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)