EU draft guideline refers to ICH Q5A, and does not distinguish between authorised products and investigational products used in first-in-human trials. This would clearly impose new hurdles for sponsors. As an example, studies like e.g. analysis of EOPC banks, or virus particle testing of more than one UBH batch are usually not performed during of a typical early development programme.

In current state-of-the art manufacturing approaches, well-established (non-human) cell lines are used to generate production cell banks, and up- and downstream processes and the product are thoroughly controlled for adventitious viruses. Importantly, there are no published reports in the literature on viral infections caused by biotechnological products. It can be questioned if ICH marketing authorisation standards are required to ensure an acceptable level of safety for subjects in early clinical phases. From a virus safety point of view, mAb products have proven to be safe, and a further increase in requirements should not be necessary. One might speculate that the very stringent attitude of some national authorities, which seems also to drive the EU guideline creation process, has its origin in the HIV blood product scandal during the 1980´s. In conclusion, virus safety requirements for IMPs are in the EU higher than in other regions (as e.g. in the US), and might even increase.

The transition from preclinical testing to first human subjects is a particularly critical step in development of a mAb. The clearly most important issue for first-in-human trials is assurance of safety of the clinical trial subjects. The Tegenero incident showed that, despite preclinical

state-of-the-art approaches, a residual risk remains that preclinical animal models claimed to be relevant might not predict a safe starting dose. Thus, the starting doses calculated with the standard NOAEL approach can be too high, which can imply risks for first human administration. As a consequence of the Tegenero disaster, a new EU guideline was issued that requires sponsors to identify potential risk factors and to apply risk mitigation strategies for first-in-human trials if needed. Special precautions are proposed if there are concerns based on the mode of action, the nature of its target, or if the IMP comprises a new type of engineered structural format. An important risk mitigation strategy is the new MABEL method for the calculation of the initial starting dose. Other risk mitigation measures concern planning and conduct of the trial, e.g. dose administration and dose escalation strategies (34). It can be expected that implementation of such strategies will aid to safety and well-being of clinical trial subjects, and lower the risk for unexpected serious clinical outcomes. However, a residual risk involved with the first human dose can never completely be excluded.

However, duration and costs of clinical trials will be negatively affected by implementation of those risk mitigation measures, which could impact the overall development time of new drugs. Furthermore, it can be expected that companies could become more cautious to develop products with a new mechanisms of action, in particular if their pharmacological activity cannot truly be mirrored in animal models. One might speculate that in the “post Tegenero era” authority expectations especially regarding preclinical data packages might become substantially higher. In worst-case, this could prolong the development of innovative drugs, and might have negative consequences on the availability of new therapeutics in the future.

With regards to strategies for pivotal clinical oncology trials, the only direct measures of patient benefit are either extension of life (measured by OS), or improvement of quality of life (using QoL questionnaires). In principle, both parameters can serve as primary endpoints, but until today no drug has been approved based on QoL measures. Many methodological issues, dependence of good patient compliance, and the need for blinding which is difficult in oncology trials might explain this. Alternative primary endpoints such as PFS, ORR or DFS might be appropriate for particular indications and disease stages, but should be regarded only as surrogate endpoints for clinical benefit. EU marketing authorisation based on such surrogate endpoints likely to predict clinical benefit is possible, but might lead to “conditional approval” (see below). However, in case of conditional approval the positive risk-benefit ratio needs usually to be confirmed by demonstration of a “real” significant clinical benefit, such as improvement of survival, in another subsequent study.

Analysis of clinical data packages for anticancer drugs submitted through the CP reveals some degree of flexibility with respect to endpoints and designs accepted by EMEA and

CHMP. Noteworthy, only the minority of anticancer drugs has been approved on the basis of survival data as primary endpoint, and even open, comparative phase II trials might be sufficient in the case of outstanding efficacy (59). With less observed efficacy, either a randomised closed comparative trial or more studies seem to be required. In case of clearly favourable safety profiles, also non-inferiority designs can be used for phase III studies to demonstrate a positive risk-benefit ratio (59). In conclusion, favourable safety and/or efficacy might contribute to a positive risk-benefit ratio, which is prerequisite for the “approvability” of a new anticancer medicine.

In situations of outstanding clinical benefit, opportunities for EU conditional marketing authorisation (63, 64) based on surrogate endpoints or phase II designs should be considered. Special phase II/III designs might also be applicable for oncology trials. EU approval under “exceptional circumstances” (65, 66) might be considered if there is no possibility to generate comprehensive clinical data, which is often the case for orphan indications. Obviously, the current EU regulatory framework provides some flexibility for acceptable registration study designs. Thus, the strategy of choice should be to seek for scientific advice at the EMEA and/or at national competent authorities before embarking on pivotal clinical trials.

In conclusion, for biotherapeutics, and in particular for monoclonal antibodies, there is only limited regulatory guidance on data requirements for the different developmental stages available. National guidelines applicable for clinical trial authorisation of NBEs are often not very detailed, and only reflect the view of the respective national agencies. Numerous EU and ICH guidance documents have been issued for biological/biotechnological products, but those clearly focus on marketing authorisation. Thus, it can be difficult to determine at which stage of development EU and ICH requirements should be fulfilled, and strategies are often based on previous experience. In general, the majority of the data should be generated already at very early stages of development, well before clinical phase III.

Due to the high diversity of biotherapeutics, development strategies that would be applicable for all types of products are usually not feasible. In order to avoid objections during clinical trial applications, critical issues that are identified during early development should be discussed with the competent authorities beforehand. As aftermath of the Tegenero incident, it can be assumed that authority expectations with regards to preclinical data packages for first-in-human trials will increase. Planning and conduct of pivotal oncology clinical trials that are suitable for registration purpose can be a particular challenge. Thus, prior interaction with regulatory agencies is highly recommended, in order to improve the chances for a successful marketing authorisation application.

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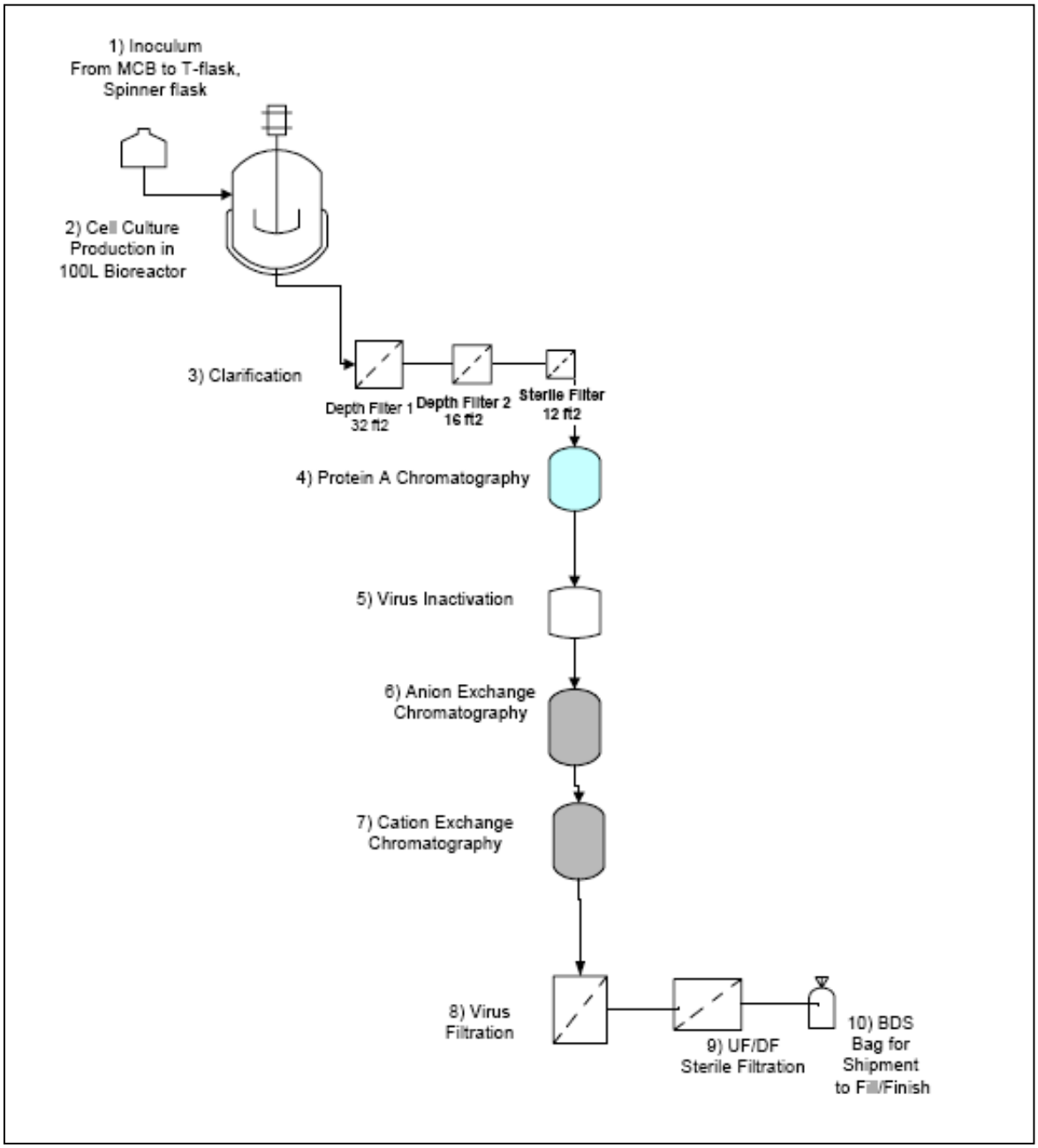
8. Annex

Flowchart: Example of manufacturing process for monoclonal antibodies



1) - 2) Upstream process (production of mAb in cell culture)
3) - 7) Downstream process (purification of mAb)

MCB Master Cell Bank
UF/DF Ultrafiltration/Diafiltration
BDS (Purified) Bulk Drug Substance

Hiermit erkläre ich an Eides statt, die Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

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Unterschrift