Preclinical Safety Evaluation of Monoclonal Antibodies

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1 Introduction 20
2 Goals of Preclinical Safety Evaluation 23
 2.1 General Considerations 23
 2.2 Start with the End in Mind 23
 2.3 Coordinate the Preclinical Safety Program in Step with the Phases of Clinical Development 23
 2.4 Plan for Process Development Changes 24
 2.5 Start Assay Development Early 25
3 Critical Issues for Success 26
 3.1 Relevant Species 26
 3.2 Science-Driven Approach 28
 3.3 Exposure 28
4 Preclinical Safety Studies 30
 4.1 Preclinical Safety Studies to Support Phase I 30
 4.2 Preclinical Safety Studies to Support Phase II 33
 4.3 Preclinical Studies to Support Phase III 34
 4.4 Preclinical Safety Studies to Support Marketing 34
 4.5 Preclinical Studies with Payload Antibodies 38
 4.6 Guidance Documents 40
 4.7 Antibody Toxicities 41
5 Summary 42
References 43

List of abbreviations

ADC, antibody drug conjugates; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; AUC, area under the serum

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Y. Chernajovsky, A. Nissim (eds.) *Therapeutic Antibodies. Handbook of Experimental Pharmacology 181.*
© Springer-Verlag Berlin Heidelberg 2008

concentration–time curve; BLA, Biologics License Application; CDC, complement-dependent cytotoxicity; C_{max}, model-predicted maximum serum concentration; CMC, chemistry manufacturing and controls; C_{min}, model-predicted minimum serum concentration; CNS, central nervous system; CRO, contract research organization; ECL, electrochemiluminescence; ELISA, enzyme-linked immuno sorbent assay; FDA, Food and Drug Administration; GLP, good laboratory practice; HNSTD, highest nonseverely toxic dose; ICH, International Conference on Harmonisation; IND, Investigational New Drug; IV, intra venous; mAb, monoclonal antibody; MS, multiple sclerosis: MTD, maximum tolerated dose; NOAEL, no adverse effect level; OECD, Organisation for Economic Cooperation and Development; PK, pharmacokinetic; PML, progressive multifocal leukoencepphalopathy; PTC, points to consider; TDAR, T cell dependent antibody response; TK, toxicokinetics; VEGF, vascular endothelial cell growth factor.

Abstract Monoclonal antibodies (mAbs) are a well-established product class of biotechnology-derived pharmaceuticals for treating multiple diseases. A growing number of mAbs are being tested in clinical trials worldwide. Many of the second generation mAbs entering the clinic today are highly engineered, produced from recombinant cell lines, and present new safety challenges for regulators and industry scientists responsible for their safety evaluation. The increasing complexity of antibodies and the variety of recombinant production cell systems used for antibody manufacturing require a well thoughtout approach for preclinical safety evaluation of mAbs. The focus of this chapter is to provide the reader with a basic framework for preparing a scientifically sound preclinical package for safety evaluation of therapeutic mAbs. We outline the general considerations for planning a preclincal program and the issues critical for success. We describe the types of preclinical safety studies and the timing for their conduct in relation to clinical trials. We also share some of the lessons learned about toxicity of mAbs from previous antibody development programs. A list of relevant regulatory documents issued by various government agencies and selected references to other useful texts and publications are also provided in the chapter. We believe that applying the principles described in this chapter will improve the quality and relevance of the preclinical safety data generated to support the future development of mAbs therapeutics.

1 Introduction

During the past 20 years, great progress has been made in developing monoclonal antibodies (mAbs) as major biotherapeutics for a wide variety of diseases, including cancers, inflammatory diseases, autoimmune conditions, and infections. There are 19 approved mAbs for therapeutic use in the US today (Table 1) and a growing number of mAbs are being tested in clinical trials worldwide (Reichert et al. 2005; Adams and Weiner 2005; Kim et al. 2005). An ever-increasing number of mAbs for diagnosis and treatment are most likely to fill the pipelines of many companies in

Table 1 Approved antibodies for therapeutic use

Approval date	Antibody	Name	Target	Antibody type	Indication	Company
1986 (US)	Muromonab-CD3	OKT3	CD3	Murine, IgG2a	A & I*	Johnson & Johnson
1984 (US)	Abciximab	ReoPro	PIIb/IIIa	Chimeric, IgG1, Fab	Hemostasis	Centocor
1987 (US) 1988 (EU)	Rituximab	Rituxan	CD20	Chimeric, IgG1	Cancer	Genentech
1997 (US) 1999 (EU)	Daclizumab	Zenapax	CD25	Humanized, IgG1	A & I*	Roche
1998 (US) 1998 (EU)	Basiliximab	Simulect	CD25	Chimeric, IgG1	A & I*	Novartis
1998 (US) 1999 (EU)	Palivizumab	Synagis	RSV	Humanized, IgG1	Infections	MedImmune
1998 (US) 1999 (EU)	Infliximab	Remicade	TNFα	Chimeric, IgG1	A & I*	Centocor
1998 (US) 2000 (EU)	Trastuzumab	Herceptin	HER2	Humanized, IgG1	Cancer	Genentech
2000 (US)	Gemtuzumab ozogamicin	Mylotarg	CD33	Humanized, IgG4, immunotoxin	Cancer	Wyeth
2001 (US) 2001 (EU)	Alemtuzumab	Campath-1H	CD52	Humanized, IgG1	Cancer	Genzyme
2002 (US) 2004 (EU)	Ibritumomab tiuxetan	Zevalin	CD20	Murine, IgG1, radiolabeled (Yttrium 90)	Cancer	Biogen Idec
2002 (US) 2003 (EU)	Adalimumab	Humira	TNFα	Human, IgG1	A & I*	Abbott
2003 (US)	Omalizumab	Xolair	IgE	Humanized, IgG1	A & I*	Genentech
2003 (US)	Tositumomab-I131	Bexxar	CD20	Murine, IgG2a, radiolabeled (Iodine 131)	Cancer	Corixa/GSK
2003 (US) 2004 (EU)	Efalizumab	Raptiva	CD11a	Humanized, IgG1	A & I*	Genentech
2004 (US) 2004 (EU)	Cetuximab	Erbitux	EGFR	Chimeric, IgG1	Cancer	Imclone
2004 (US) 2005 (EU)	Bevacizumab	Avastin	VEGF	Humanized, IgG1	Cancer	Genentech
2004 (US)	Natalizumab	Tysabri	α4-intergrin	Humanized, IgG4	A & I*	Biogen Idec
2006 (US)	Panitumumab	Vectibix	EGFR	Human, IgG2	Cancer	Amgen

*A & I = Autoimmune and inflammatory indications

the near future as a result of breakthroughs in antibody technologies coupled with identification of additional molecular targets of disease. Therefore, it is essential to conduct appropriate preclinical safety studies of mAbs to support more rapid clinical development of antibody therapeutics and ensure patient safety.

The first generations of mAb therapeutics were produced from mouse hybridomas and had limited success in the clinic. This was partially due to their inability to effectively interact with human effector cells and their rapid clearance from the system because of immunogencity (Carter 2006). With the advent of recombinant DNA technology, generation of chimeric and humanized mAbs (by grafting of the Fc portion and variable regions of the mouse antibodies with human counterparts) has alleviated some of these problems (Carter 2006). Many new technologies are now available that allow production of fully human antibodies (Hoogenboom 2005; Lonberg 2005; Carter 2006). Recombinant technology has also allowed further refinements of antibody sequences to alter the binding affinity and Fc effector functions. It is thus possible to customize mAbs for desired effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Most of the mAbs in the clinic today are genetically modified to incorporate more human characteristics aimed at reducing immunogenicity and enhancing interaction with human effector cells. The second generation of mAbs that are modified to alter glycosylation, target binding affinity, and half-life are now entering product development in an effort to improve efficacy and to increase the chances for clinical success (Adams and Weiner 2005; Carter 2006).

In parallel with the advances in antibody engineering, there has also been an evolution of the technology for generation of high titer mAb producing cell lines of both mammalian and nonmammalian origins (Carson 2005). A variety of different expression systems and production cell lines are now available for small and large-scale commercial manufacture of mAbs (Birch and Racher et al. 2006). Thus, the increasing complexity of antibody engineering and the variety of recombinant production cell systems available for antibody generation make it more critical than ever that a thorough and thoughtful approach be taken to the preclinical safety evaluation of monoclonal antibodies. In addition to their novel peptide nature, complex structure, unique biologic functions, and longer half-lives, the more routine utilization of engineered antibodies to treat chronic diseases (Kim et al. 2005) also adds to the potential safety concerns for their prolonged clinical use.

In this chapter, we provide a basic framework for preparing a scientifically sound preclinical package for safety evaluation of therapeutic mAbs. We outline the general considerations for planning a preclincal program and the issues critical for success. We describe the types of preclinical safety studies and the timing for their conduct in relation to clinical trials. We also share some of the lessons learned from previous development of antibodies. We identify the relevant regulatory documents along with selected references to other useful texts and publications. The chapter will serve as a roadmap, providing guiding principles and directing the reader to additional sources of information. We hope that application of the principles described in this chapter will improve the quality and relevance of the preclinical safety data generated to support the development of mAbs therapeutics.

2 Goals of Preclinical Safety Evaluation

The three main goals of preclinical safety evaluation of monoclonal antibodies and any biopharmaceutical are:

1. To determine a safe starting dose for the first in human Phase 1 clinical trial and subsequent dose escalation schemes
2. To identify potential target organs of toxicity and to determine whether the toxicity is reversible after a period of time following the end of treatment
3. To identify parameters that can be used to monitor safety in the clinical trials

Meeting these goals is achieved through the conduct of in vitro and in vivo nonclinical studies aimed at defining and understanding the toxicological properties of the antibody. To design an appropriate safety assessment of an antibody, it is best to have first characterized its pharmacological properties such as receptor affinity, receptor occupancy, and biological activity related to its intended therapeutic application. An understanding of the exposure response relationship is an integral part of the preclinical safety evaluation of monoclonal antibodies. Initial estimates of pharmacokinetic (PK) parameters are helpful in designing the duration of the recovery period, for example, in repeated dose-toxicity studies. Since the toxicology package is intended to support the clinical program from Phase I to approval, clearly defined clinical trials are a prerequisite for designing the supporting toxicology program. General considerations for planning a nonclincial safety evaluation program for a monoclonal antibody will be discussed below and the issues that are critical for success will be highlighted.

2.1 General Considerations

2.2 Start with the End in Mind

The most efficient way to plan a nonclincial development strategy is to start with the end in mind and to work backwards. This may seem counter-intuitive, but experience has proven time and time again that it is the most effective approach. A very useful exercise to do at the outset of the program is to write the label for the product in collaboration with clinical, regulatory, and manufacturing. This exercise will help define the key components (e.g., indication, patient population, dosing regimen, duration of treatment, route of administration, formulation, etc.) required for designing the toxicology package.

2.3 Coordinate the Preclinical Safety Program in Step with the Phases of Clinical Development

The next step in the process is to list all of the nonclinical pharmacology and toxicology studies anticipated to be conducted over the entire course of the development of

the product from the investigational new drug (IND) phase to postmarketing. Then divide the studies into categories according to when they will be conducted as follows: (1) prior to initiation of Phase I clinical trial, (2) Prior to or concurrently with Phase II, and (3) concurrently with Phase III pivotal trials. Working backwards, this will allow determination of which studies are required for registration of the product i.e., to file the Biologics License Application (BLA), but need not be completed to initiate pivotal Phase III clinical trials. Examples of such studies are chronic toxicity studies and reproductive and developmental toxicology studies. Next identify toxicology studies that are required to be completed to support initiation of Phase III and Phase II trials. Often they are one and the same. Finally, identify which studies are necessary for the IND submission.

The utility of this approach is that it allows coordination of the timing of the conduct of the safety study in step with the phases of drug development. The necessity for safety studies and the timing of their conduct is not solely governed by the clinical trials, but also by the chemistry, manufacturing and controls (CMC) development strategy that will be discussed in the next section. One of the most common occurrences in toxicology programs is need to conduct similar toxicity studies more than once because of lack of coordination with Clinical and CMC. Often the duration of treatment in the Phase I trial is shorter that the intended labeled clinical use of the product. Thus, the duration of treatment is longer in Phase II and beyond. If the IND-enabling studies are designed strictly to support the shorter duration Phase I trial then they will not be adequate to support Phase II and will necessitate the conduct of a second toxicity study of longer duration. The advantage of planning backward is that one can anticipate the need for the longer duration toxicity study beyond the initial IND and elect to conduct the longer duration toxicity study upfront. The longer duration toxicity study will provide toxicology coverage for both Phase I and II, and avoid unnecessary duplication of effort and resources.

2.4 Plan for Process Development Changes

A classic example of a CMC trigger for the need to conduct additional safety evaluations is a process change in the manufacture of the antibody. Changes can consist of upstream changes to the culture conditions of cell lines for recombinant derived mAbs or even a change in the production cell line itself, and downstream purification changes or formulation changes. Efforts will be undertaken to demonstrate comparability of the new product to the old process first using analytical methods. However, if the changes are of a sufficient magnitude that comparability cannot be assured using in vitro analytical methods alone, then a bridging toxicity study or an entirely new toxicity study will be necessary.

2.4.1 Keep Product for Comparability Studies and Bridging Strategies

A bridging toxicity study in which the old and new product is compared in a truncated study design (e.g., a short duration with a subset of endpoints) using at least one dose level of the old product to compare with the new is the most cost effective approach. It is essential that a sufficient supply of the early phase material is retained for a bridging study. It is a widespread practice to continue process development after initiation of the Phase I trial. Therefore, it is prudent to plan for a bridging study and include material for the study in the initial estimates of drug supply needs. If early phase material has not been reserved to conduct a bridging study then it may be necessary to conduct a comprehensive toxicity study de novo with antibody from the new process, essentially repeating what has done already with the early process material.

2.5 Start Assay Development Early

The final general consideration is the need to start assay development early, well in advance of the planning for the toxicity study. The lead time for assay development can be anywhere from nine months to a year. Additional time will be necessary if the assay has to be transferred to a contract research organization (CRO) and validated prior to implementation of testing specimens from the toxicity study. Product specific assays are needed to determine the stability and concentration of the antibody in a number of settings and matrices.

2.5.1 Dose Solution Analysis

It is a Good Laboratory Practice (GLP) requirement that the concentration of the antibody in the solution used for dose administration be verified. The CMC group may have an assay in place for lot release testing of the formulated antibody that can be adapted. Please be aware that the antibody will be diluted to levels considerably lower than that found in the final drug product, so additional assay development will be needed to ensure detection of antibody at low concentrations.

Demonstration of the stability of the dose solution under the conditions of use will also be necessary. Following dilution of the antibody to prepare the dosing solution, it may be held in the viviarium at room temperature for up to 8 h on the day of dose administration. Similarly, stability data are required on the formulated drug product for the duration of the dosing period, i.e., until after the last dose is administered. Quite often the toxicology studies are conducted well in advance of clinical product manufacture and initiation of formal product stability studies. It is wise to alert the CMC group or other group responsible for assay development that an assay to demonstrate antibody product stability will be needed to support the

toxicity study in advance of the formal stability program. Stability testing can be conducted concomitantly with the toxicity study.

2.5.2 PK Assay

For the purposes of assessing the PK and toxicokinetics (TK) of the antibody it will be necessary to have an assay to measure the antibody concentration in serum from animals. The assays typically employed for this purpose are enzyme-linked immuno sorbent assay (ELISA) and utilize serum as the matrix rather than plasma. Assay performance in serum from all species employed in the safety evaluation program should be examined. One of the biggest challenges in developing an assay to assess PK is the interference observed in the presence of an immune response against the antibody. It is beyond the scope of this text to discuss the assay development per se; however, it is important to be aware of the assay limitations when examining the PK data and making interpretations about exposure.

2.5.3 Immunogenicity Assay

Many antibodies, whether murine, chimeric, or humanized, are immunogenic in animals. The induction of antibody formation in animals should be included as an endpoint in PK and toxicity studies, particularly if they involve repeated dose administration. Measurement of immunogenicity using ELISA is subject to the same limitations observed with the PK assay because of cross interference between the drug (therapeutic antibody) and antidrug antibodies. The impact of antibody formation on exposure and consequently the evaluation of safety endpoints should be taken into consideration during interpretation of the overall findings of the study. Other assay platforms such as electrochemiluminescence (ECL) can be explored for measurement of immunogenicity. Given the complexities of these assays, the sooner assay development can begin the better.

3 Critical Issues for Success

3.1 Relevant Species

The single most important element in conducting a successful preclinical safety evaluation of a monoclonal antibody is choosing the most relevant animal species for toxicity testing (Chapman et al. 2007). A relevant species is one in which the antibody is pharmacologically active and expression of the target antigen is present and exhibits a similar tissue-cross reactivity profile to humans. Ideally the properties of the antigen in the animal should be comparable with those in humans

in biodistribution, function, and structure. This provides the opportunity to evaluate the toxicity arising from binding of the antibody to the target antigen, known as on-target toxicity. Furthermore, the greater the similarities in the tissue distribution of the target antigen in the animal species and in humans the more likely it is that target organs of toxicity identified in animals will be predictive for potential toxicities in humans. An animal species that expresses the target antigen, but has a somewhat different tissue distribution may still be of relevance for evaluating toxicity so long as these differences are taken into consideration for human risk assessment. Absolute equivalence of antigen density or affinity for the mAb is not necessary for an animal model to be useful. The need for a relevant animal model for safety evaluation is so critical to the overall success of the drug development program that species cross-reactivity should be included as part of the selection criteria when screening antibodies during lead selection.

Toxicity studies in nonrelevant species may not simply be uninformative, but may be misleading and are, therefore, discouraged. When no relevant model exists, there are two options, neither of which is entirely satisfactory. The first option is the use of transgenic animals that have been engineered to express the human target antigen. The utility of a transgenic animal for safety evaluation is determined by the extent to which the pharmacodymanics resulting from the antibody antigen interaction are similar to those anticipated in humans. The pharmacokinetic properties of the antibody in the transgenic mouse model are quite likely to be very different than in humans.

The second option is to consider developing a surrogate antibody to the human therapeutic antibody that is cross-reactive with the homologous antigen in animals suitable for toxicity testing. The disadvantage of this approach is that the safety evaluation will not be performed on the antibody that will be administered to humans. It should be noted that no two antibodies are exactly alike and there is inherent risk in this approach. Furthermore, the production process, impurities, pharmacokinetics, binding affinity, and mechanism of action may differ between the surrogate and therapeutic antibodies. In addition, the use of a surrogate antibody adds considerable cost to the product development because of the need to produce two antibodies for the program.

When it is not feasible to use either transgenic animal models or surrogate antibodies, it may still be advisable to conduct an assessment of the off-target toxicities of the antibody focused on evaluation of any functional effects on the major physiological systems (e.g., cardiovascular and respiratory) akin to a safety pharmacology study. Although information may be gained from these studies, the challenge is to know to what extent it is relevant to human risk assessment. The more information available on the pharmacology of the antibody intended for clinical use the better the utility of these alternative approaches can be assessed. Surrogate antibodies have been successfully used to evaluate reproductive and developmental toxicity and support licensure of monoclonal antibody products, e.g., Infliximab (Remicade®) and Efalizumab (Raptiva®).

3.2 Science-Driven Approach

Stating that taking a science-driven approach to design safety studies is the key to success may seem a little bit like stating the obvious. However, many scientists are confronted with the pressure to simply conduct whatever studies are requested of them by a regulatory authority, even if they are not relevant. Taking a "check the box" approach to the safety evaluation of a mAb may ultimately do a disservice to both the mAb product development program and the regulatory agency. First of all, a study critical to elucidating the toxic potential of the antibody may fail to be done during preclinical testing, only to be discovered later in the clinic. Typically, the sponsor will know the properties of the antibody better than the agency. The sponsor can facilitate the review process by furnishing the pharmacology and toxicology reviewer with pertinent information about the mAb so that together they can assure human safety.

3.2.1 Knowledge of the mAb and Target Antigen Biology

Knowledge of the biology of the antibody and its target antigen will allow better design of a toxicity study that will evaluate the safety and potential toxicity of the antibody. In order for mAbs to be clinically effective, a combination of mechanisms of action directed at their desired effects is typically needed. In this regard, mAbs provide multiple effector functions and other properties that make them attractive therapeutics. Some of the most relevant attributes are: (1) mAbs interact with host immune cells to induce ADCC; (2) certain isotypes of mAbs fix complement and thus induce CDC; (3) many mAbs have the potential to alter signal transduction of the target receptors thereby inducing profound changes in the target cell; (4) mAbs can also block interaction of the target antigen with its ligand(s); (5) mAbs also can enhance phagocytic ability of professional phagocytes via antibody-dependent cellular phagocytosis (ADCP); and finally (6) antibodies can also be used for targeted delivery of payloads, including radionuclides, toxins, and cytotoxic drugs.

3.3 Exposure

Antibodies typically have long half-lives compared with small molecule drugs and as a result, the antibodies may be present in the body long-after administration. In addition their pharmacological effects may last for a very long time after mAb administration (Kimby 2005). For this reason, it is important to consider the exposure–response relationship rather than the dose–response relationship during the design and interpretation of results from toxicity studies. For example, to assess if toxicity is reversible a recovery period is typically included in multiple-dose toxicity studies. The recovery period is intended to determine whether toxicity diminishes in

the absence of antibody. Although antibody is not administered during the recovery period, it takes 5 half-lives for 97% of the antibody to be eliminated. The duration of the recovery period should take into account the half-life of the antibody and ensure that exposure to the antibody is diminished or absent for a period of time to assess the potential for recovery or reversibility of toxicity.

When there is a difference of greater than tenfold in affinity of the antibody across species, it is helpful to use exposure rather than nominal dose administered to ensure appropriate design of the studies. Specifically, if the affinity of the antibody for the monkey target antigen is tenfold less than for the human target then the dose administered to monkeys should be adjusted upwards to ensure adequate exposure in the toxicity study. Exposure–response relationships are also helpful for interspecies comparisons and determination of the therapeutic index and desired safety margin for the initial starting dose in humans, and subsequent dose-escalation schema. Antibody PK parameters (e.g., clearance) will likely differ across species. Therefore, the dose levels and dose schedule or intervals between doses will need to be adjusted to achieve equivalent exposure levels across species. Failure to adjust dose levels and dose schedules based on the species may result in errors either in direction, i.e. in inadequate dosing in the toxicity studies, or more egregiously in over-dosing in humans in the clinical trials.

Exposure can be estimated by including toxicokinetic assessments in toxicity studies. The ideal approach is to conduct a single-dose PK study where multiple blood samples are collected at numerous time intervals adequate to fully describe the serum concentration–time profile of the antibody. This allows reliable estimates of the PK parameters such as area under the serum concentration–time curve (AUC), clearance, volume of distribution, and half life. If it is not possible to conduct a PK study, then collection of blood samples after the first and in particular the last dose in a multiple-dose toxicity study may provide sufficient serum concentration data to allow estimation of PK parameters. Blood samples can be collected during the recovery period including the recovery necropsy to assist in determination of the terminal elimination half-life. At a minimum it is advisable to collect peak and trough blood samples before and after each dose, respectively. This will at least provide model-predicted maximum serum concentration (C_{max}) and model-predicted minimum serum concentration (C_{min}) values, and an increase in the latter over the time course of the study will indicate dose accumulation. Dose levels in toxicity studies of antibodies usually span 1 to 2 orders of magnitude. It is not uncommon to have disproportionately higher levels of dose accumulation at the top end of the dose range or conversely nonlinear PK at the low dose levels manifest as faster clearance of the antibody and lower exposure. It is important to be aware of these differences in exposure when relating the toxicities observed to the doses administered, especially when defining the highest nonseverely toxic dose (HNSTD) and no adverse effect level (NOAEL) that will be used to determine the starting dose in humans. It is also very helpful to define the multiples of the clinical dose that were evaluated in the toxicity studies when presenting the results to regulatory agencies.

4 Preclinical Safety Studies

The preclinical safety studies described in this section are applicable to monoclonal antibody products that encompass murine, chimeric, humanized or fully human intact immunoglobulins, or any portion of immunoglobulins including fragments, single chain antibodies, and diabodies that can interact with specific target antigens. Antibodies may contain native immunoglobulin sequences or engineered sequences and be produced from hybridomas or recombinant cell lines. Antibody products also include payload antibodies carrying radionuclides, toxins, or cytotoxic drugs, where the antibody is serving as a vector for targeted delivery of the payload. The latter, known as antibody drug conjugates (ADC) or immunoconjugates, are considered as drugs products from a regulatory perspective. Preclinical safety studies that are required for drug products must be conducted in addition to the studies for antibody products to characterize the potential toxicity of the cytotoxic drug. We strongly recommend a Pre-IND meeting with the Food and Drug Administration (FDA) before initiating pivotal preclinical safety studies (Siegel 2004). In the subsections that follow we describe the various types of preclinical safety studies and the timing of their conduct in relation to the phase of clinical development.

4.1 Preclinical Safety Studies to Support Phase I

A typical Phase I IND-enabling safety package for a monoclonal will contain at a minimum: (1) a human tissue cross-reactivity study and (2) a general toxicity study in at least one relevant species. Safety packages should normally include two relevant species; however, it is not uncommon that only one relevant species can be identified, most often a nonhuman primate. All preclinical safety studies intended to support human clinical trials must be conducted in compliance with GLP.

4.1.1 Human Tissue Cross-Reactivity Studies

When the same or related antigenic determinant is expressed on human cells or tissues other than the intended target tissue, binding of the antibody may be observed. Nontarget tissue binding known as tissue cross-reactivity may result in undesired effects that raise a safety concern. Accordingly, the potential for cross-reactivity with nontarget human tissue or cells must be assessed. A panel of 32 tissues from three unrelated human donors should be evaluated by immunohistochemisty with several concentrations of antibody. There are a number of CROs that specialize in cross-reactivity studies. They can furnish the panel of human tissues, generate an appropriate protocol, and conduct any experiments necessary to optimize the conditions for the therapeutic antibody, including labeling with biotin if required, to conduct the IHC study.

4.1.2 General Toxicity Studies

As described earlier in this chapter (Sect. 4.1), the single most important element in conducting a successful preclinical safety evaluation of a monoclonal antibody is choosing the most relevant animal species for toxicity testing. Relevant animal species for testing of monoclonal antibodies are those that express the desired epitope and demonstrate a similar tissue cross-reactivity profile as for human tissues. A variety of techniques, such as immunochemical or functional assays, can be used to identify a relevant species. One of the ways to identify relevant species for toxicity testing is to conduct species cross-reactivity studies. Tissues from a variety of species commonly used for toxicity testing can be surveyed immunohistochemically using commercially available multispecies tissue microarrays. Evaluation of antibody binding to cells from animals by FACS can also be employed and is typically more sensitive than immunohistochemical analysis of tissue sections. Comparison of the DNA and amino acid sequences of the target antigen across species should be performed and the percent homology to the human sequence determined if the sequence of the animal orthologue is available. An understanding of the functional role of the target antigen and whether it is similar across species is another consideration for determining the relevance of a species for preclinical safety evaluation. As described earlier (Sect. 3.2.1) knowledge of the biology of the target antigen, antibody, and it mechanism of action will allow better selection of an appropriate species for toxicity testing. It is customary to include a justification for the relevancy of the species selected for toxicity testing in the IND submission outlining the rationale for the selection. If safety is assessed in only one species, it is wise to provide a summary of experiments conducted that demonstrated the lack of additional relevant species.

Toxicity Study Design

The toxicity study design is determined in a number of ways by the clinical trial duration, size, scope, indication, and phase of development it is intended to support. The duration of the toxicity study should equal or exceed the duration of the clinical trial and use at least the same number or more doses of antibody than will be administered to humans. The route of administration in animals should be the same as for clinical use. Antibodies are most often administered by intravenous infusion to humans. Antibodies can be administered to nonhuman primates as a 1–2 h intravenous infusion and are usually administered to rodents as a slow intravenous (IV) bolus injection rather than an infusion. The dose schedule may be identical to the human dose schedule or the intervals between doses in animals may be decreased compared with the intervals in humans. The shorter intervals may be driven by a need to compensate for faster clearance rates of the antibody in animals or to diminish the impact of immunogenicity on exposure in the study.

A typical toxicity study has three dose levels: low, mid, and high doses of the antibody and includes a control group. The vehicle the antibody is formulated in

Table 2 Typical study design for a toxicity study of a mAb in nonhuman primates

Group	Treatment	Dose Level ($mg\,kg^{-1}$)	Dose Schedule	Animal Numbers (male/female)	
				Main	Recovery
1	Vehicle control	–	Q1 wk × 4	3M/3F	2M/2F
2	Antibody	Low dose	Q1 wk × 4	3M/3F	2M/2F
3	Antibody	Mid dose	Q1 wk × 4	3M/3F	2M/2F
4	Antibody	High dose	Q1 wk × 4	3M/3F	2M/2F

is traditionally used as the control article. Dose levels should be selected to provide information on the dose–response relationship, including a toxic dose and a NOAEL dose. Toxicity testing should be performed in both male and female animals and results should be segregated according to gender for statistical analysis purposes. Thus, the numbers of animals in toxicity studies are usually quoted as the number per sex per group. An example of a multiple-dose toxicity study design for an antibody in nonhuman primates is presented in Table 2. The number of animals per group may vary depending on the species being tested. The number of animals per group is typically larger for rodents than for nonrodent species, particularly if the nonrodent species is a nonhuman primate. The number of rodents used for general toxicity studies ranges from 10 to 15 per group in the main portion of the study plus an additional 5–10 animals per group in the recovery portion of the study. Much fewer animals per group are used for nonrodent species, ranging from 3 to 4 in the main and 2–3 in the recovery portions of the study, respectively. The number of animals used per dose level determines the probability of detecting a toxic effect and should be adequate to assess potential toxicity. If toxicokinetic analyses are included in the study, additional rodents are typically added to the study for the purpose of blood collection. The number of animals required is dependent on the number of timepoints needed. Normally, sufficient blood samples can be collected from the main and recovery animals in nonrodent species without the need for additional animals dedicated for toxicokinetic analysis.

The standard endpoints assessed in a general toxicity study are listed in Table 3. Clinical signs, body weight, and changes in food consumption can serve as general indicators that the animal is not feeling well and experiencing some type of toxicity. Laboratory measurements of hematology, serum chemistry, and urinalysis parameters, collectively known as clinical pathology, provide information about the functional status of the major organ systems like the liver, kidney, hematopoietic, and immune systems. The frequency of clinical pathology assessments varies depending on the species used for toxicity testing. The blood volumes allowable for sampling for hematology and serum chemistry are greater in larger animals and thus multiple timepoints can be evaluated in life. Anatomic pathology assessments, which include macroscopic and microscopic examination of tissues and organs, allow definitive identification of the target organs of toxicity. For a very comprehensive account of the standard practices for conducting toxicity studies we highly recommend a book chapter by Roy and Andrews (2004). The standard clinical pathology parameters

Table 3 Standard endpoints in a general toxicity study

Endpoint	Frequency of Assessment
Clinical observations (cage side)	Twice daily
Detailed clinical observations	Weekly
Body weight	Weekly
Food consumption	Daily
Ophthalmology	Baseline, once during dosing phase and during recovery if changes observed
Vital signs	Every 30 min for 4 h post dose
ECG	Baseline, once during dosing phase and during recovery if changes observed (nonrodents only)
Hematology (inc. coagulation)	Periodically in-life (nonrodents) and at termination
Serum chemistry	Periodically in-life (nonrodents) and at termination
Urinalysis	Periodically in-life (nonrodents) and at termination
Gross pathology	At termination
Organ weights	At termination
Histopathology	At termination

and anatomic pathology tissues and organs examined in toxicity studies can be obtained from any CRO and are listed in the book chapter by Roy and Andrews recommended earlier.

Single and Multiple-Dose Toxicity Studies

The decision to conduct a single-dose toxicity and/or a multiple-dose toxicity study to support the initial Phase I trial is driven by the patient population, disease indication, intended number of cycles of treatment in humans, and risk benefit relationship. The duration of animal dosing for antibodies has generally been 1–3 months for repeated dose toxicity studies to support Phase I trials. For life-threatening illnesses like cancer, shorter dosing periods or acute single-dose toxicity studies may be adequate to support a short duration Phase I trial.

4.2 Preclinical Safety Studies to Support Phase II

Repeated-dose toxicity studies of longer duration than performed for the IND may be required to support Phase II. The preclinical toxicity study duration generally should meet or exceed the duration of the planned clinical trial. For example, if the Phase I study and supporting toxicity study were 1 month in duration and the proposed Phase II study is for 3 months duration, then a subchronic toxicity study of at least 3 months duration will be required to support the Phase II trial(s). It is not uncommon for a sponsor to want to obtain initial human safety on an antibody product as quickly as possible and to choose to evaluate a shorter dosing period at the early phase of development than required for ultimate licensure and use of the

product. If the timeline allows it is obviously a better use of resources (e.g., animals and money) to conduct the 3-month subchronic toxicity study at the outset to support both the 1-month Phase I and 3-month Phase II trials with a single preclinical toxicity study.

4.3 Preclinical Studies to Support Phase III

One-month and 3-month toxicity studies conducted to support the Phase I and II trials may be adequate to support initiation of Phase III trials under certain circumstances. In general, longer duration studies are usually needed owing to the increased number of patients that will be exposed to the antibody. For antibodies intended for chronic administration, studies of 6–9 months duration are required for the Biologics License Application for marketing authorization (Table 4) and may be required to support Phase III depending on the duration of the pivotal Phase III studies.

4.4 Preclinical Safety Studies to Support Marketing

The preclinical safety studies required for the marketing approval of an antibody usually include single and repeated dose toxicity studies, local tolerance studies, reproduction and developmental toxicity studies, and safety pharmacology studies. In addition, antibodies intended for chronic administration require chronic toxicology studies and for nononcology indications may require evaluation of carcinogenic potential for approval. These types of studies and their relation to the conduct of human clinical trials are presented in the International Conference on Harmonisation (ICH) M3 guidance (Table 5). Antibodies whose targets are present on immune cells or can functionally cause immune suppression or stimulation should also be evaluated for immunotoxicity. Genotoxicity studies routinely conducted for small molecule pharmaceuticals are not applicable to antibodies and, therefore, should not be conducted for antibody products.

Table 4 Duration of multiple-dose toxicity studies required for mAb marketing

Duration of Clinical Trial	Duration of Nonclinical Study	
	Rodents	Nonrodents
Up to 2 weeks	1 month	1 month
Up to 1 month	3 months	3 months
Up to 3 months	6 months	3 months
$>$3 months	6–9 months	6–9 months

Adapted from ICH M3

Table 5 Selected guidance documents for preclinical safety evaluation of mAb

Document	Title	Web site
FDA PTC	Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use	http://www.fda.gov/cber/gdlns/ptc_mab.pdf
ICH S6	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals	http://www.fda.gov/cder/Guidance/1859fnl.pdf
ICH M3	Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals	www.fda.gov/cder/Guidance/1855fnl.pdf
21CFR58	Good Laboratory Practice for Nonclinical Laboratory Studies	http://www.access.gpo.gov/nara/cfr/waisidx_03/21cfr58_03.html
OECD GLP	Good Laboratory Practice	http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html
ICH S8	Immunotoxicity Studies for Human Pharmaceuticals	http://www.fda.gov/CBER/gdlns/ichs8immuno.htm

4.4.1 Local Tolerance

Local tolerance at the site of antibody administration should be evaluated. The formulation intended for marketing should be tested. Quite often local tolerance can be evaluated in single or repeated dose toxicity studies, thus obviating the need for separate local tolerance studies.

4.4.2 Reproduction and Developmental Toxicity Studies

The aim of reproduction toxicity studies is to reveal any effect(s) on mammalian reproduction. The combination of studies selected should allow exposure of mature adults and all stages of development from conception to sexual maturity. To allow detection of immediate and latent effects of exposure, observations should be continued through one complete life cycle, i.e., from conception in one generation through conception in the following generation. A combination of studies for effects on (1) fertility and early embryonic development, (2) prenatal and postnatal development, including maternal function, and (3) embryo–fetal development should be conducted. At the earlier phases of clinical development, repeated dose toxicity studies can provide information regarding potential effects on reproduction, particularly male fertility. Evaluation of male fertility, when appropriate, should be completed before Phase III trials.

The need for reproductive and developmental toxicity studies is dependent upon the clinical indication and intended patient population. Studies should be carried out in instances in which the antibody product is intended for repeat or chronic administration to women of childbearing potential. The specific study design and dosing schedule may be modified based on issues related to antibody species specificity,

immunogenicity, biological activity, and/or a long elimination half-life. Monoclonal antibodies with prolonged immunological effects may raise specific concerns regarding potential developmental immunotoxicity. These concerns can be addressed in a developmental toxicity study design modified to assess immune function of the neonate. Developmental immunotoxicology studies can be quite challenging depending on the species used for testing and availability or lack thereof of historical data, especially for nonhuman primates. We strongly advise consultation with experts regarding the conduct of developmental immunotoxicology studies.

4.4.3 Safety Pharmacology

Safety pharmacology studies measure functional indices of potential toxicity. The aim of safety pharmacology studies is to reveal any functional effects on the major physiological systems (e.g., cardiovascular, respiratory, renal, and central nervous systems). These functional indices may be investigated in separate studies or incorporated in the design of toxicity studies. Cardiovascular assessments such as electrocardiogram, blood pressure and heart rate, and detailed clinical observations (which may reveal effects on the central nervous system (CNS) and respiratory systems) should be included in the general toxicity studies to support the IND, in particular in nonrodent species. If a safety pharmacology signal is observed in these initial toxicity studies, then specialized studies should be conducted as a follow-up. Data from unanesthetized and unrestrained animals are preferred for in vivo safety pharmacology testing. We recommend the use of telemetry for this purpose. In telemetry studies, a transmitting device implanted into test animals continuously transmits cardiac function data to a remote receiver using radio frequency communications, and allows evaluation of cardiac function in an anesthetized and unrestrained experimental animal. Investigations may also include the use of isolated organs or other test systems not involving intact animals. All of these studies may allow for a physiology-based explanation of specific organ toxicities, which should be considered carefully with respect to human use and indication(s).

4.4.4 Carcinogenicity

Standard carcinogenicity bioassays involving the conduct of long-term carcinogenicity studies in two rodent species, typically the rat and the mouse, are usually inappropriate for antibody products. In general, carcinogenicity has not been evaluated for most of the commercial antibodies on the market today with a couple of exceptions. However, assessment of carcinogenic potential should still be considered depending upon the duration of clinical dosing, patient population, and/or biological activity of the antibody (e.g., antibodies causing immunosuppression). When there is a concern about carcinogenic potential, a variety of approaches may be considered to evaluate risk. Antibody products that have the potential to support or induce proliferation of transformed cells possibly leading to neoplasia should be

evaluated for antigen expression in various malignant and normal human cells. The ability of the antibody to stimulate growth of normal or malignant cells expressing the antigen should be determined. When in vitro data give cause for concern about carcinogenic potential, further studies in relevant animal models may be needed. Incorporation of sensitive indices of cellular proliferation in long term repeated dose toxicity studies may provide useful information.

4.4.5 Pharmacokinetics

Pharmacokinetic and toxicokinetic studies are warranted to the extent necessary to understand exposure in the safety studies conducted, to allow cross-species comparisons, and to predict margins of safety for clinical trials based on exposure. The importance of the exposure–response relationship in interpretation of the results from toxicity studies was described earlier in the chapter (Sect. 3.3). Traditional small molecule distribution and excretion studies that attempt to assess mass balance are not relevant for antibodies. However, studies of biodistribution may provide the initial evidence for inappropriate tissue targeting by an mAb or explain toxicities that are observed in animals. Interpretation of the data should consider the antibody species of origin, isotype, binding to serum proteins, route of administration, and level of antigen expression in the recipient. Even if antigen is expressed in an animal model, the mAb may bind the human target antigen and its animal counterpart with different affinities. Antibody 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 therapeutic mAb may alter biodistribution. The expected consequence of metabolism of antibodies is the degradation to individual amino acids. Therefore, classical biotransformation studies as performed for pharmaceuticals are not needed for unconjugated antibodies.

4.4.6 Immunotoxicity Studies

Toxicity to the immune system encompasses a variety of adverse effects. These include suppression or enhancement of the immune response. Suppression of the immune response can lead to decreased host resistance to infectious agents or tumor cells, whereas enhancement of the immune response can stimulate the expansion of autoreactive immune cells and lead to autoimmune disease. Parameters evaluated in standard toxicity studies can indicate signs of immunotoxicity, such as changes in total leukocyte counts (white blood cells) and absolute differential leukocyte counts, gross changes in any lymphoid tissues at necropsy, and histopathological changes of the spleen and thymus. However, with standard toxicity studies, doses near or at the maximum tolerated dose can result in changes to the immune system related to stress. These effects on the immune system are most likely mediated by increased corticosterone or cortisol release. Commonly observed stress-related

immune changes include increases in circulating neutrophils, decreases in circulating lymphocytes, decreases in thymus weight, decreases in thymic cortical cellularity and associated histopathologic changes ("starry sky" appearance), and changes in spleen and lymph node cellularity. Increases in adrenal gland weight can also be observed. In situations with clear clinical observations (e.g., decreased body weight gain, decreased activity), some or all of the changes to lymphoid tissue and hematology parameters might be attributable to stress rather than to a direct immunotoxic effect. Caution needs to be exercised when attributing changes in the immune system observed in general toxicity studies to stress rather than to immunotoxicity. The evidence of stress should be compelling.

If immunotoxiciy is suspected then additional endpoints to assess immunotoxicity need to be incorporated in subsequent general toxicity studies or specific immunotoxicity studies need to be conducted. Immunophenotyping is one of the easier endpoints to incorporate into standard toxicity studies. Immunophenotyping is the identification and/or enumeration of leukocyte subsets using antibodies. Immunophenotyping is usually conducted by flow cytometric analysis or by immunohistochemistry. Immunophenotyping is not a functional assay. Studies to assess immune functions such as T cell dependent antibody response (TDAR) have been conducted to assess immunotoxicity of mAb. TDAR plus additional functional assays are described in the ICH S8 draft guidance for immunotoxicity studies for human pharmaceuticals (Table 5). Although the S8 guidance is intended for small molecule drugs and not biologics-like antibodies, it is, nonetheless, informative and relevant portions can be applied to immunotoxicity testing of antibodies.

4.4.7 Additional Comments

We described most of the different types of safety studies earlier that might be conducted for the sake of completeness. Not all of these studies may be required for every antibody product. Indications in life threatening or serious diseases without current effective therapy may warrant a case-by-case approach to the preclinical safety evaluation where particular studies may be abbreviated, deferred, or omitted to expedite development. The studies typically conducted for antibody products based on indications that are life threatening or not, are presented in Table 6

4.5 Preclinical Studies with Payload Antibodies

In addition to the studies outlined earlier for naked or unconjugated monoclonal antibodies, other studies are required for payload antibodies (Table 6). Immunoconjugates should be tested for stability ex vivo in plasma from humans and each of the animal species used for toxicity testing. Immunoconjugate stability should

Table 6 Preclincal Safety Studies of mAbs requited based on disease indication

Study type	Indication	
	Life Threatening	Nonlife Threatening
Tissue cross-reactivity	Yes	Yes
General toxicity	Yes	Yes
Immunotoxicty	Yes	Yes
Safety Pharmacology	Yes	Yes
Chronic toxicity	No	Yes
Reproductive & developmental toxicity	Yes	Yes
Carcinogenicity	No	Yes
Genetic toxicity	No	No
Additional studies for payload Abs		
PK/TK: conjugate, Ab & free payload	Yes	NA
Plasma stability	Yes	NA
Metabolism (drug payload)	Yes	NA
Distribution	yes	NA

To date antibodies carrying payloads such as radionuclides, toxin, or cytotoxic drugs have only been developed for life-threatening oncology indications

also be assessed in vivo. Individual components of an immunoconjugate should be measured during pharmacokinetic and tissue distribution studies in animals and compared with the distribution of unconjugated antibody. The target tissues for the various components and the potential toxicities should be established. 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 and release of the payload or activity in sites that are not the result of mAb targeting. The toxicity studies should contain three dose levels of the immunoconjugate and at least one dose level of the free drug and unconjugated antibody to allow comparisons of toxicities produced by the individual components. For the unconjugated antibody and the free drug, the dose level should be the molar equivalent to the high dose of the immunoconjugate if possible. If the unconjugated free drug at the equivalent high-dose level will not be tolerated then the maximum tolerated dose (MTD) can be used. 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. 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. Studies of the immunoconjugate should be performed in a species with the relevant target antigen, whenever available, and generally in rodents if a target antigen-positive species is not available. In cases where the cytotoxic drug in the conjugate is a new chemical entity, toxicity testing in two species, rodent and non-rodent, should be considered. For immunoconjugates containing radionuclides 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 in PK and TK assessments.

4.6 Guidance Documents

Table 5 lists the recommended guidance documents that provide useful information that is relevant for planning and executing a preclinical safety evaluation package for a monoclonal antibody product. We recommend starting with the ICH S6 "Pre-clinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" document. It is the primary guidance for preclincal safety evaluation of biotechnology-derived products including monoclonal antibodies. It indicates the goals of preclincal studies, outlines principles for study design, and provides an overview of the types of safety studies that are required. The FDA issued a revised version of the "Points to Consider (PTC) in the Manufacturing and Testing of Monoclonal Antibody Products for Human Use" in 1997. The PTC has a broad scope and covers manufacture and testing, and preclinical studies and clinical studies of mAbs. Reading Section III, Preclinical Studies, of the PTC document will provide the necessary information for designing a preclinical safety evaluation program. The information provided is more detailed in some aspects that in the ICH S6 document and is focused on mAbs specifically. There is a thorough description of cross-reactivity studies of mAbs. There is also a section on preclincal studies with immunoconjugates that is helpful. The ICH M3 "Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals" guidance is similar in content to S6 with more detailed information on the recommended duration of repeated-dose toxicity studies and types of reproductive toxicity studies required in relation to the phases of clinical development. There are two documents that describe good laboratory practice for the conduct of preclincial safety studies; one issued by the FDA and the second one issued by the Organization for Economic Cooperation and Development (OECD). The FDA document is published in the Code of Federal Regulations, Title 21, Part 58 (21CFR58). It prescribes good laboratory practices that are intended to assure the quality and integrity of the preclinical safety data submitted in support of initiation of clinical trials in humans. Both documents describe the conditions and process by which studies should be performed, monitored, recorded, archived, and reported. The OECD also provides information on the organization and management of multi-site studies that is helpful. Finally, the ICH S8 "Immunotoxicity Studies for Human Pharmaceuticals" guideline provides recommendations on nonclinical testing for immunosuppression induced by low molecular weight drugs. Although the S8 guidance is intended for small molecule drugs and not biologics like antibodies, it is, nonetheless, informative and relevant portions can be applied to immunotoxicity testing of antibodies.

4.7 Antibody Toxicities

Toxicity of mAbs can result from their effector functions, the antigen and associated pathways, or mechanism of action and have been encountered in previous development programs of therapeutic antibodies. More recently the potential toxicities associated with the use of super-agonist antibodies have been illustrated. We describe here selected examples of the types of toxicities observed.

A variety of side-effects and toxicities have occurred because of binding of mAbs to antigen on tissues other than the intended target organ or tumor. Binding of Cetuximab (anti-EGFR, Erbitux®) to normal skin due to expression of the target antigen causes significant skin eruptions (Robert et al. 2001; Herbst and Langer 2002). Trials for anti-CD40L antibody were discontinued because of expression of the target on platelets, which resulted in severe thromobolytic events (Sidiropoulos and Boumpas 2004). Toxicity seen with Trastuzumab (anti-Her2, Herceptin®) is the result of binding of this antibody with low levels of target antigen expressed on heart tissue (Slamon et al. 2001). Toxicity can also result from binding to the intended target. Rapid lysis of normal and tumor B cells upon binding of Rituximab (anti-CD20, Rituxan®) results in infusion related toxicity (Byrd et al. 1999). The use of anti-CTLA-4 antibodies in clinical trials demonstrated CTLA-4 pathway related toxicity, which leads to uncontrolled general activation of T cells resulting in autoimmunity (Phan et al. 2003). Treatment of patients with Bevacizumab (anti-VEGF, Avastin®) results in multiple toxicities including hypertension, bleeding, proteinuria, and thrombosis. It is very likely that these toxicities are related to disruption of the normal functions of vascular endothelial cell growth factor (VEGF) (Hurwitz et al. 2004).

Concerns arising from target-biology related toxicities have been most recently illustrated by experiences with two therapeutic mAbs, Natalizumab (α4-intergrin, Tysabri®) and TGN1412 (αCD28) (Suntharalingam et al. 2006). Shortly after accelerated approval of Natalizumab for treatment of multiple sclerosis (MS), it was recalled from the market and clinical trials were suspended because it induced a rare fatal viral demyelinating disease, progressive multifocal leukoencepphalopathy (PML), in two patients (Scott 2005; Berger Koralnik 2005; Berger 2006a). Nataltizumab is an IgG4 mAb, which lacks significant effector function but binds to α4β1-integrin and blocks migration of lymphocytes to the various tissues and organs. Lymphocytes routinely conduct immune surveillance in the body to check for infection emerging from new pathogens or previously dormant viruses. One of the most favored hypotheses is that Natalizumab inhibited the migration and homing of lymphocytes to the CNS, which resulted in the activation of a latent polyomavirus JC in the brain that led to the development of PML (Berger 2006b). The FDA approved return of Natalizumab to the market subject to a special restricted distribution program following a comprehensive review of more than 3 000 patients that revealed a total of five cases of PML.

A second highly publicized case of target-related toxicities with mAbs was the initiation of a Phase I trial with TGN1412, a new super-agonist immune system altering antibody, which targets the CD28 antigen expressed abundantly on T cells.

The first in man administration of this super-agonist mAb in six healthy volunteers led to devastating toxicities because of massive activation of T cells. Minutes after receiving the first dose of the antibody all six healthy volunteers manifested severe systemic inflammatory responses. The response started with a release of proinflammatory cytokines, which was manifested clinically as nausea, headaches, diarrhea, hypotension, vasodilatation, and fever. The condition of the subjects became very critical in next 12–16 h and was characterized by pulmonary infiltrates, renal failure, and disseminated intravascular coagulation. Patients also had unexpected depletion of monocytes and marked lymphopenia within 24 h of the initial antibody infusion (Suntharalingam et al. 2006).

It is clear from the above examples that mAbs that are functionally immunomodulatory can alter the immune response in fundamental ways. It is, therefore, recommended that mAbs that work through effector functions such as ADCC, CDC, or ADCP or have the functional ability to induce a robust biologic response should be thoroughly evaluated in relevant in vitro and in vivo models before first in man clinical trials are initiated. Furthermore, it is necessary to understand the biology of the antigen and antibody across species, especially those aspects that cannot be fully evaluated in preclinical animal models, so that human risk assessment is made in light of the limitations of the preclinical models and appropriate starting doses are selected.

5 Summary

Although therapeutic antibody products have been with us for over two decades, the complexity and diversity of antibody products entering clinical development has greatly increased in the past few years. We have a variety of recombinant antibody products (e.g., chimeric and humanized mAb, single chain and dimeric Fvs), fully human antibodies, and a host of methods for their production. Antibodies have been engineered to enhance their effector functions and alter their half lives. It is more important than ever that appropriate preclinical safety evaluations are designed to support clinical development and ensure patient safety. Preclinical testing concerns surrounding mAb products include their effector function(s), tissue cross-reactivity, immunogenicity, and stability. The critical issues for conducting a successful preclinical safety evaluation of a monoclonal antibody product are identifying a relevant species for toxicity testing, using knowledge of the biology of the target antigen and antibody to inform the design of the studies, and interpretating of the results in terms of the exposure–response relationship. Preclinical testing schemes should parallel to the extent feasible those anticipated for clinical use with respect to dose, concentration, schedule, route, and duration. 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 scheme. It is essential that they are designed appropriately to identify key toxicities and parameters for monitoring safety in the clinic. Preclinical

studies should be conducted in step with the clinical trials to ensure that appropriate studies for assessing human risk have been conducted prior to each stage of development. It is important to be very familiar with the guidance documents for preclincal safety testing and to be knowledgeable about the biology of antibodies and target antigens through continued review of the literature and interactions with the scientific community. Finally, regular communication with the FDA or other regulatory authorities is essential.

References

Adams GP and Weiner LM (2005) Monoclonal antibody therapy of cancer. Nat Biotechnol 23:1147–1157
Berger JR (2006a) Natalizumab and progressive multifocal leucoencephalopathy. Ann Rheum Dis 65:iii48–iii53
Berger JR (2006b) Natalizumab. Drugs Today 42:639–655
Berger JR and Koralnik IJ (2005) Progressive multifocal leukoencephalopathy and natalizumab–unforeseen consequences. N Engl J Med 353:414–416
Birch JR Racher AJ (2006) Antibody production. Adv Drug Deliv Rev 58:671–85
Byrd JC et al. (1999) Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. J Clin Oncol 17: 791–795
Carson KL (2005) Flexibility–the guiding principle for antibody manufacturing. Nat Biotechnol 23:1054–1058
Carter PJ (2006) Potent antibody therapeutics by design. Nat Rev Immunol 6:343–357
Chapman K et al. (2007) Preclinical safety testing of monoclonal antibodies: the significance of species relevance. Nat Rev Drug Discov 6:120–6.
Herbst RS and Langer CJ (2002) Epidermal growth factor receptors as a target for cancer treatment: the emerging role of IMC-C225 in the treatment of lung and head and neck cancers. Semin Oncol 29:27–36
Hurwitz H et al. (2004) Bevacizumab plus irinotecan fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–2342
Hoogenboom HR (2005) Selecting and screening recombinant antibody libraries. Nat Biotechnol 23:1105–1116
Kim SJ et al. (2005) Antibody engineering for the development of therapeutic antibodies. Mol Cells 20:17–29
Kimby E (2005) Tolerability and safety of rituximab (MabThera). Cancer Treat Rev 31:456–73
Lonberg N (2005) Human antibodies from transgenic animals. Nat Biotechnol 23:1117–1125
Phan GQ et al. (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci USA 100:8372–8377
Reichert JM et al. (2005) Monoclonal antibody successes in the clinic. Nat Biotechnol 23:1073–1078
Robert F et al. (2001) Phase I study of anti-epidermal growth factor receptor antibody cetuximab in combination with radiation therapy in patients with advanced head and neck cancer. J Clin Oncol 19(13):3234–3243
Roy D and Andrews PA (2004) Nonclinical testing from theory to practice. In: Teicher BA and Andrews PA (eds), Anticancer drug development guide, 2nd edn, Humana Press, Totowa, NJ, pp 287–311
Scott CT (2005) The problem with potency. Nat Biotechnol 23:1037–1039

Siegel EB (2004) The Pre-Pre-IND FDA consultation process for preclinical studies: opportunities for launching a rapid drug or biologic development program. Preclinica 2:171

Sidiropoulos PI and Boumpas DT (2004) Lessons learned from anti-CD40L treatment in systemic lupus erythematosus patients. Lupus 13:391–397

Slamon DJ et al. (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783–792

Suntharalingam G et al. (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med 355:1018–1028