Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use

U. S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
February 28,1997

ALXN2029
IPR2023-00999

ALXN2029
IPR2019-00739

Date: February 27, 1997

From: Kathryn C. Zoon , Ph.D., Director
Center for Biologics Evaluation and Research
Food and Drug Administration

Subject: Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use

To: Manufacturers of Biological Products and Other Interested Persons

This Points to Consider (PTC) document has been developed for manufacturers of monoclonal antibody products for human use. These "Points" are not regulations nor are they guidelines, but represent the current thinking that the Center for Biologics Evaluation and Research (CBER) staff believe should be considered at this time. This 1997 PTC document supersedes the 1994 PTC document of the same title, announced in the Federal Register of August 3, 1994 (59 FR 39571).

It is our intention to continuously update and revise this document in order to improve its usefulness. We invite your review and comment on the "Points". Comments should be identified with the docket number 94D-0259. Two copies of any comments should be submitted except that individuals may submit one copy. All comments should be addressed to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
12420 Parklawn Drive, Room 1-23
Rockville, MD 20857

_____-s-_____
Kathryn C. Zoon, Ph.D.

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use

[Docket No. 94D-0259]

For further information regarding this document, contact:
Sharon Carayiannis
Center for Biologics Evaluation and Research (HFM-630)
Food and Drug Administration
1401 Rockville Pike, Suite 200N
Rockville, MD 20852-1448
301-594-3074

Submit written comments on this document to:

Dockets Management Branch (HFA-305)
Food and Drug Administration
12420 Parklawn Drive, Room 1-23
Rockville, MD 20857

Comments should be identified with the docket number found in the heading of this page.

Submit written requests for additional copies of this document or any other CBER guidance to:

Office of Communication, Training, and Manufacturers Assistance (HFM-40)
Food and Drug Administration
1401 Rockville Pike, Suite 200N
Rockville, MD 20852-1448

Send one self-addressed adhesive label to assist that office in processing your request.

These documents may also be obtained by mail by calling the CBER Voice Information System at 1-800-835-4709 or 301-827-1800, or by fax by calling the FAX Information System at 1-888-CBER-FAX or 301-827-3844.

Persons with access to the INTERNET may obtain these documents using, the World Wide Web (WWW), or bounce-back e-mail. For WWW access, connect to CBER at "http://www.fda.gov/cber/cberftp.html". To receive this document by bounce-back e-mail , send a message to "ptc__mab@A1.CBER.FDA.GOV".

TABLE OF CONTENTS

Note: Page numbering may vary for documents distributed electronically.

I. INTRODUCTION 6
A. BACKGROUND 6
B. DEFINITIONS 6
C. FILING INFORMATION 7
II. PRODUCT MANUFACTURE AND TESTING 8
A. GENERAL PRINCIPLES AND DEFINITIONS 8
B. MANUFACTURE AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES 8
1. Cell lines 8
2. Production in cell culture 9
3. Production in animals or plants 10
4. Purification 10
5. Characterization of purified unmodified mAb 12
6. Anti-idiotype vaccines 13
7. Monoclonal antibodies conjugated with toxins, drugs, radionuclides or other agents (immunoconjugates) 13
C. QUALITY CONTROL AND PRODUCT TESTING 15
1. Cell line qualification 15
2. Lot-to-lot quality control monitoring of unprocessed bulk lots and purified bulk lots, and final product specifications 19
3. Stability of product 23
4. General considerations on quantitation and removal of a retrovirus contaminant 23
5. General considerations on the design and interpretation of virus clearance studies 24
6. Generic or modular virus clearance studies 24
7. Product testing requirements for mAb used as ancillary products 25
D. PRODUCT SAFETY TESTING FOR FEASIBILITY CLINICAL TRIALS IN SERIOUS OR IMMEDIATELY LIFE-THREATENING CONDITIONS 26
1. General considerations 26
2. Product safety data needed before the initiation of feasibility trials in serious or immediately life-threatening conditions 27
E. ISSUES RELATED TO MANUFACTURING CHANGES (DEMONSTRATION OF PRODUCT COMPARABILITY) 28
1. General 28
2. In vitro evidence of product comparability 28
3. Animal studies 29
4. Clinical studies to support manufacturing changes 29
III. PRECLINICAL STUDIES 29
A. TESTING CROSS-REACTIVITY OF MAB 29
1. *In vitro* testing for cross-reactivity 30
2. *In vivo* testing for cross-reactivity 30
B. PRECLINICAL PHARMACOLOGY AND TOXICITY TESTING 31
1. General considerations 31
2. Animal toxicology studies 31
3. Pharmacokinetics and pharmacodynamics 32
4. Preclinical *in vivo* studies with immunoconjugates 33
IV. CLINICAL STUDIES 34
A. CLINICAL CONSIDERATIONS FOR PHASE 1 AND 2 STUDIES 34
1. General 34

2. Dose-setting 35
B. IMMUNOGENICITY: CLINICAL CONSIDERATIONS 37
1. Monitoring the development of antibodies to mAb 37
2. Clinical consequences of immunogenicity 38
C. PRODUCT-RELATED CONSIDERATIONS FOR PHASE 3 STUDIES 39
D. ADMINISTRATION OF RADIOLABELED ANTIBODIES 39
1. Dosimetry 39
2. Early clinical development of therapeutic radiolabeled mAb 41
3. Adverse events for patients enrolled in trials of therapeutic radiolabeled mAb 43
4. Clinical development of radiolabeled mAb used as imaging agents 43
V. APPENDIX I: NORMAL HUMAN TISSUES USED IN CROSS-REACTIVITY TESTING 46
VI. APPENDIX II: MOUSE ANTIBODY PRODUCTION TEST 47
VII. APPENDIX III: ORGANS TO BE CONSIDERED IN DOSIMETRY ESTIMATES 48
VIII REFERENCES 49

Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use
February 1997

I. INTRODUCTION

A. BACKGROUND

Points to Consider documents provide a flexible approach in which FDA provides and updates its guidance on regulatory issues in many areas of drug development. Such documents are particularly useful in the rapidly evolving field of biotechnology-derived drugs and other biologics. The Center for Biologics Evaluation and Research (CBER) set out to revise the "Points to Consider (PTC) in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" with several objectives. An important goal was to facilitate initial development of monoclonal antibodies for serious or life threatening indications. Additionally, it was felt that some of the information in the 1994 document required updating and streamlining. Finally, it was necessary to review this document for consistency with current CBER policy and with International Conference on Harmonisation (ICH) documents dealing with this category of products. This updated document supersedes the 1994 version, and is designed to assist sponsors and investigators regarding monoclonal antibody (mAb) product development, including information to submit when filing Investigational New Drug Applications ("INDs") and License Applications. Although this document does not create or confer any rights for or on any person and does not operate to bind FDA or the public, it does represent the agency's current thinking on monoclonal antibody products for human use.

For mAb, as for other biologics, certain regulations contained in 21 CFR Parts 200-299 and 600-680 apply and should be consulted. In common with the other PTC, the mAb PTC are not intended to be all-inclusive. They represent recommendations on how to conduct the clinical development of a product up to and after licensure, not checklists of items to be provided before or after phase 1 trials are initiated. Specific products which raise issues that are not considered in these "Points" will be evaluated on a case-by-case basis. The discussion on abbreviated product testing for feasibility trials in serious and immediately life-threatening conditions and on generic and modular virus clearance studies does not apply to human products made in human cell substrates. Consultation with CBER is strongly advised for sponsors considering the application of abbreviated testing policies to products that have the potential to be contaminated by human pathogens. For aspects of manufacturing and of the production facility that are not included in this discussion or in applicable regulations, sponsors should consult with the Office of Therapeutics Research and Review and the Office of Establishment Licensing and Product Surveillance respectively.

B. DEFINITIONS

For the purpose of this document, the terms "**antibody**" and **"monoclonal antibody"** (mAb) may be used interchangeably and refer to intact immunoglobulins produced by hybridomas, immunoconjugates and, as appropriate, immunoglobulin fragments and recombinant proteins derived from immunoglobulins, such as chimeric and humanized immunoglobulins, F(ab') and $F(ab')_2$ fragments, single-chain antibodies, recombinant immunoglobulin variable regions (Fvs) etc. Recommendations on the manufacture of recombinant products are contained in other PTC documents from CBER (1,2). Some of these recommendations pertaining to recombinant mAb produced in cell substrates other than hybridomas are reiterated in this document for convenience of consultation. This document applies to mAb used as therapeutic or *in vivo* diagnostic agents, as well as to **ancillary products**, i.e. mAb used in the manufacture of other products for *in vivo* use. The latter include mAb that are used alone or in

conjunction with devices, for example, for *ex vivo* purging of cells to remove immune or tumor cells, for *ex vivo* cell collection (e.g. hematopoietic stem cells), or for purification of other products intended for *in vivo* administration. Generally, these mAb should meet the same criteria for safety and freedom from adventitious agents as mAb intended for direct administration to patients. Likewise, reagents that are commonly used in conjunction with mAb for *ex vivo* manipulations of cellular products intended for *in vivo* administration (e.g. complement, DNAase) should meet the same safety standards as mAb intended for direct administration to patients. However, in such cases, some procedures for virus inactivation or removal may be performed on the downstream product rather than on the mAb or other reagent (see II.C.7). Complete information on products that will be used in conjunction with the mAb, such as rabbit complement or DNAase, should be submitted before clinical studies begin. This information can be submitted as a part of the original IND submission or in the form of a Master File.

As used in this document, "**cocktails**" are defined as two or more mAb administered at a fixed ratio. Relevant targets may include multiple antigens on infectious pathogens and multiple tumor-associated antigens. The rationale for combining the products should be clear and based on the clinical context or previous clinical experience with individual products. Lack of interference among the mAb in the combination should be shown and synergistic or additive effects should be characterized. Dose-ranging for each of the components is highly desirable. In some instances, dose-setting may be based on preclinical or clinical data that show the necessity or superiority of a particular dose and ratio of mAb in the combination.

As used in this document, "**panels**" are defined as sets of mAb directed against related antigens from which one or more members would be used for an individual patient based on target antigen characterization. Such panels could be submitted for approval in a single license application. Examples of panels might include anti-idiotype mAb for lymphoma and mAb directed against different bacterial or viral serotypes. Dose-ranging for each mAb would be necessary. During the phase 3 trials to establish efficacy of the entire panel, some clinical experience with each member of the panel should be obtained.

C. FILING INFORMATION

It is not necessary to have all of the information discussed in this document available in the initial IND submission. Rather, much of the information may be developed during clinical development, with guidance from CBER or other appropriate Centers by means of frequent dialogue. At pre-IND meetings, CBER staff may provide guidance in planning clinical development and establishing the format and content of initial IND submissions. Such meetings may be particularly useful when the product is a novel molecular entity or is produced by a novel process, and when drug development plans are unusually complex.

The manufacture of mAb that are produced and controlled by similar procedures in the same facility may in some cases be documented in a single Master File. This may be particularly helpful when data from generic or modular virus clearance studies are used for multiple antibodies that differ only in the variable (v) or complementarity-determining region (CDR) and when multiple antibodies are purified by identical procedures (see Section II.C.6).

See references 3 and 4 for information on filing biologics license applications. An Establishment License Application is no longer required for mAb intended for in vivo use (3).

II. PRODUCT MANUFACTURE AND TESTING

A. GENERAL PRINCIPLES AND DEFINITIONS

Traditionally, most mAb are produced by hybridoma cell lines through immortalization of antibody-producing cells by chemically-induced fusion with myeloma cells. In some cases, additional fusions with other lines have created "triomas" and "quadromas". We anticipate an increase in recombinant mAb (e.g. chimeric or humanized mAb, single-chain or dimeric Fvs, mAbs derived from phage display libraries etc.) and human mAb in the future. These may be produced in animal cell lines (e.g. CHO, SP2/0) transfected with recombinant DNA constructs, in human cells (e.g. immortalized lymphoid cells), in bacteria, yeast, insect cells etc. Novel methods of production for mAb or mAb-derived recombinant proteins may include insects, plants or transgenic animals,.
The principles reviewed in Sections II.B. 1 through 4 may be applied, in general to all hybridoma and heterohybridoma generated products, regardless of the species of origin. All steps in manufacturing of mAb to be used in trials intended to support licensure and of licensed mAb should comply with current Good Manufacturing Practices (cGMPs), as appropriate for the stage of product development.

While manufacturing details and safety issues may be different for different expression systems, some general principles can be applied. The establishment of a reliable and continuous source from which the antibody can be consistently produced is highly recommended (e. g. master cell banks for cell cultures, seed banks for transgenic plants, founder strains for transgenic animals). If transient expression systems are used, master vector seed stocks should be generated, and the genetic stability of the expression constructs used should be tested. Appropriate in-process testing which takes into consideration the specific safety concerns of the expression system used should be instituted. Sponsors are encouraged to consult the most recent available versions of the Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, the Points to Consider in the Production and Testing of New Drugs and Biologicals produced by Recombinant DNA Technology or the Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived From Transgenic Animals (1, 2, 5), the 1996 CBER/CDER Guidance Document on the Submission of Chemistry, Manufacturing and Controls Information for a Therapeutic Recombinant DNA-derived Product or a Monoclonal Antibody Product for In Vivo Use (4), as well as relevant International Conference on Harmonization (ICH) documents (e.g. 6, 7), if applicable to their expression systems. Sponsors considering novel expression systems not specifically covered by guidance documents are encouraged to consult with CBER.

B. MANUFACTURE AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES

1. Cell lines

The following information should be provided in the IND or biologics license application:

a. Source, name, and characterization of the parent cell line, including any immunoglobulin heavy or light chains that it synthesizes and/or secretes, the fusion partner in the case of hybridomas, or the host cell line in the case of transfected cells producing recombinant mAb.

b. Species, animal strain, characterization, and tissue origin of the immune cell.

c. Description of immortalization procedures, if any, used in generating the cell line.

d. Identification and characterization of the immunogen. A complete biochemical characterization may not be possible or necessary in all cases. However, we recommend that as much information as possible be gathered on the nature and characteristics of the material used as an immunogen. Such data can be useful in choosing appropriate potency assays, as well as in evaluating potential for cross-reactivity and possible clinical usefulness. For example, a determinant which is not expressed on the surface of target cells bind necrotic cells better than intact cells.

e. Description of the immunization scheme. In the case of human mAb, any *in vitro* or *in vivo* immunization procedures should be described, as well as any relevant aspects of the subject's medical history.

f. Description of the screening procedure used. In the case of human mAb, steps performed in order to enrich antigen-specific human B cell populations should be described.

g. Description of the cell cloning procedures. If changes in cell culture process (e.g. cells adapted from serum-containing to serum-free medium) are shown not to affect product quality, it is not necessary to reclone the cells or rebank the MCB or WCB. In this context, product quality includes not just the identity, purity, potency and pharmacological characteristics of the purified product, but also its safety profile. For example, possible changes in types and/or titers of viruses detectable in the unpurified bulk material and the ability of the purification process to remove or inactivate them should be addressed).

h. For transfected animal or plant cell substrates, as well as for microbial cell substrates (bacteria, yeast), a detailed description of the vector(s) and final construct(s) generation, including whether or not extraneous amino acid sequences are introduced into the product as a result of subcloning, and description of transfection/transformation, screening and selection procedures (see refs. 1-6). Determination of cDNA sequence(s) of the predominant transcript(s) is acceptable as an indication of clonality of transfected cell lines.

i. For cell culture systems using autonomously replicating vectors (e.g. baculovirus or other transient expression systems) a detailed description of the vector system, construct generation, selection, vector banking procedures, and infection/transfection procedures should be provided (1-6).

j. For all cell substrates, description of the seed lot system for establishing and maintaining the master cell bank (MCB) and the working cell bank (WCB).

2. Production in cell culture

The following information should be provided in the IND or biologics license application:

a. A description of the culture procedures if production is entirely *in vitro* or if cells are passaged *in vitro* prior to mouse inoculation.

b. A description of the culture media used, including certification and testing. Serum additives used in hybridoma propagation should be free of contaminants and adventitious agents.

c. The steps taken to prevent or control contamination by viruses, bacteria, fungi, mycoplasma and transmissible spongiform encephalopaties (TSE) agents. These include, among other things, a description of the equipment, transfers, room classification, employee gowning procedures etc.

d. The acceptance criteria for cells or tissue culture supernatants intended for further manufacture.

3. Production in animals or plants

The following information should be provided in the IND or biologics license application:

a. A description of the cell line used as the inoculum (if any) should be provided (see 2.a. above).

b. Animal care should be in accordance with the NIH Guide for Care and Use of Laboratory Animals. For ascites production, the use of specific pathogen free (SPF) mice is recommended. To ensure manufacture of consistent, high quality ascites for production of mAb, an animal health monitoring program should be in place that encompasses quarantine procedures, sentinel animals, and an in-house health surveillance program (including screening for mycoplasma). Frequency of serological testing of sentinel mice should be established and is usually based on the incidence of virus contamination. Screening programs for known infectious agents should be updated to reflect advances in the knowledge of infectious diseases. Sponsors should be responsible for the adequacy of screening programs.

c. All protocols for ascites production should also incorporate information on: *i*) species, sex and age of animals used; *ii*) animal supplier; *iii*) volume of pristane; *iv*) volume and concentration of cell inoculum; *v*) timing of priming, inoculation, and ascites harvesting; *vi*) frequency and procedure for ascites harvesting; *vii*) definition of a batch; *viii*) animal bedding, food and water; *ix*) number of animals housed together; *x*) environmental conditions under which each procedure takes place and *xi*) number of times cells are passaged from one animal to another, if applicable.

d. For production in transgenic animals, the vectors, constructs and procedures used for gene transfer should be described. The genetic background and characterization of founder animals, the generation and selection of production herds and animal maintenance procedures should be described as well (see ref. 5 for details). Health monitoring programs for animal herds or colonies should be in place, including screening for zoonoses known to exist in captive animals of the relevant species in North America. Programs for screening and detection of known infectious agents should be tailored for the animal species and periodically updated to reflect advances in the knowledge of infectious diseases. Sponsors should be responsible for the adequacy of screening programs (see paragraph b above). When initially establishing transgenic animal strains, the following considerations should be kept in mind: *i*) non-transgenic animals to be used for breeding or gene transfer procedures should be obtained from closed herds or colonies that are serologically well characterized and as free as possible of pathogens of concern for the animal species or for humans; *ii*) the use of imported animals or first generation offspring from imported animals is discouraged and *iii*) animals from species in which TSE have been documented should be obtained from closed herds with documented absence of dementing illnesses and controlled food sources.

e. For production using autonomously replicating vectors (e.g. baculovirus) in live insect larvae, larvae maintenance procedures should be described in detail, including procedures used to control and monitor bioburden.

f. For production in plants (e.g. transgenic plants or autonomously replicating vectors using plants as bioreactors), early consultation with CBER staff is recommended.

4. Purification

Purification schemes for mAb should be described in the IND or biologics license application. We

recommend that mAb purification schemes incorporate the following characteristics:

a. Production techniques that will prevent the introduction of and eliminate contaminants, including animal proteins and materials, DNA, endotoxin, other pyrogens, culture media constituents, components that may leach from columns, and viruses.

b. Incorporation of one or more steps known to remove or inactivate retroviruses in excess of the endogenous particle load, whenever applicable (see Reference 8 and discussion of virus clearance studies in Section II.C.). As a general guidance, we recommend that each purification protocol include at least two orthogonal (i.e. based on different mechanisms) robust virus removal steps (see below). Including these steps would not obviate the need for virus clearance studies, except in the case of products intended for use in feasibility trials in serious or life-threatening conditions (see Section II.D.2.)

> *i.* **Robust virus removal/inactivation steps** are defined as those that have been shown to work well under a variety of conditions (e.g. pH or ionic strength of column buffers) with a variety of mAb. Robust steps include low pH, heat, solvent/detergent treatments, and filtration (see Table III). Sponsors have the option of providing adequate evidence indicating that a step different from these is robust, or is reliably effective for removal/inactivation under the conditions employed. An estimate of the efficiency of robust steps in removing virus may be demonstrated by: (a) cross-referencing master files or reliable scientific literature published in peer-reviewed journals or (b) generic or modular clearance studies (see Section II.C.6. for definition).

c. Demonstration of the ability of the purification scheme to remove adventitious agents and other contaminants, by means of a clearance study. For some contaminants, e.g. DNA, pristane or protein A, such a clearance study, if appropriately carried out, may be an acceptable alternative to routine testing for the contaminant. In the case of virus clearance studies, we recommend the use of several model viruses encompassing large and small particles, DNA and RNA genomes, as well as chemically sensitive and resistant lipid enveloped and non-enveloped strains. Human blood products should be avoided in production of other biologicals. When human blood products that may be contaminated with hepatitis C virus (HCV) or other infectious agents must be used in production (e.g. as media additives), such schemes should include viruses that are acceptable models for HCV, such as bovine viral diarrhea virus (BVDV) or Sindbis virus. Retrovirus clearance studies should be performed prior to phase 1 trials, except for products intended for use in the setting of serious or life-threatening conditions in feasibility trials (see Section II.D.). Clearance studies for other viruses and/or other contaminants should be carried out prior to production for phase 2/3 trials and may need to be repeated if the final manufacturing process has changed. ICH guidelines are currently being drafted to address in further detail the viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.

d. Limits should be prospectively set on the number of times a purification component (e.g. a chromatography column) can be reused. Such limits should be based upon actual data obtained by monitoring the component s performance over time.

e. As a product is developed, retention samples from each production should be saved under appropriate conditions so that side-to-side comparisons may be made to determine product comparability (see Section II.E.).

f. A description of the purification room(s) design features, HVAC and other support systems, equipment, transfers and personnel should be provided. Emphasis should be placed on operational features that minimize the risk of contamination from the environment or cross-contamination from other products.

5. Characterization of purified unmodified mAb

Before a mAb is studied in humans, a precise and thorough characterization of antibody structural integrity, specificity, and potency should be conducted and described in the IND. The mAb should be as free as possible of non-Ig contaminants. A properly qualified in-house reference standard with known characteristics, specificity, and potency, and that is stored under appropriate conditions and periodically tested to ensure its integrity, should be used for lot-to-lot comparisons. Reference standards should be updated as a product evolves but should be finalized by the start of phase 3 trials. Appropriate standard operating procedures (SOPs) should be developed for qualification of a new reference standard.

a. Structural Integrity

A combination of SDS-PAGE, IEF, HPLC, mass spectrometry, or other appropriate physicochemical methods should be used to show that the purified antibody is not fragmented, aggregated, or otherwise modified (e.g. loss of carbohydrate side chains). Side-by-side comparisons of production lots to the in-house reference standard should be performed.

b. Specificity

Assays should provide evidence that the binding of the mAb to the target antigen is specific. Once the specificity of the antibody is characterized, it should be screened for cross-reactivity with human tissues (see Section III.A.). The following are some suggestions on the design of specificity studies:

i. Direct binding assays should include both positive and negative antibody and antigen controls. At least one isotype-matched, irrelevant (negative) control antibody should be tested. Negative antigen controls should include a chemically similar, antigenically unrelated compound, if available (e.g. similar chemical nature, size, charge, and charge density).

ii. Whenever possible, the protein, glycoprotein, glycolipid, or other molecule bearing the reactive epitope, should be biochemically defined, and the antigenic epitope, itself, determined. If the antigenic determinant is a carbohydrate, the sugar composition, linkage, and anomeric configuration should be established.

iii. If possible, fine specificity studies using antigenic preparations of defined structure (e.g. oligosaccharides or peptides) should be conducted to characterize antibody specificity by means of inhibition or other techniques. For complex biological mixtures, the lots of test antigen and/or inhibitors used for direct binding tests should be standardized. Inhibition of antibody binding by soluble antigen or other antibodies should be measured quantitatively.

iv. Once the specificity of an antibody has been determined, it is important to quantitate antibody binding activity by affinity, avidity, immunoreactivity, or combinations of these assays, as appropriate. A number of published methods are suitable for measurement of antibody binding activity (9, 10).

c. Potency Assays and Potency Specifications

Potency assays are used to characterize the product, to monitor lot-to-lot consistency, and to assure stability of the product. Potency may be measured by a binding assay, a serologic assay, activity in an

animal model, and/or a functional assay performed *in vitro* or *in vivo*. It is desirable that the assay(s) bear the closest possible relationship to the putative physiologic/pharmacologic activity of the product and be sufficiently sensitive to detect differences of potential clinical importance in the function of the product. In particular, when the performance of the antibody depends not only upon antigen binding but also on other critical functions, it is desirable that the potency assay(s) measure all such functions. Documentation of the potency assay's performance, including sensitivity, intra- and inter-assay variation and robustness, should be provided.

i. Antibody binding activity may be quantitated by ELISA, RIA, radioimmune precipitation, cytotoxicity, flow cytometry, or any other standard, appropriate method. Activity should be expressed as specific antigen-binding units per mg or :g of antibody. Product should be compared to an in-house reference standard. Appropriate measurements of antibody affinity, if established, may be a useful adjunct to other assays. Parallel line bioassay or a similar, valid statistical procedure should be used in calculating potency.

ii. The potency of a mAb may also be tested by measurement of *in vivo* function in animal models, although such assays are often cumbersome and difficult to standardize and should not be the sole measure of potency.

iii. The permissible range of values in potency assays that reflects adequate biological activity of a product should be based on experience with a particular antibody. Ideally, potency assays should be correlated with *in vivo* activity in order to develop control tests which will ensure an effective product. This implies that multiple production lots should be used during the clinical development program and potency assay results should be correlated with clinical performance. When clinical performance is measured by *in vitro* tests used as surrogates of efficacy, such tests should be validated in a phase 3 clinical trial of appropriate design.

6. Anti-idiotype vaccines

The following issues should be addressed for anti-idiotype vaccines:

a. In the case of an anti-idiotype vaccine (Ab2 vaccine), the Ab2 immunogen should be characterized as to the Ab2 type, e.g. classical type (Ab2") or antigen mimic (Ab2$) (11).

b. Ab2$ vaccines should be shown to be reactive with the appropriate population of human Ab1 (antibody to nominal antigen) if such antibodies are available.

c. The Ab2 preparation should be studied for the appropriateness of response (to target antigen) in xenogeneic as well as syngeneic animals (12).

7. Monoclonal antibodies conjugated with toxins, drugs, radionuclides or other agents (immunoconjugates)

Immunoconjugates are typically produced by chemical processes using specific reagents to link the unconjugated antibody with a non-antibody agent. Alternatively, immunoconjugates can be obtained as chimeric recombinant proteins containing non-immunoglobulin and immunoglobulin sequences in the same polypeptide chain. In addition to previously discussed recommendations for unconjugated (naked) mAb, manufacturers of immunoconjugates should address the following:

a. Construction of the Immunoconjugate.

A full description of the reagents and the process used to construct an immunoconjugate should be submitted, including:

i. A description of components such as toxins, drugs, enzymes, and cytokines that are linked to the mAb, including: the source, structure, production, purity (including demonstration of freedom from adventitious agents), and characterization of all components (if components are purchased, a certificate of analysis should be supplied).

ii. A description of chemical components, such as linkers and chelating agents, that will be used in preparing the immunoconjugate. These should include documentation of the sources of reagents and method of preparation and determinations of residual impurities from synthesis or purification. Charts of the synthetic reaction pathways and any relevant published or in-house data concerning the toxicity of chemicals used in the production of an immunoconjugate should be provided.

iii. The average ratio of coupled material to antibody and the number of conjugated moieties per antibody should be determined as the first step in establishing lot release criteria for the final product and developing the relationship between immunoglobulin substitution number, potency, and stability.

iv. Products prepared using recombinant DNA technology (e.g., derived from transfected cell lines or microbial cell substrates, chimeric, reshaped, complementarity determining region [CDR] grafted, single chain Fv antibodies, and recombinant immunoconjugates) should follow recommendations discussed in references 1-7, as appropriate. The stability of recombinant immunoconjugates should be studied carefully, as such chimeric proteins may have altered conformational stability, solubility or tendency to aggregate compared to their component polypeptides in their native structures. Loss of specific immunoreactivity due to denaturation or formation of aggregates (e.g. diabodies formed by recombinant Fvs) may lead to altered pharmacokinetics and/or binding to non-target tissues.

b. Purity of the Immunoconjugate

i. Special care should be taken to ensure that the antibody preparations are as free as possible of extraneous immunoglobulin and non-immunoglobulin contaminants as such contaminants could react with nuclides, toxins or drugs during the construction of the immunoconjugate.

ii. The amount of free antibody and free components in the final product should be determined with limits set for each. Reactive intermediates should be inactivated or removed.

c. Immunoreactivity, Potency and Stability of the Immunoconjugate

Coupling of toxin or drug to an antibody may alter the activity of either component.

i. Immunoreactivity before and after coupling should be assessed using appropriate methodology (9, 10).

ii. Activity of the non-immunoglobulin component of immunoconjugates, should be assessed by a potency assay whenever appropriate (e.g., toxins, cytokines or enzymes, but not radio-

immunoconjugates intended for use in imaging)

iii. Limits on the percent change in immunoreactivity resulting from construction of the immunoconjugate should be established as part of product specifications.

iv. The immunoconjugate should be tested for stability *in vitro* by incubation in pooled human serum at 37° C under sterile conditions. Plasma may be used instead of serum, provided that the anticoagulant used does not affect the stability of the immunoconjugate (e.g., chelating agents may react with some radioisotopes, heparin may interact with basic proteins, etc.). Aliquots should be analyzed at timed intervals for the concentration of intact immunoconjugate and degradation products. The conditions under which product stability is evaluated and the positive and negative controls used should be fully described. Stability in human serum or plasma is not relevant for topically administered immunoconjugates which are demonstrated not to be absorbed into the bloodstream. It should be established whether or not such immunoconjugates or their components are detectable in plasma after topical administration, and whether or not they elicit an immune response.

d. Specific Issues Related to mAb Coupled to Radionuclides

The preparation of the radioimmunoconjugate should be performed in a standardized, well-controlled, and validated manner. Methods should be developed to estimate the percent radioactivity in each of the three species of concern: free isotope, conjugated mAb, and labeled, non-mAb substances.

i. It is recommended that the initial IND submission for a radiolabeled mAb contain analytical results from two to three radiolabeling runs that demonstrate the preparation of an immunoreactive, sterile, and pyrogen-free product. These radiolabeling runs should be performed by the same personnel who will radiolabel the mAb for the study, using the reagents that will be used for the study.

ii. Radiopharmaceutical grade isotopes should be used when preparing immunoconjugates. The sterility and pyrogen-free nature of each isotope should be documented by submission of a certificate of analysis and letters of cross-reference for manufacturing information.

iii. The concentrations of covalently-bound and free isotope in the final product as well as residual levels of labeling reagents and their decomposition products should be determined during the trial labeling runs.

iv. Quality control tests that will be performed before and/or after each patient administration should be described.

v. When appropriate, colloid formation by the radio-immunoconjugate should be determined and limits set for it.

C. QUALITY CONTROL AND PRODUCT TESTING

1. Cell line qualification

Qualification of the cell line for production of a mAb to be used as a biologic therapeutic should include screening the master cell bank (MCB) and the working cell bank (WCB), at least on a one-time basis, for endogenous and adventitious agents utilizing the tests outlined in Table I and described in the Points to

Consider in the Characterization of Cell Lines Used to Produce Biologicals (1). Because the WCB is derived from the MCB and propagated for only a few additional passages in tissue culture, abbreviated testing for detecting newly introduced contaminants is acceptable. Any virus contaminant should be quantified and, wherever possible, identified in order to establish the extent of virus clearance that the purification process should achieve (see also Section II.C.5.). The tropism of virus contaminants for human cells should be determined by appropriate infectivity assays. In the case of tissue culture or fermenter production, end-of-production cells (EPC) should be tested at least once to evaluate whether new contaminants are introduced or induced by the growth conditions. EPC should also be re-examined when there are changes in culture medium or in the scale of production. Cells at the limit of *in vitro* age used for production can be tested in lieu of EPC to allow for possible extensions in the length of time cells are kept in culture as manufacturing schemes are developed. The term EPC will be used throughout this text for ease of consultation. For cell lines which are known not to be susceptible to infection with mammalian viruses (e.g. plant cells, some insect cells), bacterial and fungal sterility testing, and in some cases testing for Mycoplasma or other mollicutes such as Spiroplasma, will be the most important concerns. Consultation with CBER is advised before using these cell substrates.

Table I
Cell Line Qualification

Tests	MCB	WCB	EPC
Sterility	+	+	+
Mycoplasma	+	+	+
Virus			
Adventitious	+	-	+
Species-specific*	+	-	-
Retrovirus	+	-	+
Authenticity	+	+	+

* Tests for rodent, primate, or human viruses (other than retroviruses), as appropriate
Retrovirus testing is not required for murine hybridomas. All other cell substrates should be tested as described in Section II.C.1.d.

a. Cell lines should be free from bacterial and fungal contamination as demonstrated by sterility testing. Recommended testing procedures for mycoplasma (cultivable and non-cultivable) are described in Ref. 1.

b. Screening for adventitious viruses (other than retroviruses) should include routine *in vivo* and *in vitro* tests (1).
c. Screening for species-specific viruses (other than retroviruses):

i. The mouse antibody production (MAP) test for murine cell lines (see Appendix II), the HAP test for hamster lines, and the RAP test for rat lines should be used. *In vivo* testing for lymphocytic choriomeningitis (LCM) virus, including non-lethal strains, is recommended. Testing of hamster cell lines should include minute virus of mice (see III.B.1.c.).

ii. Material that is contaminated with LCM, reovirus, Sendai virus, or Hantaan virus should not be used for mAb production.

iii. Cell lines from non-human primates should be screened for the following: herpes viruses (simiae and SA-8), cytomegalovirus (sCMV), encephalomyocarditis virus, simian hemorrhagic fever virus (SHF), varicella virus of simians (sVZV), adenovirus, SV-40, monkeypox, rubeola, and Ebola virus. Any other zoonotic agents suggested by the cell line derivation history should be screened for.

iv. Human cell lines should be screened for Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B (HBV) and HCV, human herpes virus 6 (HHV-6), and any other viruses that are suggested by the medical history of the donor and type of tissue used to establish the original line. Cells from patients who are known to have developed Creutzfeld-Jakob disease (CJD) or other TSE, or from persons with two or more genetically related family members with CJD, should not be used.

*v***.** Heterohybridomas using cells from 2 different species should be tested as appropriate for both species of origin.

vi. For cell lines of other species please consult with CBER.

d. Retrovirus testing of cell lines: Retrovirus contamination of cells from different species varies. The following should be considered when designing studies to detect retrovirus:

i. Murine cells used to produce monoclonal antibodies should be considered inherently capable of producing infectious murine retrovirus. The amount of retrovirus in the unprocessed bulk should be quantitated on a series of bulk harvests and shown to be consistent from lot to lot (1). Endogenous virus particle burden should be determined at the end of a typical fermentation, prior to purification, preferably by thin-section EM on material pelleted by ultracentrifugation. Particle burden determination is preferable to infectivity assays at this stage of production because it does not depend upon the susceptibility to infection of the cell lines used for virus amplification and it provides a "worst case scenario" of the level of viral contamination. Thin-section EM also allows morphological observation of viruses. Other, novel methods of equal or superior sensitivity and general applicability may be acceptable, if appropriately validated. Sufficient retrovirus removal by the purification scheme should be demonstrated (see also Section II.C.4.).

ii. Rat myeloma cell lines and hybridomas may not express retrovirus (13).The absence of detectable retrovirus, however, should be demonstrated by co-cultivation of the test article with a cell line(s) susceptible to a wide range of retroviruses combined with a sensitive detection assay, including examination of EPC and several production lots. If retrovirus is not detected by infectivity assays or electron microscopy, further clearance studies may not be needed. It is suggested that purification schemes for mAb produced by rat cell lines include one or more robust retrovirus inactivation or removal step.

iii. CHO cell lines express defective retrovirus particles (14). Whether hamster cell lines express infectious retroviruses has not been shown. Sponsors should demonstrate the lack of infectious hamster retroviruses by means of the most sensitive infectivity assays available. These include co-cultivation of the test article with a cell line(s) susceptible to a wide range of retroviruses combined with a sensitive detection assay. As a product moves into pivotal clinical trials, it may be necessary to make additional attempts to detect potential infectious virus by utilizing a wider range of indicator cells, including human cell lines (15, 16). Because of uncertainty about the validity of infectivity assays for hamster retroviruses, sufficient retrovirus particle removal by the purification scheme should be demonstrated (see also Section II.C.4-5). It is suggested that purification schemes for mAb produced by hamster cell lines include one or more robust retrovirus inactivation or removal step.

iv. Hybridomas or transfected clones produced from cells of non-human primate or human origin should be examined for the presence of retrovirus. Generic assays, such as transmission electron microscopy (TEM) or reverse transcriptase (RT) can be used to assess the presence of retrovirus. Other assays may be used, as long as they are appropriately validated. In addition, all primate cell lines should be screened for simian immunodeficiency virus (SIV), simian T lymphotropic virus (STLV), Foamy virus, human T lymphotropic virus (HTLV), and human immunodeficiency virus (HIV). Cell lines from non-human primates should be additionally tested for presence of simian retroviruses (SRV).

e. Each cell clone generated by stable transfection of widely used parental lines (e.g. CHO) should be considered as a new cell line from the standpoint of viral safety. Such clones may have significantly

different genetic characteristics compared to the parental line as a result of the transfection procedure itself, the clonal selection process and positional effects due to random integration of vector DNA into the cellular genome. Each clone should be screened for retroviruses and for adventitious viruses as described above and in Table I. Screening for species-specific viruses can be done once, on the MCB of the parental line.

f. Authenticity testing should confirm the cell line's species of origin, identity, and lack of cell-line cross-contamination.

2. Lot-to-lot quality control monitoring of unprocessed bulk lots and purified bulk lots, and final product specifications

Quality control monitoring should be performed on each lot of product, as defined in 21 CFR 600.3(x). Table II summarizes lot-to-lot product safety testing.

Table II
Lot-to-lot product safety tests

Tests	Unprocessed Bulk	Purified Product	Final Product
Sterility	+*	+	+
Mycoplasma	+	-	-
Virus			
Adventitious	+	-	-
Species-specific	+	-	-
Retrovirus	+	-	-
Polynucleotide	-	+	-
Endotoxin	-	-	+

*Bioburden testing with acceptable limits is sufficient at this stage.

In vitro testing with three indicator cell lines should be performed routinely for non-ascites material. *In vivo* testing is generally done once for non-ascites material but should be repeated when production methods change.

MAP, RAP or HAP testing for ascites only..

Quantitation of retrovirus (preferably by TEM) in the unprocessed bulk is important for murine hybridomas. For other hybridomas, generic assays for detection and quantitation of retrovirus, such as TEM coupled with appropriate co-cultivation assays are important if MCB or EPC are positive.

a. Unprocessed Bulk Lots

i. There should be set limits for bacterial bioburden in unprocessed bulk material. If bioburden testing of pooled ascites harvests shows the presence of viable contaminants, they should be quantified, and allowable limits for bacterial contamination should be set based on manufacturing experience. The identity of the bacterial species should be determined on a periodic basis and whenever the allowable limits for contamination have been exceeded. Filtration of ascites

harvests through a # 0.45 :m filter prior to storage is recommended (see also II.C.2.a.*ii*.).

ii. Tests for cultivable and non-cultivable mycoplasma should generally be performed on unprocessed bulk hybridoma supernatants, prior to any clarification by filtration (1). The filtration of unprocessed bulk ascites through a 0.45 :m filter followed by storage at #-60° C prior to testing for mycoplasma is acceptable if samples of unfiltered material are retained for testing. If mycoplasma contamination of animals or unpurified bulk ascites or hybridoma supernatants is detected, these should not be used or processed further.

iii. *In vitro* virus testing with three indicator cell lines (e.g. Vero, MRC5, 3T3) should be performed routinely. *In vivo* testing is generally done once (as part of cell line qualification, Section II.C.1) but should be repeated when production methods change (1). Bioreactors containing hamster cells can become contaminated with minute virus of mice that may escape detection in routine *in vitro* assays. MAP testing or PCR testing for this virus appear to be more sensitive. In all cases, the frequency of monitoring should be specified in SOPs and justified based on actual experience when using continuous production in contrast to batch production. When contamination with a particular virus is encountered in a facility, consideration should be given to modifying the routine testing program in order to detect that virus.

iv. Species-specific virus testing should be performed (see Table II and II.C.1.c.).

v. Murine retrovirus contamination should be quantitated routinely for bulk ascites harvests. This may not be necessary if the sponsor can provide data showing that little variation exists in the concentration of mAb and retroviral load of their bulk ascites over several consecutive manufacturing runs, and the purification scheme used can remove substantially more than the highest load observed. If ascites production uses different groups of mice, periodic serologic monitoring for species-specific viruses should be performed on each group prior to their use for producing ascites. For tissue culture harvests, retrovirus contamination should be quantitated on three clinical grade production lots in order to establish the level of virus contamination for the specific cell line and manufacturing process (1) (see II.C.4). This quantitation of retrovirus should be done preferably by generic assays such as TEM or alternatively by sensitive infectivity assays (see also Section II.C.1.d.). Quantitation should be repeated when changes in tissue culture media, duration or scale of culture are made.

vi. For hybridomas of non-murine origin or other cell substrates, see section II.C.1.d. for appropriate assays to determine whether retrovirus is present. In those cases where MCB or EPC are positive for retrovirus, each lot of unpurified bulk should be examined for detection and quantitation of retrovirus by generic assays, such as TEM, coupled with appropriate co-cultivation assays.

b. Purified Bulk Lots (Drug Substance)

In addition to lot-to-lot safety testing summarized in Table II, routine testing on purified bulk lots of unmodified and modified mAb product should include the following determinations (for discussion of immunoconjugates see Section II.B.7.):

i. If a cell bank containing a known infectious agent is used, CBER staff should be consulted before proceeding with development, and clearance studies should be conducted to demonstrate the removal/inactivation of this agent by the mAb purification scheme. Testing for murine

retrovirus during clearance studies should employ infectivity assays which detect ecotropic recombinant murine retrovirus (ERV) and the polytropic or mink-cell focus-forming murine retrovirus (MCF). The infectivity assay should be comprised of an amplification period on a cell line sensitive to infection by these murine retroviruses (for example, Mus dunni cells, 17) coupled with an appropriate indicator assay (for example, PG4 S+L- assay for MCF virus, immunofluorescence assay with appropriate antibodies for detection of ERV). Assays which do not rely on infectivity, such as PCR-based assays, may be substituted, provided they have been validated for sensitivity and specificity, and that their results are correlated with those of infectivity assays. The first consecutive 3-5 lots of purified bulk should be tested to confirm that the contaminant was removed by the purification scheme. For cell lines containing viruses or virus-like particles, the absence of detectable virus should be confirmed for purified bulk by means of suitable methods having high specificity and sensitivity for the detection of the virus in question. For the purpose of marketing authorization, data from at least 3 lots of purified bulk manufactured at pilot-plant or commercial scale should be provided. However, for cell lines for which the endogenous particles have been extensively characterized, such as CHO cells, and adequate clearance has been demonstrated, it is not usually necessary to assay for the presence of non-infectious particles in purified bulk. Should a human infectious agent be identified in the cell bank, every lot of purified product should be tested and consultation with CBER staff is recommended before extensive product development.

ii. Chemical purity including the residual amounts of extraneous animal proteins, e.g., albumin, immunoglobulin or other contaminants in the final product. An SDS-PAGE analysis, under both reducing and non-reducing conditions, of increasing amounts of purified material should be provided. Generally, silver staining methods are more sensitive but less quantitative than Coomassie blue for SDS-PAGE.

iii. Molecular integrity, including the presence of aggregated, denatured or fragmented product.

iv. Immunoglobulin class or subclass, if used as a test of identity.

v. IEF pattern of the antibody (or its heavy and light chains) in each bulk lot with comparison to the in-house reference standard.

*vi***.** Sterility.

vii. Lot-to-lot testing for DNA content, prior to any excipient addition, is recommended as a way to monitor purification efficiency and reproducibility. DNA content in the final product should be as low as possible, as determined by a highly sensitive method. Low cell viability at harvest may contribute to high DNA levels in unprocessed bulk. It is suggested that, whenever possible, the final product contain no more than 100 pg cellular DNA per dose (18). It is suggested that a method with a sensitivity of 10 pg be used to determine DNA levels (2). An appropriately conducted clearance study for DNA removal may be an acceptable substitute for lot-to-lot testing.

viii. Tests for detection and quantitation of potential contaminants or additives (e.g., antibiotics, other media components, host cell proteins, chromatography reagents, preservatives, or components that may be leached from affinity chromatography columns such as protein A). Whenever possible, contaminants introduced by the recovery and purification process should be below detectable levels using a highly sensitive analytical method. However, for many of these potential contaminants, depending upon their potential for toxicity or immunogenicity, an

appropriately conducted clearance study may be an acceptable substitute for lot-to-lot testing. For products intended for marketing, at least 3 exhibit lots should be tested to confirm the removal of the contaminant(s) for which clearance studies have been conducted. Such clearance studies for product contaminants may have to be repeated when manufacturing schemes are changed. We recommend that antibiotics, particularly penicillin or other beta lactams, not be used. However, if they are used, their removal must be demonstrated by an adequate clearance study. The acceptability of trace contaminants that cannot be removed by standard methods should be discussed with CBER prior to the submission of an IND. Pristane, if used in the propagation of ascites fluid, should be shown to be undetectable by a sensitive test.

ix. A brief description of the formulation process and areas to be used for it should be provided. This description should incorporate information on the processing area, support systems, personnel and product transfers in sufficient detail to highlight the design or operational features utilized to minimize contamination or cross-contamination.

c. Final-Filled Product (Drug Product)

The following tests should be performed on the contents of final containers from each filling of product as defined in 21 CFR 600.3(y). In certain situations (e.g. user-radiolabeling), special approaches to final container testing may need to be developed on a case-by-case basis after discussion with CBER:

i. Protein quantity.

ii. Potency (21 CFR 610.10).

iii. Purity (21 CFR 610.13). Electrophoretic migration of the product in both the native and reduced states on polyacrylamide gels with comparison to the in-house reference standard can be used as a test for purity.

iv. Sterility (21 CFR 610.12).

v. A test for endotoxin. The Limulus Amebocyte Lysate (LAL) assay may be an acceptable equivalent method as allowed in 21 CFR 610.9 when validated by the rabbit pyrogen test as described in 21 CFR 610.13. A U.S. licensed test system should be used to perform the LAL assay. Comparative testing should be repeated when LAL lots made by a different manufacturer are used. Conditions necessary for comparative testing of the rabbit pyrogen and LAL assay procedures should be discussed with CBER on a case-by-case basis. See also "Guidelines on Validation of the LAL Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices", December , 1987 (19).

vi. An appropriate identity test (21 CFR 610.14).

vii. Moisture (21 CFR 610.13) testing, when appropriate.

viii. Preservative (21 CFR 610.15) testing, when appropriate.

ix. Excipients, when appropriate

x. pH, when appropriate.

xi. The areas used for final fill of drug products should follow the recommendations provided in the Guidelines on Sterile Drug Products Produced by Aseptic Processing (20). For information on validation of equipment used in aseptic processing, refer to the Guidelines for Submitting Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products (21).

3. Stability of product

Product stability should meet the demands imposed by the clinical protocol. Accelerated stability testing data may be supportive but do not substitute for real-time data for product approval and labeling.

a. A stability testing program should be developed that includes tests for physico-chemical integrity (e.g., fragmentation or aggregation), potency, sterility, and, as appropriate, moisture, pH and preservative stability, at regular intervals throughout the dating period. See also "Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics" (22) and the relevant ICH document (7). For products that are in clinical trials, significant changes that occur during storage should be reported to CBER. For license applications, and preferably prior to each stage of development, stability tests that support the proposed dating period should be performed on the final-filled product, using the container and closure configuration intended for distribution. For biologics license applications, storage of production intermediates (e.g. unpurified bulk, purified bulk) also should be supported by stability testing.

b. Stability tests for assuring biological activity (e.g. quantitative *in vitro* potency assays) should include a manufacturer's in-house reference standard. Whenever possible, a single lot of test antigen (e.g. purified antigen, cells or tissue) should be used throughout the study. A quantitative potency assay(s) should be used to permit a meaningful comparison of activities.

c. Accelerated stability studies, i.e. stability testing after storage at temperatures exceeding routine storage temperatures, may help to identify and establish which tests are stability-indicating. Specific parameters that indicate stability should be monitored by trend analysis on every lot on the stability testing program.

4. General considerations on quantitation and removal of a retrovirus contaminant

The amount of any retrovirus contaminant in the unprocessed bulk product should be estimated. If a retrovirus is detected, any possible or suspected tropism for human cells should be explored (e.g. by co-cultivation assays). This should be followed by demonstration of removal of the contaminant, using the contaminant itself or a representative analogue of the known contaminant (e.g. a model retrovirus) in a model purification system (1).

Unprocessed bulk supernatant concentrates or ascites should be assayed prior to any manipulation other than clarification by low speed centrifugation, unless it can be shown that virus testing would be made more sensitive by initial partial processing. We recommend that retroviruses contaminating ascites or supernatants produced by rodent cell lines be quantitated by TEM of concentrated samples of supernatant. If TEM results are negative, it should be assumed that the titer of retrovirus is equivalent to the lowest limit of detection ($1x10^6$/ml). Ascites and supernatants produced by non-rodent or hybrid cell substrates should be assayed as described (see Section II.C.2.b.*vi*.). Demonstration that retroviruses are removed or inactivated by the purification scheme should occur prior to phase 1 studies, except for mAb intended for use in feasibility trials in serious or life-threatening conditions (see Section II.D.). References 1, 2 and 8,

and paragraphs 5 and 6 below, also discuss the design of studies to demonstrate retrovirus removal. The goal of such studies is to demonstrate that the purification process is able to remove substantially more virus than is estimated to be present in a single dose equivalent of starting material.

5. General considerations on the design and interpretation of virus clearance studies

These considerations are provided here solely for guidance purposes in the design of virus clearance (i.e., removal/inactivation) studies.

a. General Experimental Design

The objective of a virus clearance study is to provide a quantitative estimate of the level of virus reduction provided by the removal/inactivation procedures. Thus, the study should be designed, conducted and analyzed in a manner that will provide accurate information to reliably assess the ability of these procedures to remove/inactivate viruses. The study, including virus infectivity assays, should be designed according to good scientific practice to yield data with accuracy and precision that are amenable to statistical analysis. Each clearance study should include an appropriate control experiment performed in parallel to the experimental condition to assess virus inactivation caused by experimental manipulation (dilution, concentration, filtration, storage etc.). Any observed difference should be used to adjust the virus reduction/inactivation values for each removal/inactivation procedure. It is preferable that the study be conducted under current Good Laboratory Practices (21 CFR, Part 58).

b. Statistics

Virus clearance studies should be supported by appropriate statistical analysis demonstrating that the study is valid and reliable. Virus infectivity assays used to quantitate the virus titer should be sensitive, reproducible and conducted with sufficient replicates to demonstrate statistical accuracy. Sufficient sample volumes should be tested to ensure that there is a high probability of detecting virus in the sample if present. The experimental variability present in virus titration should be determined in virus infectivity assays and reported using confidence intervals. Within assay variability should be calculated using conventional means (standard deviation, standard error of the mean, etc.). Between assay variation can be monitored by the inclusion of an appropriate virus reference preparation run in parallel with unknown samples. The 95% confidence intervals for within assay variation should be established and reported for each virus infectivity assay, and should be in the order of " 0.5 $\log_{10}$ of the mean. The quantitative estimate of virus reduction for each procedure should be reported as the reduction factor. This is defined as the $\log_{10}$ of the ratio of the virus load in the starting material to the virus load in the post reduction material ($\log_{10}$ (volume x virus titer of starting material / volume x virus titer of post-reduction material)). In general, this virus reduction factor should be based upon the amount of virus detected in the spiked starting material. The 95% confidence interval for each reduction factor is calculated from the 95% confidence interval of the virus infectivity assays at the beginning (+s) and end (+a) of each procedure using the formula " $\sqrt{a^2+b^2}$. Such confidence intervals should be calculated whenever possible in studies of clearance using relevant or specific model viruses (with respect to viruses actually detected in the cell line as opposed to non-specific model viruses). The reduction factors for each procedure are summed to calculate an overall reduction factor for the entire process. Additivity of reduction factors assumes that different steps are independent, i.e. have different mechanisms of action so that virus forms (mutant, aggregates etc.) escaping one step do not have a higher likelihood of also escaping the other. Reduction factors from two steps with the same mechanism of action (e.g. two incubations at low pH) are not necessarily additive. Due to the high intrinsic variability of infectivity assays, reduction factors of one log or less are considered negligible and are not included in the overall reduction factor.

6. Generic or modular virus clearance studies

a. A generic clearance study is one in which virus removal and inactivation is demonstrated for several steps in the purification process of a model antibody. These data may then be extrapolated to other antibodies following the same purification and virus removal/inactivation scheme as the model antibody.

b. A modular clearance study is one that demonstrates virus removal or inactivation of individual steps during the purification process (column chromatography, filtration, pasteurization, solvent/detergent, low pH, etc). Each module in the purification scheme may be studied independently of the other modules. Different model mAb may be used to demonstrate viral clearance in different modules, if necessary. If the purification process of a product mAb differs at any of the virus removal or inactivation modules from the model mAb, this module must be studied independently from the model. The other, identical modules in the procedure may be extrapolated to the product mAb.

c. Applicability: For monoclonal antibodies manufactured at one site, generic or modular clearance data may be extrapolated to other monoclonal antibodies of the same species and H and L chain class and, generally, subclass, derived from the same source (e.g. ascites or tissue culture) and cell substrate. Generic and/or modular clearance will apply only when the mAb have similar biochemical properties and are purified by identical methods. Particular attention should be paid to column elution buffer conditions, including pH and ionic strength, the sequence of columns, protein concentration, dwell times, flow rate, pressure, temperature, and potential problems associated with scale-up, at all steps of virus inactivation and removal. In some cases, sponsors may demonstrate virus removal/inactivation for a particular module at two different values of a given parameter (e.g. ionic strength, dwell time, temperature) and use any values of that parameter falling within this range. In order to apply generic or modular clearance algorithms to a specific product, it is necessary to determine the virus load in the unpurified bulk for each specific product using at least 3 lots (see Section II.C.2.). This information is then used to determine whether the total reduction/inactivation factor provided by the purification procedure ensures that substantially more virus is removed or inactivated than the estimated unpurified bulk titer. For example, generic clearance might apply to a series of murine mAb of different specificities but of the same H and L chain isotypes that are purified in an identical manner, or to a series of humanized mAb of the same H and L chain isotype but with different CDRs, that are purified by identical methods. The concepts of generic and modular virus clearance studies do not apply to products of entirely human origin or to products that have the potential to be contaminated by human pathogens. Consultation with CBER is advised before applying generic or modular virus clearance studies.

d. Virus Clearance Master Files: A sponsor purifying mAb for a variety of applications or a manufacturer producing mAb for a variety of sponsors may submit a Master File containing the data demonstrating virus removal or inactivation for different purification schemes. This Master File may then be cross-referenced in INDs/IDEs or license applications using mAb purified by these schemes if applicability criteria are met.

7. Product testing requirements for mAb used as ancillary products

Testing requirements for mAb used as ancillary products (see I.B. for definition) vary depending on the clinical indication and the stage of product development. From a safety standpoint, they should be characterized in the same way as products intended for *in vivo* administration. However, production steps which follow purification of the mAb can be used as part of the virus removal/inactivation scheme. These include, for example, conjugation of mAb to solid phases for affinity purification, sanitation of affinity columns etc. The concepts of generic and modular clearance studies apply to ancillary products as well. Purity of the ancillary product may not be as critical as for products intended for *in vivo* administrations, provided that the performance of the ancillary product in the production of the final product is acceptable

and reproducible, limits are set for impurities and the nature of impurities is known. Leaching of mAb or impurities from the mAb preparations into the final product should be taken into consideration in testing the final product, as appropriate. Removal of mAb or impurities from the mAb preparation may be demonstrated by means of a clearance study. Labeling for the final product may need to carry precautionary statements about potentially toxic or immunogenic residual impurities.

D. PRODUCT SAFETY TESTING FOR FEASIBILITY CLINICAL TRIALS IN SERIOUS OR IMMEDIATELY LIFE-THREATENING CONDITIONS

1. General considerations

The extent of product testing necessary before a particular clinical trial is initiated depends on the source and nature of the product, the stage of product development and the clinical indication. Abbreviated testing needs described in this Section (II.D.) apply to feasibility clinical trials in serious or immediately life-threatening conditions for which no effective alternative treatment exists. Abbreviated testing does not apply to human products made in human cell substrates, but may apply to recombinant products made from transfected human genes, depending on the cell substrate. Consultation with CBER is strongly advised for sponsors considering the application of abbreviated testing in this setting to products that have the potential to be contaminated by human pathogens. For the purpose of this document, the following definitions should be considered:

> *i*) **Feasibility clinical trials.** These are pilot studies whose objectives include, among others, early characterization of safety and initial proof of concept in a specific patient population. These trials are limited in scope, and are generally conducted in a single center, with a small number of patients (e.g. 5-20). These trials cannot be used by themselves to support licensure of a product. Studies conducted in normal volunteers are not included in this definition.
>
> *ii)* **An immediately life-threatening condition** is "a stage of a disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment" (21 CFR 312.34).

See 21 CFR 312.34 and the Federal Register vol. 52, No. 99 (May 1977) for a discussion of serious or life-threatening conditions. Application of abbreviated testing requirements to serious conditions which are not immediately life-threatening as defined in 21 CFR 312.34 will depend upon an assessment of the potential risks and benefits to the patient(s). Factors that should be considered in such a risk-benefit analysis include, among others: *i*) the nature and manufacture of the product; *ii*) the nature and severity or stage of the disease; *iii*) the anticipated effect(s) of product administration (e.g., diagnosis, palliation or cure); *iv*) the availability of comparable or satisfactory alternate treatments *v*) characteristics of the patient population (e.g. age, response to previous therapy); *vi*) the number of patients involved and *vii*) the design of the clinical trial (e.g. patient follow-up, safety monitoring etc.). Pre-IND consultation with CBER staff is strongly recommended for sponsors planning to use the abbreviated testing described below for serious but not immediately life-threatening diseases. The guiding principle for these trials is that sufficient information should be provided before testing in human subjects to assure that patients and their contacts will not be put at unacceptable risk. Informed consent issues for these trials are discussed in Section IV.A.1.e. The limited testing described below should not be used to support development beyond the stage of feasibility trials. Therefore, sponsors are encouraged to plan for additional testing and characterization as described in Section II.C. when they intend to pursue advanced clinical development and seek licensure. In designing the purification process, it is advisable to include at least two orthogonal robust virus inactivation/removal steps. This would further reduce the testing necessary to begin initial clinical trials.

2. Product safety data needed before the initiation of feasibility trials in serious or immediately life-threatening conditions

a. Sterility (bacteria and fungi) testing should be performed on the final product. Three vials of final product should be tested. Routine methods in use in the sponsor's hospital accredited clinical diagnostic laboratory can be used for these tests. Mycoplasma and endotoxin testing are strongly encouraged.

b. *In vitro* and *in vivo* testing for adventitious viruses : If the unpurified bulk product is free of adventitious viruses by *in vitro* and *in vivo* tests or if the purification scheme does include at least two orthogonal robust steps (see Table III), these tests are not required.
Table III shows ranges of retrovirus removal (expressed in decimal logs) that might be expected with various robust inactivation/removal steps and is presented to aid manufacturers in the design of mAb purification schemes.

Table III

Inactivation Step	Reported log virus removal
pH # 3.9	**3-4**
heat	**4**
solvent/detergent	**5**
filtration (15-40 nm)	**4-8**

If the mAb is produced as an ascites fluid and the mice used have been MAP tested and found free of species-specific viruses, adventitious virus testing of the final product is not necessary. When testing is necessary, we recommend that a minimum amount of product equal to 3 maximum human doses be used for these tests. For the purpose of these trials, MAP testing can be limited to known human pathogens: Hantaan, LCM, Reovirus and Sendai virus. For mAb produced in primate cell lines or in non-murine cell lines whose potential for contamination by human pathogenic viruses in unknown, the cell lines or the EPC or the unpurified bulk product should be tested as described in Section II.C.1. c and d.

c. Murine retrovirus testing of the final product is needed on the final-filled product only when the antibody is produced in a murine cell substrate and the purification scheme does not include at least two robust orthogonal virus inactivation/removal steps (see Table III). We recommend that a minimum amount of product equal to 3 maximum human doses be used for these tests. Testing of the final product, when necessary, should be done by a highly sensitive infectivity assay, such as amplification in Mus dunni cells followed by detection in PG4 cells or by other sensitive means as outlined in II.C.2.b.*vi*.

d. MAb used as ancillary products (see I.B. for definition) in feasibility clinical trials in serious or life-threatening conditions. Two cases can be distinguished:

> *i*. When the final product can be tested and the results are available prior to administration (e.g. purified recombinant proteins or cells that can be stored frozen), safety testing can be carried out on the final product itself, as determined during review of the final product.

ii. When the final product is administered prior to or without any safety testing and/or processing, testing of the mAb should be performed as described above in paragraphs D.2. a. through c. In this case, amounts of mAb comparable to those used in one run of final product purification should be used.

The same safety considerations apply to complement, DNAase and other biological reagents used as ancillary products for cell depletion in conjunction with mAb.

E. ISSUES RELATED TO MANUFACTURING CHANGES (DEMONSTRATION OF PRODUCT COMPARABILITY)

1. General

Changes in the product manufacturing scheme frequently occur during clinical development of mAb. Sponsors should develop a plan for demonstrating that the products made by the old and new schemes are comparable, particularly when preclinical or clinical data developed prior to the production changes will be used to support further clinical trials and/or marketing applications (23). Similar considerations apply in the case of significant scale up in the manufacturing process (with or without modification of the general manufacturing scheme) implemented during or after completion of phase 3 trials.

When changes in manufacturing occur during early clinical development, plans for evaluation of product comparability should be incorporated into product development strategies. Such plans should be discussed with CBER and, when appropriate, submitted to CBER for review (see ref. 23).

In-process specifications may be affected by manufacturing changes or process scale-up and appropriate revisions should be undertaken. Similarly, process validations (e.g., virus clearance studies, removal of contaminants or leachables) for all affected steps should be repeated after any significant manufacturing change. In the case of process scale-up, it is recommended that, whenever possible, a column geometry and a ratio between sample volume and bed volume as close as possible to that of the original process be maintained. This is particularly important for those steps (e.g., size-exclusion chromatography) where these parameters are critical to the chromatographic process. Ref. 23 should be consulted for more details on demonstrating product comparability.

2. In vitro evidence of product comparability

In general, when a product is obtained by a modified or scaled-up manufacturing scheme, the results of a rigorous physico-chemical characterization and *in vitro* functional comparison (see Product Manufacture and Testing, Section II.B.) will dictate whether additional data (e.g., pre-clinical and/or clinical data) will be needed. A protocol for demonstrating physico-chemical, immunochemical and biological comparability of two products should prospectively define acceptable variation in the results of individual assays and acceptance criteria for product comparability. For quantitative assays (specific biological assays) accurate estimates of inter and intra-assay variations should be provided. Assays with high intrinsic variability are poorly suited for the evaluation of product comparability. Comparisons should test a number of separate product lots in parallel in order to demonstrate the reproducibility of the new manufacturing scheme. An *in vitro* biochemical characterization of mAb comparability should include a side by side comparison of the two products by a number of different techniques. Properly stored retention samples from previous lots should be used for such side by side comparison. A list of techniques could include SDS-PAGE under reducing and non-reducing conditions, Western blot, size-exclusion analytical chromatography, reverse phase high performance liquid analytical chromatography, isoelectrofocusing, mass spectrometry, an

analysis of glycosylation including carbohydrate content and composition, peptide mapping or other appropriate tests. *In vitro* functional comparison should include assays aimed at the characterization of the biological function of the antibody (e.g., binding, cytotoxicity, epitope modulation, etc.). Whenever possible, a comparison of the affinity constants of the two products is highly recommended.

3. Animal studies

Depending on the quality of the data and the type of *in vitro* assays, the nature of the manufacturing change and the types of product differences observed or anticipated, a program of comparative testing (pharmacokinetics, etc.) in appropriate animal models may be considered in lieu of human clinical data when biochemical testing shows differences or cannot exclude significant differences in two products. In some cases, pharmacokinetic studies are complementary to *in vitro* studies. Pharmacokinetic studies in animals may be informative, even in the absence of the target antigen, depending upon the question to be addressed and the expected contribution of antigen binding to the biodistribution of specific mAb in humans. The extent of animal toxicity testing that may be needed to assess comparability will depend upon the safety profile of each specific product, the magnitude of the changes in manufacturing, the presence or absence of detectable differences in purity, structure or *in vitro* activity. Sponsors are encouraged to discuss plans for comparative testing of the two products in animals with CBER or to submit proposal for such testing to CBER for review and comment. The proposed program should be appropriate in view of biochemical data and include statistical considerations.

4. Clinical studies to support manufacturing changes

Comparative clinical evaluation of the products produced by different or scaled-up manufacturing schemes may be needed in certain situations:

a. Product activity cannot be adequately characterized by analytical testing.

b. Biochemical or biological testing show differences in the products.

c. Animal testing reveals significant pharmacokinetic or other differences in the products.

d. The formulation of the product has been changed in a way that can affect its bioavailability. The latter changes generally dictate a need for clinical pharmacokinetic studies.

Pharmacokinetic, safety and/or efficacy data may be required depending upon the nature and magnitude of the observed changes in the biochemical and or biological properties of the product.

Additional information on product comparability testing can be found in ref. 23.

III. PRECLINICAL STUDIES

A. TESTING CROSS-REACTIVITY OF MAB

When the same or related antigenic determinant is expressed on human cells or tissues other than the intended target tissue, binding of the antibody to this tissue may be observed. Non-target tissue binding may have serious consequences, particularly when pharmacologically active antibodies or cytotoxic immunoconjugates are used. Accordingly, cross-reactivity studies with human tissues (or cells if

applicable) should always be conducted prior to phase 1 to search for cross-reactions or non-target tissue binding. In the special case of bispecific antibodies, each parent antibody should be evaluated individually, in addition to testing of the bispecific product.

1. *In vitro* testing for cross-reactivity

Human cells or tissues are presently surveyed immunocytochemically or immunohistochemically. Appropriate newer technologies should be employed as they become available and validated.

a. Reactivity of the antibody or immunoconjugate should be determined with the quick-frozen adult tissues listed in Appendix I. Surgical samples are preferred. Post-mortem samples are acceptable with adequate tissue preservation. Tissues from at least three unrelated human donors should be evaluated in order to screen for polymorphism. The effect of fixatives on tissues that are known to be positive should be evaluated to ensure that the target antigen is preserved during tissue processing.

b. In special situations it may be appropriate to assay cross-reactivity on representative cultured cell lines, stem cells, and embryonic/fetal tissue.

c. Several concentrations of the product should be tested. The ability to detect cross-reactions may depend on antibody concentration. Antibody affinities as well as expected achievable peak plasma concentrations should be considered when choosing the proper concentrations for tissue binding studies. An "ideal" concentration for these studies may be the lowest mAb concentration that produces maximum (plateau) binding to the target antigen. An attempt should also be made to compare the ratio of specific binding to target tissue to specific binding to cross-reactive tissue. Because non-specific as well as Fc-mediated binding may be observed, it should be distinguished from specific cross-reactions using inhibition assays with purified antigen, when available.

d. Positive and negative controls are essential for interpreting study results. Controls confirm the acceptable condition of the tissues and adequacy of the assay. Anti-transferrin receptor mAb may be a useful positive control, since transferrin receptor is a common and abundant molecule on the surface of growing normal and tumor cells.

e. If a conjugated, chemically modified antibody or antibody fragment is to be used clinically, it should be tested in that form if at all feasible. The substitution of antibodies of similar specificity for cross-reactivity testing is discouraged.

f. When cross-reactions are encountered and there is a reason to suspect genetic polymorphism of the target antigen, studies should be expanded to a larger panel of tissues to better characterize this polymorphism.

g. A comparison of *in vitro* cross-reactivity in tissues from different species is important in determining the most relevant animal for subsequent toxicology studies.

2. *In vivo* testing for cross-reactivity

Cross-reactivity of a monoclonal antibody with non-target human tissues should dictate a comprehensive *in vivo* investigation in animals, when appropriate models are available. This finding, particularly with cytolytic immunoconjugates or antibodies with ADCC activity, generally indicates the desirability of more extensive preclinical testing, including studies in more than one animal species over a range of doses and

repeat dose animal studies. Localization to non-target tissues should be kept in mind when designing clinical trials.

B. PRECLINICAL PHARMACOLOGY AND TOXICITY TESTING

1. General considerations

a. Preclinical safety testing of mAb is designed to identify possible toxicities in humans, to estimate the likelihood and severity of potential adverse events in humans, and to identify a safe starting dose and dose escalation, when possible. Preclinical testing concerns surrounding mAb products include their immunogenicity, stability, tissue cross-reactivity, and effector function(s). Species differences may complicate the design and interpretation of preclinical studies. CBER recognizes that animal models expressing the antigen of interest or a closely related, highly cross-reactive epitope are not always available. Pharmacokinetic and pharmacodynamic properties of mAb that are dependent upon specific antigen binding may not be evident in animal studies conducted in species which do not express the antigen of interest. In some cases, xenograft models can be developed by introducing cells expressing the antigen of interest into immunodeficient mice (e.g. SCID or nude mice). Such models can provide information on specific targeting of desired cells, especially with radiolabeled mAb or immunoconjugates. Transgenic models expressing the antigen of interest are another possibility, if available. Whenever they are available, parallel models which explore the effects of mAb against the animal homolog of the antigen of interest can be informative. *In vivo* activity models have proven valuable in providing data which support a rationale for the proposed product use and in defining safety and toxicity. Animal disease models are available to study the effects of mAb on many inflammatory and autoimmune diseases, and allograft rejection. The extent of preclinical safety testing and the results of such testing will influence safety considerations for initial clinical trials (e.g. starting dose, dose escalation scheme, etc.).

b. Preclinical testing schemes should parallel to the greatest extent feasible those anticipated for clinical use with respect to dose, concentration, schedule, route, and duration. The range of doses selected for study should include at least one dose that is equivalent to and one dose that is a multiple of the highest anticipated clinical dose, with appropriate adjustments for interspecies differences in body size. A broad dose range should be explored. The highest doses tested should elicit adverse effects, whenever possible. Dose ranges are best established with a minimum of three doses. The linearity and overall shape of the dose response curve should also be defined by investigation of several doses and dosing intervals. If changes in manufacturing and/or formulation are made subsequent to conduct of preclinical studies, the decision to repeat some or all preclinical studies should depend on an assessment of the impact or likely impact of these changes on the product (see Section II.E.).

2. Animal toxicology studies

When planning toxicity testing for mAb, the following should be considered:

a. If the test article is an unconjugated antibody and there is no animal model of disease activity or animal that carries the relevant antigen, and cross-reactivity studies with human tissues are clearly negative, toxicity testing may not be necessary.

b. When a relevant animal model is available, an attempt should be made to study the dose-dependence of pharmacodynamic effects. The use of a broad range of doses, including high doses may allow a better prediction of the therapeutic index.

c. The properties of a relevant antigen in the animal should be comparable to those in humans in

biodistribution, function, and structure. For example, studies of $CD34^+$ progenitor cells in the baboon are useful because the same cell fractions in both species express the $CD34^+$ antigen and produce hematopoietic engraftment. Absolute equivalence of antigen density or affinity for the mAb, however, is not necessary for an animal model to be useful. Differences in binding, for example, may be compensated for by alterations in the dose or dosing frequency. Differences between the animal and human in antigen number, the affinity of a mAb for the antigen, or the cellular response to mAb binding, should be identified. This will allow more accurate extrapolation of safe human starting dose and estimation of the margin of safety.

d. Routine assessments of mutagenicity are not generally needed for mAb.

e. Reproductive and developmental studies including teratogenicity in an appropriate animal species should be carried out in instances in which the product is intended for repeat or chronic administration to women of childbearing potential. Results of such peri- and post-natal developmental studies should be submitted for marketing approval. Evaluation of male fertility, when appropriate, should be completed before phase 3 trials.

3. Pharmacokinetics and pharmacodynamics

A pharmacokinetic model may aid in the interpretation of preclinical activity and toxicity, and in the recommendation of an appropriate dosing regimen and thereby improve the design of clinical trials. Such studies should aim at determining pharmacokinetic and pharmacodynamic endpoints. Of particular importance to the selection of clinical dosage is determining the relationship activity to area under the curve (AUC) of tissue or blood concentration over time. In considering the relationship of activity to AUC, factors related to the pharmacodynamics of the monoclonal antibody should be used in evaluating potential clinical effects. These factors include pathophysiologic status, threshold for effects as well as molecular events like the rates of association and dissociation for the site of action. Studies of biodistribution may provide the initial evidence for inappropriate tissue targeting by a mAb or explain toxicities that are observed in animals. Interpretation of data should consider species of origin, isotype, whether the mAb is an intact immunoglobulin, a fragment or an immunoconjugate, method of labeling, stability of the immunoconjugate, level of antigen expression in the recipient, binding to serum proteins, and route of administration. Even if antigen is expressed in an animal model, the mAb may bind the human target antigen and its animal counterpart with different affinities. MAb half-life may also be affected by glycosylation, susceptibility to proteases, presence of circulating antigen, and host immune response. The presence of antibodies to the product may alter biodistribution and elimination. In some cases, informative pharmacokinetic studies may be obtained in animal models which do not express specific antigenic determinants, depending upon the role played by antigen binding in product biodistribution, biotransformation and excretion.

a. Selection of the animal species for pharmacokinetic and pharmacodynamic testing should be guided by the following considerations:

> *i.* Preference should always be given to study of a mAb in an animal model in a species that shares a cross-reactive or identical target antigen with humans, whenever such a species is available. For unconjugated mAb directed at human antigens not expressed in animal models or foreign antigens (bacterial, viral, etc.), studies in animal species lacking the target antigen may not be necessary unless they are designed to address manufacturing issues (see Section II.E. on Product Comparability).
> *ii.* Study of non-human primates is appropriate for unconjugated mAb when there are antigen

binding data that indicate that primates are the most relevant species.

iii. Normal rodent and murine xenograft models should be critically evaluated for their likelihood of predicting accurately human pharmacokinetic behavior of mAb. Xenograft models may be more useful in evaluating the ability of mAb to bind to human tumors *in vivo*.

b. Changes in manufacturing or formulation may result in significant changes in biological activity. Therefore, it is recommended that the material used in the preclinical studies be manufactured using the same procedures as used or intended for use in manufacturing material for clinical trials. In some cases it may be appropriate to modify the components of the formulation for preclinical testing. For example, substitution of the homologous animal serum albumin for human serum albumin that is used as a carrier will prevent the formation of anti-albumin antibodies in animal studies and thereby increase the relevance of preclinical testing.

c. Pharmacokinetic parameters should be defined using one or more assay methods (e.g., a radiolabeled mAb should be assayed by ELISA and by measurement of radioactivity). In the case of immunoconjugates of any type, intact conjugate should be distinguished from free mAb and free ligand (e.g. toxin, drug, or radionuclide). Pharmacokinetic parameters that are most important for product characterization, as well as most useful for determining product comparability include T_{max}, C_{max}, $T_{1/2}$ and AUC.

d. The development of anti-immunoglobulin antibodies greatly complicates study and interpretation of the effects of repeat dosing in animals. Murine antibodies are non-immunogenic in mice but are immunogenic in humans, making it difficult to extrapolate the results of repeat dose studies in mice to planned repeat dose administration in humans. The reciprocal problem will occur with fully human, chimeric or "humanized" mAb. Repeat dose studies in rodents in this case may be of little value.

4. Preclinical *in vivo* studies with immunoconjugates

a. Immunoconjugates should be tested for stability *in vivo*.

i. Individual components of an immunoconjugate should be measured during pharmacokinetic and tissue distribution studies in animals and compared to the distribution of unconjugated antibody.

ii. The target tissues for the various components and the potential toxicities that they may cause should be established.

b. Immunoconjugates containing radionuclides, toxins, or drugs should undergo animal toxicity testing even when the target antigen is not present in an animal species, because of possible conjugate degradation or activity in sites that are not the result of mAb targeting. Depending upon the nature of the components of the immunoconjugate and the stability of the conjugate itself, separate studies of the components may be warranted. The toxicity profile of each component should adequately describe the incidence and severity of possible adverse effects. Results should be correlated closely with studies of conjugate stability. Studies of the immunoconjugate should be performed in a species with the relevant target antigen or disease model, whenever available and generally in rodents if a target antigen-positive species is not available. Toxicity testing of free toxin or nuclide may be performed in a different species.

c. For immunoconjugates containing radionuclides:

i. Animal biodistribution data may be used for initial human dose estimation.

ii. Animal models that express the targeted antigen, whenever such models are available, are more likely to reveal the effects of antigen "sinks" or tissues with unexpected antigen expression on biodistribution and/or toxicity.

iii. Xenograft models may evaluate tissue targeting and antigen non-specific radioimmunoconjugate distribution problems, but are not helpful at identifying areas of normal tissue cross-reactivity.

iv. An adequate number of animals should be studied to achieve radiation dose estimates with an acceptable coefficient of variation (usually less than 20%).

v. There should be complete accounting of the metabolism of the total dose of administered radioactivity and an adequate number of time points to determine early and late elimination phases.

vi. Radioimmunoconjugates should be tested for stability *in vitro* by incubation in serum or plasma (see Section II.C.7.). Methods should be developed to estimate the percent radioactivity in each of the three species of concern: free isotope, conjugated mAb, and labeled, non-mAb substances.

IV. CLINICAL STUDIES

MAb administered to humans have usually been well-tolerated. Instances of serious or fatal adverse events have generally resulted from intended or unintended binding of mAb to specific antigens. These events emphasize the importance of screening tissues for mAb cross-reactivity, particularly when relevant-antigen animal models are not available. The results of preclinical tests may alert physicians to potential toxicities and may indicate that more conservative dosing schemes are justified during dose escalation.

A. CLINICAL CONSIDERATIONS FOR PHASE 1 AND 2 STUDIES

1. General

a. Different approaches to Phase 1 and 2 studies may be warranted depending on the nature of the mAb. Initial studies of therapeutic mAb in phase 1 are generally escalation studies of single-doses of the mAb. The goal should be to determine a presumed optimal biologic dose (OBD) that is usually defined by pharmacokinetics or pharmacodynamic measurements (e.g., degree of antigen binding or saturation or target blood levels, determined on the basis of preclinical studies) and, where appropriate, by the tolerability to the agent (e.g., the maximally tolerated dose [MTD]) (24-26). In the case of a therapeutic unconjugated mAb, studies that identify the MTD may not be necessary. Instead, determination of a presumed OBD may be a more appropriate goal. Immune activation, when relevant to the mechanisms of action or toxicity of the mAb, should be evaluated. In the case of radiolabeled therapeutic mAb or immunotoxins, undesired tissue targeting and release of conjugate due to degradation are major safety concerns. Patients receiving immunotoxins should be monitored for capillary leak syndrome and for hepatic, renal, and muscle toxicities.

Some antibody-specific side effects are more likely to occur with certain subclasses of immunoglobulins.

These antibodies (e.g., human IgG1 and IgG3, mouse IgG2a) are more likely to activate complement or activate antibody-dependent cell-mediated toxicity (ADCC) via their Fc regions, leading to lysis of bound cells. MAb may also cause desired or adverse effects by blocking or inducing functions of target cells (e.g., cytokine release syndrome following stimulation of T-cell receptors by anti-CD3).

b. In general, subjects in clinical trials of therapeutic and diagnostic products, including mAb, should be representative of the population targeted for eventual product use. Because of the potential immunogenicity of mAb, healthy volunteers may not be appropriate candidates for phase 1 trials. The nature of the mAb, the target antigen, and the proposed clinical application should be considered before deciding to enroll healthy volunteers in a trial. Situations in which healthy volunteers might be used in early trials include the following:

> *i.* When the risks of studying a new agent initially in the index population are too high, such as when the index population expresses abnormally high levels of antigen (raising specific toxicity concerns) or when the index population may be particularly vulnerable to toxicity because of serious illness or significant organ dysfunction.
>
> *ii.* When the index population is so ill that safety data are confounded and difficult to interpret.

When healthy volunteers are considered for inclusion in initial studies of a mAb, the informed consent should reflect the absence of direct medical benefit. For healthy volunteers, as for patient volunteers, the informed consent process should also illustrate the potential immediate and long-term risks of receiving xenogeneic proteins. These include possible toxicity, allergic reactions, and, in the case of murine and other non-human mAb, potential future inability to receive or benefit from a diagnostic or therapeutic mAb because of the development of an immune response against the foreign protein.

c. Sponsors and investigators should carefully consider whether single doses of the mAb, multiple doses of the mAb in a single course, or multiple courses of therapy will be most likely to optimize benefit over risk. Concomitant therapies or repeat administration of the mAb may alter its safety and efficacy profiles. Changes in antigen mass (e.g., due to binding and clearance, or to antigen modulation by the mAb) and immune responses to the mAb, for example, may prevent extrapolation of single dose data to multiple-dose schedules. Furthermore, repeat administrations in the face of an antibody response against the therapeutic agent may lead to toxicity and/or loss of therapeutic benefit.

d. Subjects with prior parenteral exposure to xenogeneic proteins or with a history of xenogeneic protein allergies should be excluded from phase 1 studies of mAb products that have been derived from the same or a closely related species.

e. Informed consent issues in feasibility clinical trials in serious or immediately life-threatening conditions:
If applicable, informed consent forms for these trials should clearly state the following in language understandable to the patients:

> *i.* only a limited characterization of the processes used to prepare the product for their ability to remove endogenous or exogenous infectious/toxic agent(s) was performed, and
>
> *ii.* there may be potential health risks, including hitherto unknown risks, derived from exposure to such agents if they are present in the product.

2. Dose-setting

a. Whenever possible, the selection of the phase 1 starting dose should be based on safety and toxicity information derived from testing in a relevant animal model. When extrapolating from animal doses to human doses, information about the relative affinity of the mAb for the human antigen as compared to its animal analogue may be of great value. The target *in vivo* dose or concentration range should be based both on *in vitro* studies of cells for which antibody-antigen affinity and functional activity (e.g., immune modulation, cytotoxicity) have been measured, as well as on study of a relevant-antigen animal model, if available, to assess *in vivo* activity. If animal studies are judged to be impossible or of no relevance and initial *in vivo* studies are to be performed in humans, testing should begin at a low dose that is based on extrapolation from tissue culture studies or from available information gathered in clinical trials of a similar mAb.

b. Initial studies of radioimmunotherapeutic mAb should also employ escalating single-doses of the mAb, with the lowest and highest doses based on animal dosimetry and on the projected tolerance of normal organs to radiation. Both the elimination half-life of the mAb and the elimination half-life of the radioactivity should be characterized.

c. If a multiple-dose regimen of a mAb is anticipated, multiple-dose schedules should be explored in late phase 1 or phase 2 trials, after basic data on toxicity, peak levels, clearance, distribution, and biologic effects are available from single-dose studies. The time required for recovery from the biologic effects of single doses (e.g., immune recovery after $CD4^+$ cell depletion or modulation, return of bone marrow function after radioimmunotherapy) should also be well understood prior to initiation of multiple-dose regimens. The rationale for dosing schedules should be provided. The rationale should be based on dose tolerance, available pharmacokinetic and pharmacodynamic data in humans, and on relevant animal models of safety and efficacy. Modified dosing regimens to compensate for a high antibody response against the agent or circulating antigen may need to be studied. Pharmacokinetic studies to determine the relationships of human anti-mAb antibody titers and circulating antigen levels with organ distribution, clearance, and toxicity may be necessary.

d. Before repeat administration of a radioimmunotherapeutic or immunotoxin, the investigator should characterize all organ toxicities and pathology resulting from single dose administration. The timing of recovery from all toxic effects should be determined. Intra-patient dose escalation may confound interpretation of safety data because it may be difficult to determine whether toxic effects (e.g., to bone marrow) are due to prolonged therapy or to increased dosing levels. Intra-patient dose escalation may be appropriate if no toxicity is seen at the initial dose levels or if it is possible to use initial safe "test" doses and if cumulative toxicity is deemed unlikely. If intra-patient dose escalation is performed, consideration should be given to threshold and carryover effects, as well as to the reversibility of clinical and laboratory adverse events.

e. Design of pharmacokinetic studies should include consideration of the species in which the immunoglobulin is produced, the immunoglobulin class and subclass, and the structure of the antibody (e.g., whole mAb, Fab fragment) or immunoconjugate. A relevant study population will have the appropriate antigen and antigen mass. If antigen mass is likely to alter the bioavailability of the mAb, this should be determined in pharmacokinetic studies so that its impact on dose setting and on stratitifcation and analysis of later trials can be considered. Aside from obtaining estimates of common pharmacokinetic parameters, pharmacokinetic studies may also be very useful in situations in which the comparability of different products or formulations is to be determined (see Sections II.E. and III.A.). Pharmacokinetic studies optimally include the following:

i. Determination of plasma concentration profiles, distribution, and clearance of the mAb.

ii. Determination of doses for further study based on dose-concentration effect relationship and correlation with desired concentrations estimated from *in vitro* studies.

iii. Determination of peak and trough mAb levels and elimination rate constants.

iv. Determination of the organs and sites where the mAb is distributed, metabolized, and eliminated.

v. Determination of the fate of immunoconjugates by assaying the whole molecule and its components.

vi. Investigation of the relationships between the elimination rate and the method of administration, antigen load, and presence of a circulating antigen or of an antibody response against the therapeutic agent.

B. IMMUNOGENICITY: CLINICAL CONSIDERATIONS

Monitoring of antiglobulin titers and immune activity is of great importance in evaluating the safety and efficacy of mAb and in designing protocols involving repeat administration. Immune responses to mAb may have little or no effect, or may interfere significantly with the safety and/or efficacy of a mAb.

1. Monitoring the development of antibodies to mAb

Depending on the source of the mAb, assays for anti-immunoglobulins will need to be developed to detect human anti-mouse antibodies (HAMA), human anti-rat antibodies (HARA), human anti-human antibodies (HAHA), human anti-chimeric antibodies (HACA), and anti-idiotype antibodies. As appropriate for the mAb, assays should be developed to detect human immunoglobulins directed against humanized or primatized antibodies, immunonuclides and immunotoxins, their individual components (e.g., ricin), and neoantigens formed by the linked antibody/toxin/nuclide.

a. The timing of sample collection for evaluating the presence of an anti-mAb antibody should take into account whether the mAb is intended to be given as single or as multiple doses. Titers of the anti-mAb antibody should always be established at baseline to account for pre-existing antibodies (including anti-globulin or anti-conjugate antibodies, when appropriate), and also before readministration of the mAb. Post-administration samples may be drawn early (e.g., two weeks after administration), but should also be drawn at later times (e.g., at six-eight weeks).

b. The assay(s) used to detect the anti-mAb antibody should be standardized to the greatest extent possible. Aliquots of a "reference" preparation of antibody, e.g. anti-mouse antibody for a HAMA assay, with defined specificity from a human or primate source should be aliquoted and frozen to facilitate future intra- and inter-study comparisons. The reference preparation can also be used to establish a standard curve for routine testing. The assay(s) should be validated by establishing sensitivity, specificity, precision, and accuracy. Inhibition or competition studies with both negative and positive controls should be used to demonstrate that the assay detects antibodies to the mAb product. Studies should assess the range of reactivity of normal individuals and should evaluate potential interference by serum components such as bilirubin and lipids.

c. The specificity of the immune responses to the mAb should be identified and characterized in a sample of patients. These studies should establish whether the responses are generated against a heavy-chain isotype determinant, a light-chain, constant (C) region, variable (V) region, idiotypic epitope(s), immunoconjugate, or neoantigen. These data will demonstrate whether it is possible to use an anti-mAb antibody test with broad specificity (e.g., detecting human antibodies reactive with antigens of both heavy and light chain constant regions of all foreign immunoglobulin classes), or whether a more restricted anti-mAb antibody test that is idiotype-specific is necessary. In certain instances the anti-mAb antibody assay should include the actual mAb product as the detection antigen.

d. The choice of the appropriate assay for anti-mAb antibody depends on the proposed use and labeling of the product. Development and validation of the assay should accompany the clinical development of the new mAb. The results of the anti-mAb antibody testing should be correlated with product efficacy and adverse events.

e. A license application submitted for a mAb to be administered in a repeated dosing regimen should include a clinically available, validated test that reliably measures human antibody responses to the mAb, if an anti-mAb response may affect the safety, efficacy or dosing of the product. If a commercially available HAMA (or other anti-mAb antibody) test kit, is available, it may be used provided it has been demonstrated to reliably detect antibody response against the new mAb product. In most cases, humanized mAb require an assay specific for the product itself. If no appropriate anti-mAb antibody test is available, a properly validated test system should be developed by the sponsor.

2. Clinical consequences of immunogenicity

a. When a patient is found to have developed an antibody response against the therapeutic or diagnostic mAb, adverse events should be anticipated and appropriate precautions taken. MAb are generally given in facilities where acute resuscitative care is immediately available. The use of non-hospital settings for mAb administration (e.g., clinic or home) should be justified by clinical safety data. Vital signs should be observed closely for at least one hour after completion of the mAb administration. The possibility of delayed adverse effects from immune responses to mAb should be considered and reflected in the trial design, including appropriate clinical and laboratory testing.

b. Anaphylaxis, anaphylactoid and other immune reactions

i. True IgE-mediated anaphylaxis to whole mouse immunoglobulins is infrequent. It is theoretically possible that anti-human allotype responses of an allergic nature could occur but they have not been reported to be of clinical importance to date. If the mAb is conjugated to chelating agents or toxins, the likelihood of allergic reactions may be greater. In all cases, repeated administration of a mAb increases the likelihood of a hypersensitivity response. Immediate hypersensitivity reactions may range from mild to severe. Skin testing is not advised because it is a poor predictor of sensitivity to mouse immunoglobulins and can cause sensitization.

ii. Infusional reactions such as chills, rigors, aches, and low grade fever, are common during or immediately following mAb administration (the incidence is approximately 5% with several antibodies). The mechanism of these reactions is not clear. The frequency and intensity of such reactions can often be controlled by using slower infusion rates or by pre-medication.

iii. Serum sickness is unusual following mAb administration but has been described. Unlike anaphylaxis and infusional reactions, which occur during or immediately after antibody treatment,

serum sickness is delayed by several hours. The correlation between circulating levels of soluble antigen and immunocomplex-mediated adverse events such as serum sickness should be explored if such adverse events are observed.

c. Anti-mAb antibodies can interfere directly with some antibody-based clinical tests for antigens, such as CA-125 and CEA, by binding to the murine detection antibody. Indirect interference with diagnostic assays is theoretically possible if mAb administration induces anti-idiotypic responses that mimic the antigen. When appropriate, evidence for either type of interference should be systematically sought using well designed *in vitro* studies. Ideally, attempts should be made to circumvent such interference and alternative clinical assays should be validated.

d. When subjects are selected for testing a mAb, the risk that future therapy with a monoclonal antibody may be compromised by elicitation of an antibody response against the therapeutic agent or other mAb should be considered and reflected in the informed consent form.

C. PRODUCT-RELATED CONSIDERATIONS FOR PHASE 3 STUDIES

When planning manufacturing changes or scale-up programs during phase 3 clinical trials, sponsors should consider that product comparability may have to be demonstrated (see ref. 23 and Section II.E.). This may or may not require additional clinical studies depending upon the adequacy of preclinical data (see ref. 23 and Section II.E.). Thus, it is suggested that scale-up programs and contemplated changes in product manufacture be anticipated prior to the initiation of phase 3 trials. Sponsors should study a number of separate product lots during drug development to demonstrate that a safe and effective product can be prepared reliably.

D. ADMINISTRATION OF RADIOLABELED ANTIBODIES

1. Dosimetry

Grade 3 and grade 4 organ toxicities have been reported with therapeutic radioisotopes. Therefore, dosimetry estimates for human subjects are required prior to the initiation of phase 1 studies. The dosimetry estimates should be developed with simulation models utilizing an appropriate diagnostic radioisotope label on the antibody. If no diagnostic radiolabel for the antibody is available for simulation, animal studies with the therapeutic radiolabel may be utilized for dosimetry estimates. The actual dosimetry data for the therapeutic radiolabeled antibody, itself, should be acquired concurrent with the initial phase 1 study and reported prior to the initiation of a phase 2 study.

For diagnostic radiolabeled antibodies, as for the therapeutic isotopes, the investigator should provide estimates of the organ dosimetry prior to the first phase 1 study. Final dosimetry calculations from human studies should be completed prior to the submission of the license application.

a. General considerations: Sufficient data from animal or human studies should be submitted to the IND, to allow a reasonable calculation of radiation-absorbed dose to the whole body and to critical organs upon administration to a human subject [21 CFR 312.23(a)(10)(ii)]. See Appendix III for a list of organs to be included in dosimetry estimates.

The amount of radiation delivered by internal administration of radiolabeled antibodies should be calculated by internal radiation dosimetry. The absorbed fraction method of radiation dosimetry is described in two systems [21 CFR 361.1 (b)(3)(iv)]:

i. The Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine

ii. The International Commission on Radiological Protection (ICRP).

The investigator should specify which methodology is used. The mathematical equations used to derive the radiation doses and the absorbed dose estimates should be provided. Sample calculations and all pertinent assumptions should be listed and submitted.

Safety hazards for patients and health care workers during and after administration of the radiolabeled antibody should be identified, evaluated, and managed appropriately.

b. Calculation of radiation dose to the target organ

Investigators should determine the following, based on the average patient:

i. The amount of radioactivity that accumulates in the target tissue/organ.

ii. The amount of radioactivity that accumulates in tissues adjacent to the target tissue/organ.

iii. The residence time of the radioactive mAb in the target tissue/organ and in adjacent regions.

iv. The radiation dose from the radioisotope, including the free radioisotope and any daughter products generated by decay of the radioisotope.

v. The total radiation from bound, free, and daughter radioisotopes associated with the radioimmunoconjugate, based upon immediate administration following preparation and upon delayed administration at the end of the allowed shelf life.

c. Maximum absorbed radiation dose

The amount of radioactive material administered to human subjects should be the smallest radiation dose that is practical to perform the procedure without jeopardizing the benefits obtained.

i. The amount of radiation delivered by the internal administration of radiolabeled antibodies should be calculated by internal radiation dosimetry using both the MIRD and ICRP systems. The higher estimate of the absorbed dose determined from either of these systems should be used in the radiation dosimetry safety assessment.

ii. Because of known or expected toxicities associated with radiation exposure, dosimetry estimates should be obtained as delineated in IV.D.1.a and b.

iii. Calculations should be provided that anticipate changes in dosimetry that might occur in the presence of diseases in organs that are critical in radioimmunoconjugate metabolism or excretion (e.g., renal dysfunction causing a larger fraction of the administered dose to be cleared via the hepatobiliary system or vice versa).

iv. Possible changes in dosimetry resulting from patient to patient variations in antigen mass should also be considered in dosimetry calculations (e.g., a large tumor mass may result in a larger than

expected radiation dose to a target organ from a radiolabeled anti-tumor mAb).

v. The mathematical equations used to derive the estimates of the radiation dose and the absorbed dose should be provided. Sample calculations and all pertinent assumptions should be listed.

vi. Calculations of dose estimates should be done assuming freshly labeled material to account for maximum amount of label as well as at the maximum shelf life of the radiolabeled antibody to allow for the upper limit of radioactive decay contaminants and should: (a) Include the highest amount of radioactivity to be administered; (b) Include the radiation exposure contributed by other diagnostic procedures such as roentgenograms or nuclear medicine scans that are part of the study; (c) Be expressed as Gray (Gy) per megaBequerel (MBq) or per millicurie of radionuclide; and (d) Be presented in a tabular format and include doses of individual absorbed radiation for the target tissues/organs and the organs listed in Appendix III.

2. Early clinical development of therapeutic radiolabeled mAb

a. Evaluations that should be conducted prior to Phase 1 studies.

Prior to phase 1 studies, investigators of therapeutic applications of radiolabeled antibodies should conduct the following evaluations for the average adult to be entered into the study:

i. The therapeutic radiolabeled antibody should be evaluated for *in vitro* stability and composition of the radioactive material to be administered. The expected and acceptable levels of the percent of free radioisotope, the percent of radioisotope bound to immunoreactive antibody, and the percent of bound radioisotope to non-immunoreactive antibody should be established. Calculations of the estimates should be at the maximum planned shelf life of the radiolabeled antibody to allow for the upper limit of radioactive decay contaminants and should be based upon the maximum dose of radioactive material to be administered to patients.

ii. The expected biodistribution and routes of clearance of the administered radiolabeled antibody dose fractions in tissues/organs should be defined.

iii. The expected biodistribution and routes of clearance that might occur in the presence of diseases in organs that are critical in radioimmunoconjugate metabolism or excretion should be described.

iv. The expected biodistribution and routes of clearance that might occur in the presence of immune responses (e.g., HAMA, HAHA, HARA) should be described.

With reference to the radioactive fractions of the administered radiolabeled antibody dose and the patterns of biodistribution, the following issues should be addressed:

v. From the biodistribution estimation, the expected residence time of the radiolabeled antibody fractions in the target tissues/organs and non-target tissues/organs should be determined.

vi. Based on the estimated residence times in each organ, the radiation exposure for each tissue/organ should be estimated.

vii. Based on the radiation exposure for each tissue/organ, the potential toxicity should be

described.

viii. Based on the potential radiation toxicity to tissues/organs, toxicity monitoring protocols should be developed and incorporated into the clinical trial.

ix. If the study has increasing doses of radioactive materials (e.g., a study of the maximum tolerated dose), the radiation exposure for tissues/organs and the associated potential toxicities should be estimated for each radiation dose level.

b. Selection of patients for phase 1 trials of therapeutic radiolabeled mAb.

Patients should be entered into phase 1 trials with therapeutic radiolabeled antibodies only after consideration of the following:

i. To reduce the potential for alterations in biodistribution, patients enrolled in early studies should not have prior or concurrent exposure to investigational or approved antibodies.

ii. Patients should be evaluated for immune response to the appropriate species of monoclonal antibody (e.g., HAMA, HARA). Patients demonstrating evidence of immune response should generally be excluded from phase 1 studies.

iii. For proper evaluation of potential adverse events in early studies, patients should be in adequate health to allow follow-up for three months without adjunctive chemotherapy or radiation therapy. Generally, patients should have a Karnofsky score greater than 70.

iv. Consultation with CBER is strongly recommended when considering the inclusion of pediatric patients in early radiolabeled antibody trials.

c. Study design issues for phase 1 studies of therapeutic radiolabeled mAb

Phase 1 studies should be designed to address the following points:

i. The prepared therapeutic radiolabeled antibody should be evaluated for *in vivo* stability. The previously estimated expected and acceptable levels of the percent of free isotope, the percent of bound radioisotope to immunoreactive antibody, and the percent of bound radioisotope to non-immunoreactive antibody should be confirmed and demonstrated to be reproducible. Calculations should be with the routine shelf life of the prepared radiolabeled antibody and based upon the maximum dose of radioactive material administered to patients.

ii. The pharmacokinetics and biodistribution in the patient population should be studied.

iii. The residence times with radiation dosimetry for tumor (when applicable), tissues, and organs should be determined.

iv. The pattern of toxicity, its relationship to administered dose and the organs of concern for acute and delayed radiation injury should be established.

v. Any apparent evidence of response of tumor to the administration of the radiolabeled antibody should be documented.

vi. The trial design should incorporate patient imaging with a diagnostic radiolabel on the antibody to confirm the expected biodistribution and residence times prior to the administration of the therapeutically radiolabeled antibody.

3. Adverse events for patients enrolled in trials of therapeutic radiolabeled mAb

The mechanism for follow-up of patients and reporting of adverse events should be described in the protocol prior to initiation of the trial. Complete evaluation and reporting of the adverse events potentially associated with the therapeutically radiolabeled antibody should be assured. If patients are referred to their attending physicians during the follow up phase, the investigator should plan and control the follow up of the treated patients for complete and timely reporting of adverse events potentially associated with the administration of the therapeutic radiolabeled antibody.

Patients removed from a trial should be continued in follow up for three months. All adverse events during that time interval should be reported, even if they are not thought to be related to the administered radiolabeled antibody.

4. Clinical development of radiolabeled mAb used as imaging agents

a. Prior to the initiation of phase 3 studies

Investigators of diagnostic applications of radiolabeled antibodies should collect stability, safety and pharmacokinetic information for the average adult expected to be entered into phase 3 studies.

i. The expected percent of free radioisotope, the expected percent of radioisotope bound to immunoreactive antibody, and the percent of the radioisotope bound to non-immunoreactive antibody should be determined. If unlabeled antibody is to be administered in conjunction with radiolabeled antibody, the ratio and amounts of the labeled and unlabeled antibody should be defined.

ii. The biodistribution of the administered radiolabeled antibody dose fractions in tissues/organs should be delineated, and from the biodistribution, the expected residence time of the radiolabeled antibody fractions in the target tissues/organs and non-target tissues/organs should have been estimated. The routes of clearance of the radiolabeled antibody should be determined.

iii. The changes in pharmacokinetics of the radiolabeled antibody with organ impairment, antigen load in the circulation, and tumor burden should be evaluated. The potential for clearance artifact to degrade patient imaging should be explored.

iv. Estimates of appropriate imaging times and techniques should be developed. Adjunctive imaging aids (e.g., enemas, emptying of the urinary bladder) should be evaluated.

v. Evidence of image quality should be gathered. The ability of the radiolabeled antibody to image known and/or occult disease should be documented. These data should be compared to imaging data obtained using standard diagnostic techniques whenever possible.The incidence of false positive localization of the radiolabeled antibody and the incidence of misinterpretation of the images to produce false positive and false negative interpretations should be explored. Disease specific factors (e.g., stage of disease, tumor burden, and co-morbid illness) should be evaluated for impact on technical procedures in the imaging protocol (e.g., time of imaging).

vi. Phase 2 trials should be designed to define the appropriate patient populations for phase 3 trial(s), to define the technical procedures used for imaging in the anticipated patient populations and to identify potential clinical utility of the test to be further explored in later studies.

vii. Multiple clinical sites should be employed in phase 2 studies to assess the reproducibility of the imaging techniques, and of the preparation and administration of the radiolabeled antibody.

b. Pivotal efficacy studies of radiolabeled mAb used as imaging agents

CBER staff should be consulted for review of and comments on Phase 3 protocol(s), prior to the initiation of the phase 3 study(ies). The following elements should be incorporated into each clinical protocol:

i. A prospectively defined and detailed primary efficacy endpoint and analytical plan.

ii. A study population consisting of those patients for whom the imaging agent is intended after licensure. The performance and utility of an imaging test may vary substantially based on the stage, extent, or severity of the disease, determined in part by the results of other diagnostic tests. Therefore, the study population and subpopulations to be analyzed should be carefully defined in terms of stage of disease as well as in terms of diagnostic tests performed and test results prior to study imaging. The protocols should be designed to assess the imaging performance and the utility in the populations.

iii. A plan for acquisition and storage of imaging data for radiolabeled antibody in the confirmatory studies.

iv. A prospective plan for evaluation of imaging performance:

(a). On-site image interpretation and reporting should be defined and documented. To the extent possible, the information available to the on-site reader should be defined by protocol and recorded on the case report forms.

(b). The off-site image interpretation should be the basis of the principal analysis of imaging performance in the phase 3 clinical trial. The off-site image interpretation and reporting of all radiolabeled antibody image findings and all confirmatory imaging should be defined and documented prospectively. The information available to the off-site reader should be defined by protocol and recorded on the case report form. In general, the off-site reader should have little information beyond the entry criteria of the study and specifically should not be aware of the on-site reading, the results of other diagnostic tests, or patient outcome data.

(c). Planned sample size should be sufficient to determine imaging performance measures to a predetermined precision (i.e., 95% confidence interval width). Imaging performance may vary with the stage, extent, and/or site of disease as defined by pre-imaging evaluations, and this should be accounted for prospectively in planning analysis of imaging performance. To determine performance, imaging results should be compared with another indicator of disease, usually results of standard imaging, biopsy, exploration, patient follow-up or some combination of these.

(d). If the planned use of the test is in conjunction with other diagnostic tests, its imaging performance should be determined and reported in groups of patients defined by the results of the other tests. For example, in some cases it will be important to know the imaging performance in patients with positive CT scans and the imaging performance in patients with negative CT scans.

v. A prospective plan for evaluation of clinical utility:

(a).When an agent has a significant incidence of false negative and false positives or significant toxicities, it is particularly important that the clinical utility be assessed to determine whether the value of the diagnostic information outweighs the potential adverse consequences of incorrect information or the toxicities. In this context, clinical utility means the extent to which information obtained by use of the mAb agent in a defined clinical setting can be expected to contribute to outcome, to contribute to the convenience or appropriateness of patient management, or to provide accurate prognostic information.

(b). Based on phase 2 and other data, the protocol should indicate the specific manner in which clinical utility is to be explored. The following issues should be addressed:

(1) The stage and severity of the disease in which the test is to be indicated should be specified.

(2) The protocol should specify whether the test is to be used in conjunction with or in lieu of other diagnostic tests. Because radiolabeled mAb image by a mechanism distinct from that of radiopharmaceuticals or diagnostic devices, the information obtained from a monoclonal antibody image may be complementary to that obtained by those means. For example, a mAb agent may not be as sensitive overall as an accepted standard test, but may be able to detect disease accurately under conditions where the standard technique fails.

(3) How the various results of the test are hypothesized to be clinically useful (for management or prognosis) should be clearly delineated. For example: positive results together with a positive CT scan are sufficiently diagnostic to avoid further diagnostic evaluation including biopsy; positive results are useful to guide biopsy; positive uptake is predictive of response to a specific therapeutic modality.

V. APPENDIX I: NORMAL HUMAN TISSUES USED IN CROSS-REACTIVITY TESTING

1. Adrenal
2. Bladder
3. Blood cells
4. Bone Marrow
5. Breast
6. Cerebellum
7. Cerebral cortex
8. Colon
9. Endothelium
10. Eye
11. Fallopian tube
12. Gastrointestinal tract
13. Heart
14. Kidney (glomerulus, tubule)
15. Liver
16. Lung
17. Lymph node
18. Ovary
19. Pancreas
20. Parathyroid
21. Pituitary
22. Placenta
23. Prostate
24. Skin
25. Spinal cord
26. Spleen
27. Striated muscle
28. Testis
29. Thymus
30. Thyroid
31. Ureter
32. Uterus (cervix, endometrium)

VI. APPENDIX II: MOUSE ANTIBODY PRODUCTION TEST

The following tests for murine viruses (mouse antibody production test (27) should be performed on any MCB and EPC derived from murine cell lines and on all lots of mAb derived from mouse ascites fluid:

1. Ectromelia
2. EDIM
3. GD VII virus
4. Hantaan virus
5. LCM virus, including challenge for non-lethal strains
6. LDH-elevating virus
7. Minute virus of mice
8. Mouse adenovirus
9. Mouse encephalomyelitis
10. Mouse hepatitis
11. Mouse salivary gland (murine CMV)
12. Pneumonia virus of mice
13. Polyoma
14. Reovirus type 3
15. Sendai
16. Thymic virus

VII. APPENDIX III: ORGANS TO BE CONSIDERED IN DOSIMETRY ESTIMATES

1. all target organs/tissues
2. bone
3. bone marrow
4. liver
5. spleen
6. adrenal
7. kidney
8. lung
9. heart
10. urinary bladder
11. gall bladder
12. thyroid
13. brain
14. gonads
15. gastrointestinal tract
16. adjacent organs of interest

VIII REFERENCES

1. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, CBER, FDA, 1993.

2. Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology, FDA, CBER, April 10, 1985 and Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability, CBER, FDA, April 6, 1992.

3. Elimination of Establishment License Application for Specified Biotechnology Products and Specified Synthetic Biologics. Final rule. (May 14, 1996; 61 Federal Register 24227).

4. Guidance for Industry for the Submission of Chemistry, Manufacturing and Controls Information for a Therapeutic Recombinant DNA-derived Product or a Monoclonal Antibody Product for In Vivo Use, CBER/CDER, FDA, August, 1996.

5. Points to Consider in the Production and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals, CBER, FDA, 1995.

6. International Conference on Harmonisation; Final Guideline on Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products, (February 23, 1996; 61 Federal Register 7006).

7. International Conference on Harmonisation; Final Guideline on Stability Testing of Biotechnological/Biological Products, (July 10, 1996; 61 Federal Register 36466).

8. Marcus-Sekura, CJ. Validation of removal of human retroviruses. In Developments in Biological Standardization, volume 75, "International Symposium on Virological Aspects of the Safety of Biological Products", edited by the International Association of Biological Standardization, pp. 133-143, Karger, New York, New York, USA, 1991.

9. Rubinstein, LJ and Stein, KE. Murine immune response to the Neisseria meningitidis Group C capsular polysaccharide. J Immunology 1988; 141:4352-56.

10. Lindmo, T, Boven, E, Cuttitta, F, et al. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. J Immunol Meth 1984; 72:77-89.

11. Nisonoff, A, Lamoyi, E. Implications of the presence of an internal image of the antigen in anti-idiotype antibodies: possible application to vaccine production. Clin Immunol Immunopathol 1981;21:397-406.

12. Stein, KE and Epstein, S. [letter] Mol Immunol 1991;28:193.

13. Burtonboy, G, Delferriere, N, Bodeus, M, and Mousset, B. Absence of Viral Particles in Rat Hybridomas. An Ultrastructural Study In Rat Hybridomas and Rat Monoclonal Antibodies (Ed. H. Bazin), pp. 465-473, CRC Press, Boca Raton, Florida, USA.

14. Dinowitz, M, Lie, YS, Low, MA, et al. Recent studies on retrovirus-like particles in Chinese hamster

ovary cells. In Developments in Biological Standardization, volume 76, "Continuous Cell lines, An International Workshop on Current Issues", edited by the International Association of Biological Standardization, pp. 201-207, Karger, New York, New York, USA, 1992.

15. Miller, DG and Miller, AD. Tunicamycin treatment of CHO cells abrogates multiple blocks to retrovirus infection, one of which is due to a secreted inhibitor. J Virol 1992;66:78-84.

16. Eglitis, MA, Kadan, MJ, Wonilowicz, E, Gould, L. Introduction of human genomic sequences renders CHO-K1 cells susceptible to infection by amphotropic retroviruses. J Virol 1993;67:1100-1104.

17. Lander, M R and Chattopadhyay, S K, A Mus dunni cell line that lacks sequences closely related to endogenous murine leukemia viruses and can be infected by ecotropic, amphotropic, xenotropic and mink cell focus-forming viruses. J Virol. 1984;52:695-698.

18. Report of a WHO Study group (1987). Acceptability of cell substrates for production of biologicals. World Health Organization Technical Report Series 747.

19. Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices, CDER/CBER/CDRH/CVR, FDA, December, 1987.

20. Guidelines on Sterile Drug Products Produced by Aseptic Processing, Center for Drugs and Biologics, FDA, June, 1987.

21. Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products, CDER/CVM, FDA, November, 1994.

22. Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics, Center for Drugs and Biologics, FDA, February, 1987.

23. FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products, CBER, FDA, April, 1996.

24. Goldsmith, MA, Slavik, M, Carter, SK. Quantitative prediction of drug toxicity in humans from toxicology in small and large animals. Cancer Res 1975;35:1354-64.

25. Collins, JM, Zaharko, DS, Dedrick, RL et al. Potential roles for preclinical pharmacology in phase I trials. Cancer Treat Rep 1986; 70:73-80.

26. EORTC Pharmacokinetics and metabolism group: pharmacokinetically-guided dose escalation in phase I clinical trials. Eur J Clin Oncol 1987; 23:1083-87.

27. Nicklas, W, Kraft, V, and B Meyer. Contamination of transplantable tumors, cell lines, and monoclonal antibodies with rodent viruses. Lab Anim Sci 1993;43: 296-300.