Monoclonal antibodies (mAbs) are a well established class of therapeutics as evidenced by a large number of FDA approved mAbs for the treatment of cancers and autoimmune diseases. Monoclonal antibodies that are molecularly engineered for enhanced functions and pharmacokinetic properties are routinely being considered for development by many biotechnology companies. Safety evaluation of current generation of mAbs poses new challenges due to the highly complex nature of engineering aspects and variability induced by the diverse recombinant cell systems to generate them. This review provides a basic outline for nonclinical safety evaluation of therapeutic antibodies. Important considerations for planning a preclinical program, the types of nonclinical safety studies, and a general timeline for their conduct in relation to clinical trials are described. A list of relevant regulatory documents issued by government agencies is also provided. Adoption of these principles will greatly enhance the quality and relevance of the nonclinical safety data generated and will facilitate future development of mAb therapeutics.

Introduction

Today, monoclonal antibodies (mAb) represent a class of biotherapeutics for a wide variety of disease indications, with over 20 mAbs approved for therapeutic use in the US and a large number of mAbs in clinical trials worldwide.1-3 The first generation of mAb therapeutics, which were produced from mouse hybridomas, achieved little clinical success due, in part, to an inability to effectively interact with human effector cells and to their rapid clearance from the system due to immunogenicity.4 Most therapeutic mAbs in development today are chimeric or humanized to incorporate more human characteristics aimed at reducing immunogenicity and enhancing interaction with human effector cells.4-7 More recently, fully human antibodies are also being developed that are generated using transgenic animals or display technologies.8-10 In addition to favorably altering binding affinities, customized mAbs that have enhanced effector function e.g., antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are even possible. To improve efficacy and increase the chance for clinical success, mAbs that have been modified to alter glycosylation, target binding affinity and half-life are now increasingly considered for clinical development.2,3,10 Concurrently, the technology for the generation of high-titer mAb-producing cell lines from mammalian and non-mammalian origins has also evolved.11,12 Thus, a thorough nonclinical safety evaluation of these mAbs is very important due to the increasing complexity of antibody engineering aspects and the variability induced by the diversity of recombinant production cell systems for generation of antibodies. Furthermore, their complex structure, unique biologic functions and the longer half-lives of mAbs compared with traditional small molecule drugs add to the safety considerations in addition to concerns due to prolonged clinical use of mAbs for the treatment of chronic diseases.3

In this review, we discuss the general considerations for planning a nonclinical program and describe the types of nonclinical safety studies that should be performed. The timing for the conduct of these studies in relation to clinical trials and relevant regulatory documents as well as selected references to other useful texts and publications are also provided. The application of guiding principles described in this review will ultimately improve the quality and relevance of the nonclinical safety data generated and will help to
prepare a scientifically-sound nonclinical package for safety evaluation of therapeutic mAbs.

**General Considerations for Nonclinical Development**

The overall goal of the nonclinical studies for mAbs is to define the toxicological properties of the mAb in question and provide information for product development (Table 1). The main objectives of the nonclinical evaluation are (1) identification of target organs for toxicity and to determine whether the toxicity is reversible following the treatment, (2) identification of a safe starting dose for human Phase I clinical trials and subsequent dose escalation schemes, (3) provide information to monitor safety parameters in the clinical trials and (4) provide safety data to support claims on the product label.\(^\text{13}\)

In order to achieve these goals, both in vitro and in vivo nonclinical studies aimed at defining and understanding the pharmacological properties of the antibody are conducted (Table 2). The most effective approach for designing a nonclinical development strategy is to start with the end in mind and work backwards. Writing the product label in conjunction with clinical, regulatory and manufacturing functions at the start of the project is critical for designing a sound toxicology package. This should include key components such as, indication, patient population, dosing regimen, duration of treatment, route of administration, formulation, etc. After preparation of a draft product label and development strategy, the next step is to develop a list of all potential nonclinical pharmacology and toxicology studies that will be conducted during development of the product from those that enable the first-in-man studies to post-marketing. The studies can be categorized into those conducted: (1) prior to initiation of Phase I clinical trials, (2) prior to or concurrently with Phase II trials and (3) concurrently with Phase III pivotal trials. This organization will allow determination of the optimum timing of nonclinical studies (Fig. 1), especially those that are required prior to submission of a US Biologics License Application (BLA) or European Marketing Authorization Application (MAA). These types of studies include chronic toxicity studies and reproductive and developmental toxicology studies. The next step is to identify toxicology studies that are required for the initiation of Phase III and Phase II trials, and studies for enabling IND submission.

The studies and their timing are influenced not only by the clinical trials but also by the chemistry, manufacturing and controls (CMC) development strategy. Unfortunately, failing to coordinate the nonclinical safety assessment with clinical and CMC aspects of development may lead to repetition of toxicity studies. For example, suppose the duration of treatment in the Phase I trial is shorter than the intended labeled clinical use of the product, and the Phase II and later trials have a longer duration of treatment. If the IND-enabling studies are designed with only the shorter Phase I trial in mind, a second, longer toxicity study will be required to support Phase II and beyond. Thus, the obvious advantage of planning backward is the ability to anticipate and plan for longer toxicity studies at the outset of the project. A longer duration toxicity study will provide toxicology coverage for both Phases I and II, and avoid unnecessary duplication of effort and resources.

A process change in the manufacture of the antibody often necessitates additional safety evaluations. Such changes include upstream modifications to the culture conditions, changes in the production cell line itself and downstream purification or formulation changes. Analytical methods will initially be employed in order to demonstrate comparability of the new product to the old; however, significant changes may preclude comparability using in vitro analytical methods alone and then a bridging toxicity study or an entirely new toxicity study will be necessary. The most cost effective approach for comparing a new product with the old is to conduct a bridging toxicity study design with at least one dose of the old product. A sufficient supply of the early phase material is essential for the success of the bridging study. Since continued process development is the norm after initiation of the Phase I trial, planning for a bridging study in the initial design of the program is appropriate.

All nonclinical safety studies intended to support human clinical trials must be conducted in compliance with good laboratory practice (GLP). This includes performance of assays used to support the toxicity study discussed in the next section and bioanalytical testing of serum for pharmacokinetic purposes or anti-therapeutic antibodies for immunogenicity assessment.

### Table 1  Aims of the nonclinical safety evaluations of mAbs

| 1. | Identify target organs for toxicity and determine if toxicity is reversible |
| 2. | Determine starting dose for Phase I and determine dose escalation scheme for Phase I |
| 3. | Identify parameters for safety monitoring in humans |
| 4. | Provide data for safety information on the label |

### Table 2  Important factors for designing nonclinical safety studies

- Knowledge of antigen target biology
- Pharmacological properties of the antibody
  - Receptor binding affinity
  - Receptor occupancy
  - Biological properties of the antibody
    - ADCC
    - CDC
    - ADCP
    - Signaling potential
- Exposure-response relationship
- Initial estimate of PK parameters
  - Useful for the determination of recovery period
- Clearly defined clinical trial design

Assay Development

A number of product-specific assays to determine the stability and concentration of the antibody are needed to support safety studies. As assay development can take several months, particularly, if a contract research organization (CRO) is used to validate the assays prior to implementation of testing toxicity samples, assay development should begin well before the toxicity study planning. Most common assays include dose solution analysis, PK assays and
immunogenicity assays (Table 3). It is beyond the scope of this text to discuss assay development; however, recognizing the assay limitations is important during data examination and interpretation.

**Dose solution analysis.** A verification of the concentration of the antibody solution used for dose administration is required. As the antibody will be diluted considerably for administration, additional assay development to ensure detection of the antibody at low concentrations is required. In addition, stability of the dose solution under the conditions of use must be demonstrated since the diluted antibody dosing solution may be stored in the vivarium at room temperature for up to eight hours on the day of administration. Data is similarly required for the stability of the formulated drug product for the duration of the dosing period. These assays are needed in a number of settings and biologic matrices. Since the toxicology studies are often conducted well in advance of clinical product manufacture and initiation of formal product stability studies, the group responsible for assay development should be advised of the requirement for an antibody product stability assay in support of the toxicity study.

**PK assays.** Assays are also required to assess the antibody concentration in the serum of treated animals. Both PK and toxicokinetics (TK) of the antibody and its concentration in the serum of treated animals must be determined. For this purpose, an enzyme-linked immunosorbent assay (ELISA) is most often employed and utilizes serum as the matrix. Those responsible for assay development should be aware that interference from an immune response against the antibody is a significant challenge to PK assay development.

**Immunogenicity assays.** Since antibodies are often immunogenic in animals, the induction of anti-drug antibodies (ADA) in animals and its impact on exposure and safety endpoints should be included as an endpoint in PK and toxicity studies, particularly if they involve repeated dose administration. Cross-interference between the therapeutic antibody and ADA confounds the measurement of immunogenicity using ELISA similarly to the PK assay. Electrochemiluminescence (ECL) is another type of assay that can be utilized to measure immunogenicity that can help to reduce some of the cross-interference. Given the complexities of these assays, assay development should commence as early in the process as possible.

### Successful Approach to Nonclinical Safety Evaluation

**Selection of relevant species.** For successful nonclinical safety evaluation of a mAb, the most relevant animal species should be chosen for toxicity testing. A relevant species is one in which the antibody is pharmacologically active, the target antigen should be present or expressed and tissue cross-reactivity profile should be similar to humans. Using immunochemical or functional assays, a relevant animal species that expresses the desired epitope and demonstrates a tissue cross-reactivity profile similar to human tissues can be identified. Species cross-reactivity studies, which are useful in this process, involve an immunohistochemical survey of tissues from a variety of species using commercially available multi-species tissue microarrays. Alternatively, evaluation of antibody binding to cells from these animals by flow-activated cell sorting (FACS) is typically more sensitive than immunohistochemical analysis of tissue sections. DNA and amino acid sequences of the target antigen should be compared across species; the homology between species should be determined.

In addition, the biodistribution, function and structure of the antigen should be comparable between the relevant animal species and humans to allow evaluation of toxicity arising from antibody binding of the target antigen, which is referred to as on-target toxicity. Furthermore, strong similarities in target antigen tissue distribution in the animal species and humans make it more likely that target organs of toxicity identified in animals will predict potential toxicities in humans. A lack of similarity in antigen tissue
distribution between the animal species and humans does not entirely preclude use of the animal species for toxicity studies, but these differences must be taken into consideration for human risk assessment. As for antigen density or affinity, absolute equivalence between the animal model and humans is similarly not required. Justification for the relevancy of the species selected for toxicity testing should be included in the regulatory submission. If only one species is used for safety evaluation, a summary of experiments that demonstrate the absence of additional relevant species is warranted.

Two acceptable options have been considered in the event of a lack of relevant animal models. The first option is the use of transgenic animals engineered to express the human target antigen.21 The success of using this type of animal model for safety evaluation relies on the extent to which the pharmacodynamics resulting from the antibody-antigen interaction are similar to those expected in humans. The second option is the development of a surrogate antibody to the human therapeutic antibody that cross-reacts with the homologous antigen in animals suitable for toxicity testing;16,22,23 however, the safety evaluation in this case will not be performed on the therapeutic antibody to be administered to humans. An inherent risk of this approach stems from the fact that no two antibodies are exactly alike. Not only does this approach add significant cost to product development, but in addition many parameters including the production process, presence of impurities, pharmacokinetics, binding affinity and mechanism of action may differ between the surrogate and therapeutic antibodies.16 Indeed, studies of surrogate antibodies have been successfully performed for the evaluation of reproductive and developmental toxicity and licensure of mAb products such as infliximab and efalizumab.16,24

When neither of these options is available, evaluation of the off-target toxicities of the antibody including functional effects on the major physiological systems may still be warranted. In addition, in vitro systems using human cells that expresses target antigen could in some cases be used to determine on-target toxicity and to determine the effective biologic dose of the therapeutic antibody. However, these types of studies present a challenge to predicting the relevance to human risk assessment, although more information on the pharmacology of the antibody intended for clinical use allows a better assessment of these alternative approaches.

Knowledge of the mAb and target antigen biology. Knowledge of the mAb and target antigen biology will permit better toxicity study design and interpretation. Often, mAbs have multiple effector mechanisms that make them clinically effective therapeutics; however, toxicity can also result from these functions. A variety of side-effects could result from binding of mAbs to antigen on tissues other than the intended target organ. For example, significant skin eruptions have been described in patients treated with cetuximab (anti-EGFR) due to binding of the mAb to antigen expressed on normal skin.25,26 Likewise, trials for anti-CD40L antibody were discontinued due to severe thrombolytic events in patients induced by binding of the mAb to the target on platelets.27 Toxicity is also seen in patients treated with trastuzumab (anti-Her2) due to binding of this antibody to low levels of target antigen expression on heart tissue.28 On the other hand, toxicity can also result from binding of the therapeutic mAb to the intended target as illustrated by rapid lysis of normal and tumor B cells by binding of rituximab (anti-CD20) to its target resulting in tumor lysis syndrome.29 Similarly, anti-CTLA-4 antibodies lead to uncontrolled general activation of T cells resulting in autoimmunity in some patients30 and treatment of patients with bevacizumab (anti-VEGF) results in multiple toxicities associated with binding to their normal targets.31

Concerns arising from target-biology related toxicities have been highlighted by recent experiences with two therapeutic mAbs, natalizumab (α4-intergrin) and TGN1412 (anti-CD28). Natalizumab for the treatment of multiple sclerosis was recalled from the market because it induced a rare fatal viral demyelinating disease, progressive multifocal leukoencephalopathy (PML), in two patients.32-34 The FDA approved return of natalizumab to the market subject to a special restricted distribution program following a comprehensive review of a large number of patients. Similarly, first in man administration of TGN1412, a super-agonist mAb, in six healthy volunteers led to devastating toxicities due to massive activation of T cells.35 It can be deduced from the above examples that mAbs with immunomodulatory properties have the potential to induce unexpected toxicities. Thus, thorough evaluation of mAbs in relevant in vitro and in vivo models before first-in-man clinical trials are initiated is necessary, especially, if the mAbs work through effector functions, have the functional ability to induce a robust biologic response, or act by ablation of certain classes of immune cells. It is also important to understand the biology of the antigen and antibody across species, especially those aspects that cannot be fully evaluated in nonclinical animal models. This will allow human risk assessment in light of the limitations of the nonclinical models, and help to appropriately select the starting dose for clinical studies.

Importance of exposure of mAbs. Since antibodies typically have long half-lives compared with small molecule drugs, therapeutic antibodies may persist in the body for extended periods following administration. Thus the pharmacological effects of the antibody may last well beyond initial dosing.36 As a result, the exposure-response relationship rather than the dose-response relationship should be considered during both the design and interpretation of toxicity studies, and a recovery period is included to determine whether toxicity is reversible in the absence of antibody.20,27 Despite cessation of antibody administration during the recovery period, five half-lives must elapse before almost all of the antibody has been eliminated from the animal. Thus, the half-life of the mAb influences the duration of the recovery period and the subsequent toxicity evaluation. A difference in affinity of greater than 10-fold between species mandates the use of exposure rather than nominal administered dose for appropriate study design. Specifically, the dose in the animal model should be adjusted to reflect the difference in affinity between the animal and humans in order to ensure adequate exposure in the toxicity study. Exposure-response relationships also allow interspecies comparisons, determination of the therapeutic index, evaluation of the desired safety margin for the initial starting dose in humans, and calculation of the dose escalation scheme. Antibody PK parameters will likely differ across species; therefore, in order to achieve equivalent exposure in all species, the dose levels and schedule need to be adjusted. Failure to make necessary adjustments based on species differences may result in inadequate dosing in the toxicity studies or even overdosing in humans in clinical trials.

A single-dose PK study where multiple blood samples are collected at various time intervals is ideal to fully describe the antibody serum concentration-time curve. From these data, PK parameters such as
Table 4 Typical endpoints in a general toxicity study

| Frequency of Assessment | Endpoint                                      |
|------------------------|-----------------------------------------------|
| Baseline               | • Ophthalmology                               |
|                        | • EKG                                         |
| Every 30 min for 4 h post dose | • Vital signs                                 |
| Twice daily            | • Clinical observations (cage side)           |
| Daily                  | • Food consumption                            |
| Weekly                 | • Detailed clinical observations              |
|                        | • Body weight                                 |
| Once during dosing phase | • Ophthalmology                               |
|                        | • EKG                                         |
| During recovery if changes observed | • Ophthalmology                               |
|                        | • EKG (non-rodents only)                      |
| Periodically in-life   | • Hematology (non-rodents only)               |
|                        | • Serum chemistry (non-rodents only)          |
|                        | • Urinalysis (non-rodents only)               |
| At termination         | • Hematology                                  |
|                        | • Serum chemistry                             |
|                        | • Urinalysis                                  |
|                        | • Gross pathology                             |
|                        | • Organ weights                               |
|                        | • Histopathology                              |

area under the serum concentration-time curve (AUC), clearance, volume of distribution and half-life can be reliably estimated. In the absence of a PK study, collection of blood samples after the first and last doses in a multiple-dose toxicity study may provide sufficient serum concentration data to allow estimation of PK parameters. During the recovery period and necropsy, collection of blood samples can assist in determining the terminal elimination half-life. Peak and trough blood samples collected before and after each dose will, at the least, provide maximum ($C_{\text{max}}$) and minimum ($C_{\text{min}}$) serum concentration values. An increase in the $C_{\text{min}}$ values over time will indicate dose accumulation. While antibody dose levels in toxicity studies usually span 1 to 2 orders of magnitude, disproportionately higher levels of dose accumulation at the top end of the dose range or conversely non-linear PK at the low dose levels are not uncommon and manifest as slower clearance of the antibody and lower exposure, respectively. These differences in exposure should be considered when relating the observed toxicities to the administered doses in order to define the highest non-severely toxic dose (HNSTD) and the no adverse effect level (NOAEL) that will be used to determine the starting dose in humans. For presentation of the results of toxicity studies to regulatory agencies, it is also helpful to define the dose used in the toxicity studies in relation to the doses used or planned in the clinical studies.38

Toxicity study design. Clinical trial duration, size, scope, indication and phase of development are considered in the determination of the toxicity study design. The duration of the toxicity study should equal or exceed the duration of the clinical trial and use at least the same number of antibody doses that will be administered to humans. In the toxicity study, the route of administration should be the same as for clinical use. While antibodies are most often administered by intravenous (IV) infusion to humans, they can be administered to non-human primates as a 1–2 hour IV infusion and to rodents as a slow IV bolus injection. On the other hand, the dose schedule may mimic the human dose schedule or the intervals between doses may be shorter in the experimental animals compared to humans, since shorter intervals may be needed to compensate for faster clearance rates of the antibody in animals or to diminish the impact of immunogenicity on exposure. In a typical toxicity study, a control group that receives the vehicle in which the antibody is formulated is included along with three dose levels (low, mid and high). The dose-response relationship, including a toxic dose and a NOAEL dose, should dictate the dose levels used in toxicity testing.

Toxicity testing should be performed in both male and female animals and the results should be segregated according to sex for statistical analysis. As a result, animal numbers are typically reported as the number of animals per sex per group. The number of animals per group may vary depending on the species being tested as this number is typically larger for rodents than for non-rodent species, especially non-human primates. Typically, 10–15 rodents per sex group in the main study plus an additional 5–10 animals per sex group in the recovery portion of the study are used. In non-rodent species, 3–4 animals per sex group are used in the main study, and only 2–3 animals are used in the recovery portions of the study. The number of animals per sex group used per dose level determines the probability of detecting a toxic effect and should be adequate to assess potential toxicity. For TK evaluations, the number of time points desired determines the total number of animals required. In rodent studies additional animals may be added for blood collection if TK analyses are to be performed. In non-rodent species, sufficient blood samples for TK analyses can typically be collected from the main and recovery study animals, without addition of animals dedicated for TK assessments.

A general toxicity study incorporates various measurements that include multiple end points as illustrated in Table 4. Clinical signs, body weight and changes in food consumption serve as general indicators about whether the animal is experiencing some type of toxicity. Clinical pathology measures such as hematology, serum chemistry and urinalysis parameters offer information about the functional status of major organ systems, including the liver, kidney and hematopoietic and immune systems. Timing and number of clinical pathology assessments depends on the species used for toxicity testing. Because greater blood volumes can be acquired from larger animals, multiple time points can be evaluated. Anatomic pathology assessments, which include macroscopic and microscopic examination of tissues and organs, are used to identify the target organs of toxicity. Standard practices for conducting toxicity studies as well as standard clinical pathology parameters and anatomic pathology tissues and organs examined have been comprehensively reviewed (ref. 39).

Nonclinical Safety Studies to Support Clinical Development

Here, we describe nonclinical safety studies for mAb products including murine, chimeric, humanized, fully human intact antibodies and antibody derived fragments. This includes antibodies that contain either native immunoglobulin or engineered sequences, and are produced from hybridomas or recombinant cell lines. Antibody
products may also include payload antibodies, which deliver radio-
nuclides, toxins or cytotoxic drugs to the appropriate targets. These
mAbs, which are known as antibody drug conjugates (ADC) or
immunocytokines are considered drug products from a regulatory
perspective. Thus, as for other drugs, nonclinical safety studies must
be conducted that include the payload portion of the conjugate e.g.,
studies must be done on the cytotoxic drug, the antibody portion,
and the combined ADC itself. A Pre-IND meeting with the Food
and Drug Administration (FDA) or other regulatory agencies prior
to initiation of nonclinical safety studies is highly recommended.40
In the following sections, we describe the types of nonclinical safety
studies and their timing with regard to clinical development.

Studies to Support Phases I to III

The Phase I IND-enabling safety package for a mAb will include a
human tissue cross-reactivity study and a general toxicity study in at
least one relevant species, most likely a non-human primate, although
two relevant species should be included, if possible. Non-target
binding or cross-reactivity occurs when the same or related anti-
genic determinant is expressed on human cells or tissues other than
the intended target tissue. This cross-reactive binding may result
in undesired effects and safety issues. The cross-reactivity potential
must be evaluated by immunohistochemically (IHC) using several
concentrations of the labeled antibody on a panel of 32 tissues from
two unrelated human donors. Fortunately, several CROs specialize
in cross-reactivity studies, and can furnish the panel of human tissues
and generate appropriately labeled reagents.

In support of an initial Phase I trial, a single- and/or multiple-
dose toxicity study may be conducted depending on the patient
population, disease indication, intended number of cycles of treat-
ment in humans and risk-benefit relationship. A period of one
to three months is a typical duration for repeated-dose toxicity
studies, while shorter duration studies or acute single-dose toxicity
studies may be adequate to support a short duration Phase I trial for
life-threatening illnesses such as cancer. When evidence of clinical
benefit is observed in oncology patients, additional doses may be
administered during the Phase I study without the need for addi-
tional testing in animal safety studies. This situation is similar to that
with small molecule cytotoxic cancer drugs where the number of
cycles of treatment in early clinical trials may exceed the nonclinical
toxicity study coverage.

Phase II trials often require repeated-dose toxicity studies of
longer duration than performed for the IND. The duration of the
nonclinical toxicity study should meet or exceed the duration of
the planned clinical trial. The one-month and three-month toxicity
studies performed in support of the Phase I and II trials may be suffi-
cient for the initiation of Phase III trials under certain circumstances;
however, longer studies are generally required since the number of
patients that will be exposed to the antibody is increased.41 For
example, six- to nine-month chronic toxicity studies are required for
BLA for marketing authorization of antibodies intended for chronic
administration. Details of the requirements for duration of multiple
dose toxicity studies in rodents and non-rodents required for market
approval is given in the International Conference on Harmonization
(ICH) M3 guidance document.42

Safety Studies to Support Marketing

Marketing approval of an antibody usually requires single- and
repeated-dose toxicity studies, local tolerance studies, reproduction
and developmental toxicity studies, and safety pharmacology studies.
If the antibody is intended for chronic administration, chronic
toxicity studies are required, and in the case of non-oncology indi-
cations, evaluation of carcinogenic potential may also be necessary.
In addition, antibodies that have potential to cause immune suppres-
sion or stimulation, or have targets present on immune cells should
also be evaluated for immunotoxicity. Genotoxicity studies are not
applicable to antibodies and should not be conducted. Local toler-
ance at the site of antibody administration as well as the formulation
intended for marketing can also be evaluated by obtaining data from the
single- or repeated-dose toxicity studies without the need for separate studies. Various studies needed to support marketing mAb
therapeutics are given in the ICH S6 guidance document.20

Safety pharmacology. To measure functional affects on major
physiological systems, safety pharmacology studies are conduct-
ed 54,55 To address this, separate studies may be performed or these
measures may be incorporated in the design of toxicity studies. One
simple approach is to perform cardiovascular assessments, especially
in non-rodent species in the general toxicity studies to support the
IND. Examples of such assessments are electrocardiogram, blood
pressure and heart rate. Detailed clinical observations may uncover
central nervous systems and respiratory effects. Specialized studies
can then be performed to follow up any safety issues identified in
these initial toxicity studies. Data from unanesthetized and unre-
strained animals can be obtained by telemetry, where a transmitting
device is implanted to facilitate transmission of continuous cardiac
function data to a remote receiver using radio frequency communica-
tions. Additional examination of isolated organs or other test systems
not involving intact animals may also be performed.

Developmental and reproductive toxicity studies (DART).
Reproduction toxicity studies are designed to identify the effects of
the antibody on mammalian reproduction and include exposure of
mature adults, as well as all stages of development from conception
to sexual maturity.56 The studies should address the effects of the
antibody on fertility and early embryonic development; prenatal
and postnatal development, including maternal function; and
embryo-fetal development.20,43,56 The repeated-dose toxicity studies
performed during the earlier phases of clinical development offer
information regarding potential effects on reproduction, particularly
on male fertility. DART studies should be completed for licensure.
EU and Japanese regulatory agencies require these studies to be
completed before Phase III. The clinical indication and intended
recipient population dictate the required reproductive and devel-
opmental toxicity studies. These studies should be conducted if the
antibody product is intended for repeat or chronic administration
to women of childbearing potential. The study design and dosing
schedule may be modified based on antibody species specificity,
immunogenicity, biological activity or a long elimination half-
life. Immunological effects that are long-lasting may raise specific
concerns regarding potential developmental immunotoxicity and a
developmental toxicity study designed to assess the neonatal immune
function can address these concerns. Consultation with experts
before study initiation is highly recommended since developmental
immunotoxicology studies can be quite challenging due to the target species and availability or lack thereof of historical data, especially for non-human primates.

Carcinogenicity studies. Standard carcinogenicity bioassays are inappropriate for antibody products, and as a result, currently marketed antibodies have not been evaluated for carcinogenicity. However, assessment of carcinogenic potential should be considered if a potential for this risk exists. If a risk is identified, ability of the antibody to stimulate growth of normal or malignant cells expressing the antigen should be determined. Studies in relevant animal models and in vitro cellular proliferation should be evaluated in long-term repeated-dose toxicity studies in order to address carcinogenicity of the antibody.

Pharmacokinetics studies. PK and TK studies are implemented in order to understand exposure in the safety studies, to allow cross-species comparisons, and to predict margins of safety for clinical trials based on exposure. We have already described the importance of the exposure-response relationship in the interpretation of the toxicity studies. While traditional small molecule distribution and excretion studies are not relevant for antibodies, biodistribution studies of mAbs may provide evidence for inappropriate tissue targeting or may explain the observed toxicities in animals. The antibody isotype, binding affinity, binding to serum proteins, route of administration and level of antigen expression in the recipient animal should all be taken into consideration when interpreting results of these studies. As catabolism of antibodies results in their degradation to individual amino acids, pharmaceutical biotransformation studies are not required for unconjugated antibodies.

Immunotoxicity studies. The purpose of immunotoxicity studies is to determine the adverse effects of therapeutic mAbs on the immune system. Stimulation and expansion of immune cells leading to an autoimmune disease, immune suppression resulting in decreased host resistance to infectious agents or tumor cells are considered as main adverse events affecting the immune system. In standard toxicity studies, evaluation of total leukocyte counts, absolute differential leukocyte counts, lymphoid tissues at necropsy, and histopathology of the spleen and thymus may indicate toxicity to the immune response. These changes in immune parameters may also result from stress due to doses near or at the maximum tolerated dose, and these effects are most likely mediated by increased corticosterone or cortisol release. The following stress-related immune changes are commonly observed: increases in circulating neutrophils, decreases in circulating lymphocytes, decreases in thymus weight, decreases in thymic cortical cellularity and associated histopathologic changes, changes in spleen and lymph node cellularity and increases in adrenal gland weight. Clinical observations, such as decreased body weight gain and activity, may suggest that the changes to lymphoid tissue and hematologic parameters are a result of stress rather than to a direct immunotoxic effect. However, compelling evidence of stress is necessary in order to discount the immunotoxic effects of the antibody. If evidence for immunotoxicity is uncovered in the initial toxicity studies, then specific endpoints should be included in subsequent general toxicity studies or in additional specific immunotoxicity studies. Immunophenotyping, which is the identification and/or enumeration of leukocyte subsets with specific antibodies, is one of the easier endpoints to incorporate into standard toxicity studies. This assay is usually conducted by FACS or IHC. Since immunophenotyping is not a functional assay, other parameters such as the T cell-dependent antibody response are measured to assess mAb immunotoxicity. Functional assays, are described in the ICH S8 guidance for immunotoxicity studies for human pharmaceuticals.

Different types of safety studies described above may not be required for every antibody product. Products intended for treatment of life-threatening or serious diseases without current effective therapy may warrant a case-by-case approach to the nonclinical safety evaluation. In these cases, particular studies may be abbreviated, deferred or omitted to expedite development. Table 5 lists the studies typically conducted for antibody products in the cases of life-threatening diseases and other less serious conditions.

Guidance Documents by Regulatory Agencies

Documents containing useful information for planning and executing mAb nonclinical safety evaluation are provided by various regulatory agencies (Table 6). The primary guidance for nonclinical safety evaluation of biotechnology-derived products, the ICH S6 “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals” document, describes the goals of nonclinical studies, outlines principles for study design, and provides an overview of the types of required safety studies. In 1997, the United States FDA issued a revised version of “Points to Consider (PTC) in the Manufacturing and Testing of Monoclonal Antibody Products for Human Use.” This document provides a broad description of manufacture and testing, including nonclinical and clinical studies of mAbs. The information necessary for designing a nonclinical safety evaluation program specifically for mAbs is included in this document. Specifically, this document thoroughly describes cross-reactivity studies of mAbs and preclinical studies with immunonjugates. The ICH M3 “Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals” guidance is similar to the S6 document and provides detailed information on the recommended duration of repeated-dose toxicity studies and types of reproductive toxicity studies required with regard to the phases of clinical development. Two documents describe GLP for the conduct of nonclinical safety studies: an FDA-issued document in the Code of Federal Regulations, Title 21, Part 58 (21CFR58) and a document issued by the Organization for Economic Co-operation and Development (OECD). The FDA document promotes practices to ensure the quality and integrity of the nonclinical safety data submitted in support of initiation of clinical trials in humans. The process of performing, monitoring, recording, archiving and reporting

| Table 5 | Nonclinical safety studies based on disease indication |
|----------|-----------------------------------------------------|
| **Life-threatening** | **Non-life-threatening** |
| • Tissue cross-reactivity | • Tissue cross-reactivity |
| • General toxicity | • General toxicity |
| • Safety Pharmacology | • Immunotoxicity |
| • Chronic toxicity | • Safety Pharmacology |
| • Reproductive and developmental toxicity | • Chronic toxicity |
| • Carcinogenicity | |
Table 6  Selected guidance documents for preclinical safety evaluation of mAb

| Type of document | Document Title                                                                 | Year Published and Reference |
|------------------|-------------------------------------------------------------------------------|------------------------------|
| Book             | Cavagnaro JA: Preclinical safety evaluation of biopharmaceuticals, Hoboken, NJ: Wiely | 2008 (38)                    |
| Book chapter     | Teicher BA, Andrews PA, Anticancer Drug Development Guide, Preclinical Screening, Clinical Trials and Approval, 2nd ed. Humana Press, Part III, pages 287-335 | 2004 (50)                    |
| International Guidance Documents |                                                                                 |                              |
| ICH S6           | Preclinical safety evaluation of biotechnology-derived pharmaceuticals (ICH S6) | 1995 (20)                    |
| ICH M3           | Non-Clinical Safety Studies For The Conduct Of Human Clinical Trials For Pharmaceuticals (ICH M3) | 1995 (42)                    |
| ICH S3A          | Toxicokinetics: the assessment of systemic exposure in toxicity studies (ICH S3A) | 1995 (52)                    |
| ICH S4           | Duration of Chronic Toxicity Testing in Animals (Rodent and Non-Rodent Toxicity Testing) (ICH S4) | 1998 (53)                    |
| ICH S7A          | Safety pharmacology studies for human pharmaceuticals (ICH S7A) | 2000 (54)                    |
| ICH S7B          | The Non-clinical Evaluation of the Potential for delayed Ventricular Repolarisation (QT Interval Prolongation) by Human Pharmaceuticals (ICH S7B) | 2000 (55)                    |
| ICH S5(R2)       | Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility | 1993 (Core) (56)  
|                  |                                                                                | 1995 (Addendum)              |
| ICH M4           | Organisation of The Common Technical Document for The Registration of Pharmaceuticals for Human Use | 2003 (57)                    |
| US Guidance Documents |                                                                                   |                              |
| FDA PTC          | Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use | 1997 (46)                    |
| CFR              | 21CFR58: Good Laboratory Practice for Nonclinical Laboratory Studies | 2004 (47)                    |
| FDA Guidance for Industry | Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers | 2005 (49)                    |
| European Guidance Documents |                                                                                 |                              |
| EMEA Guideline   | Strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products | 2007 (50)                    |
| Position paper   | Position Paper on the non-clinical safety studies to support clinical trials with a single micro dose | 2002 (58)                    |

ICH = International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. CFR = Code of Federal Regulations.

the studies are described thoroughly in both documents. Although the ICH S8 “Immunotoxicity Studies for Human Pharmaceuticals” guideline provides recommendations on clinical testing for immunosuppression induced by low molecular weight drugs, this information is applicable to antibody immunotoxicity testing.37 Two other important guidance documents issued by regulatory agencies include one by US FDA “Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers”49 and the other by EMEA “Strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products”50 are useful for designing first-in-man trials in healthy human volunteers. A recently published comprehensive text “Preclinical safety evaluations of biopharmaceuticals”38 could also serve as an excellent reference source for various aspects of nonclinical safety studies. A selected list of additional regulatory documents and other useful texts for designing sound non-clinical safety studies are also provided in Table 6.

Summary

While therapeutic antibody products have been marketed for many years, the complexity of mAbs, recombinant method to produce them and diversity of antibody products entering clinical
development has greatly increased recently. Today’s therapeutic mAbs are engineered for improved functional capabilities and half-lives. Thus, it is very important to design appropriate nonclinical safety studies to support clinical development and ensure patient safety. Effector functions, tissue cross-reactivity, immunogenicity and stability are major safety concerns for mAbs products. Importantly, identification of a relevant species for toxicity testing, knowledge of the biology of the target antigen and antibody and interpretation of the results in terms of the exposure-response relationship are critical elements underlying the design of a successful nonclinical safety evaluation. These studies should be designed to identify potential toxicities and should parallel the anticipated dose, concentration, schedule, route and duration to be used in clinical studies. In order to ensure that the appropriate studies have been conducted at the optimum time, nonclinical studies should be conducted in step with the clinical trials. Design and implementation of these studies requires familiarity with the guidance documents describing preclinical safety testing. Furthermore, interactions with appropriate scientific leaders and regular communication with the FDA or other regulatory authorities is paramount to successful toxicity testing and subsequent clinical trials of mAbs.

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