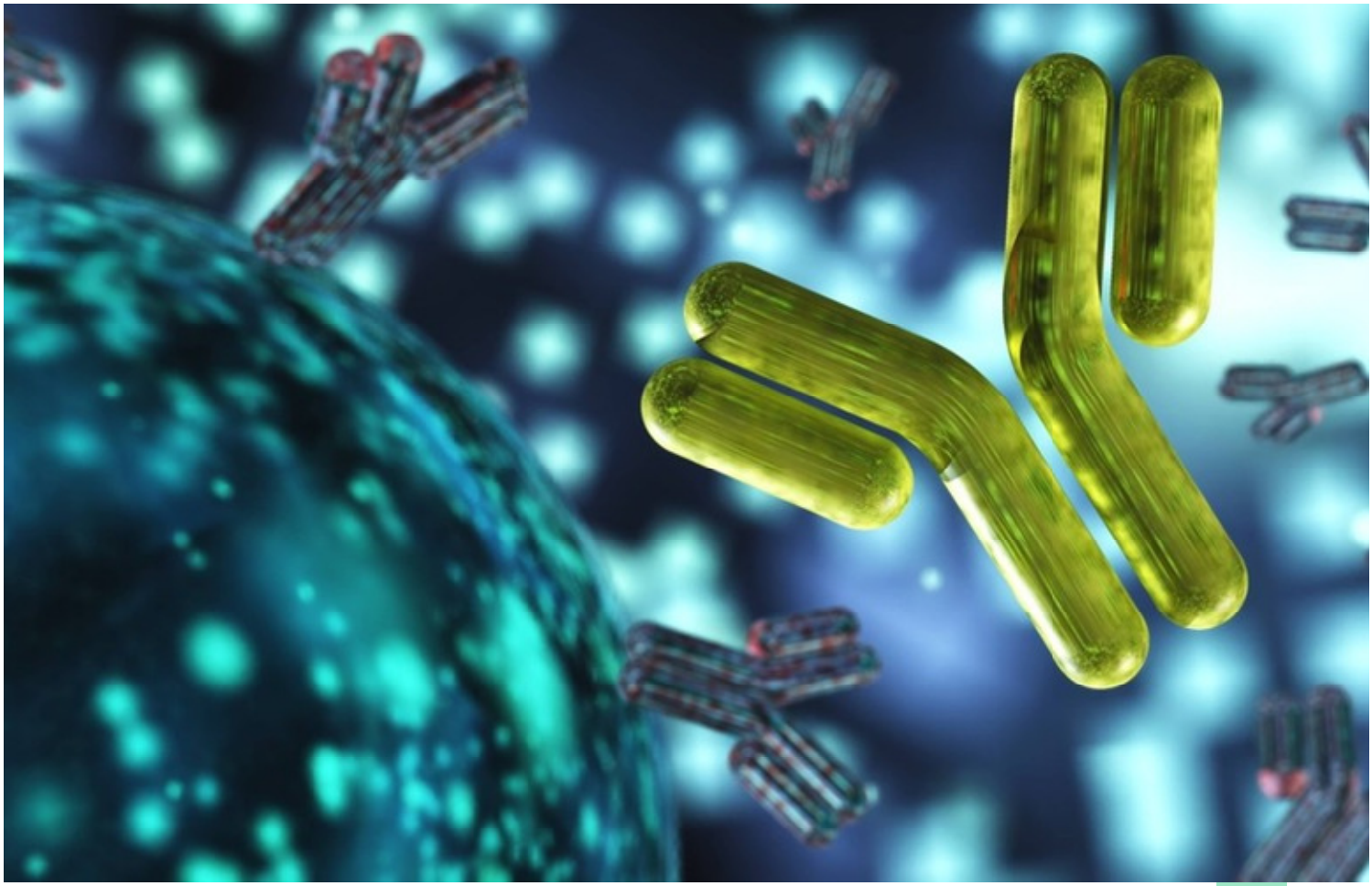

Opportunities for Nonclinical Safety Evaluation of Therapeutic Monoclonal Antibodies

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Executive Summary

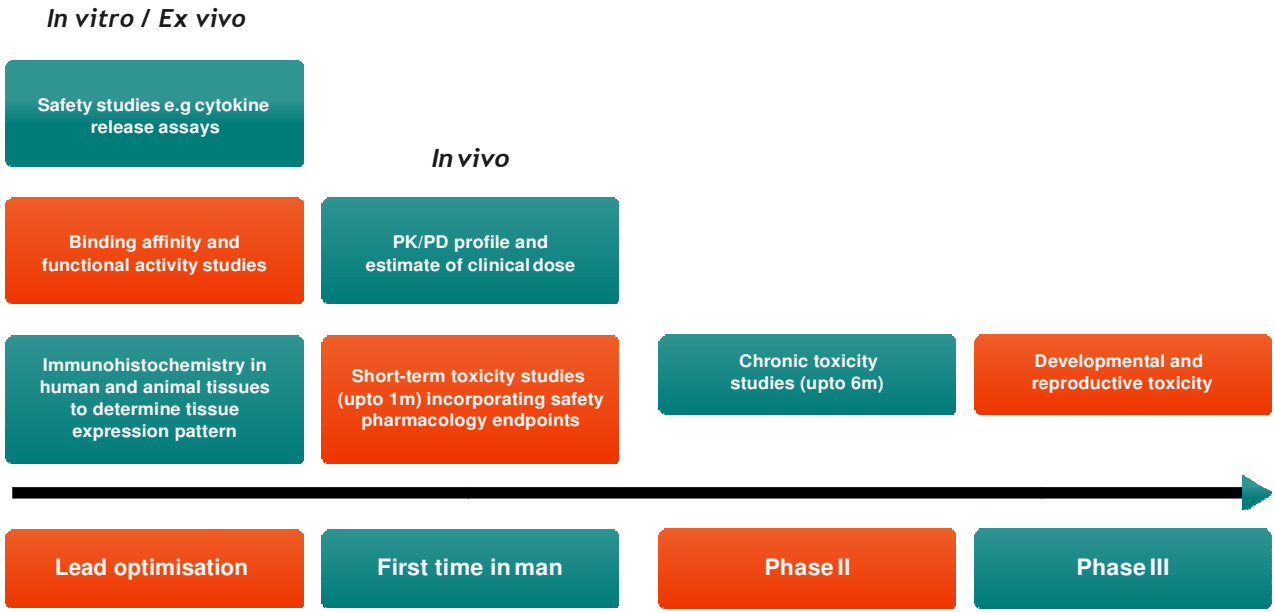
The development of monoclonal antibodies (mabs) remains high on the therapeutic agenda for the majority of pharmaceutical and bio-technology companies. Effect or functions, tissue cross-reactivity, immunogenicity and stability are major safety concerns for MAB products. Thus it is very important to design appropriate nonclinical safety studies to support clinical development and ensure patient safety. 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, identification of a relevant species for toxicity testing, knowledge of the biology of the target antigen/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. Design and implementation of these studies requires familiarity with the appropriate regulatory guidance documents, timing for the conduct of studies, 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.

Introduction

Biotherapeutic monoclonal antibodies (mAbs) are a well established class of therapeutics as evidenced by a large number of FDA approved mAbs and its market is growing rapidly. Most therapeutic mAbs in development today are chimeric or humanized to incorporate more human characteristics aimed at reducing immunogenicity and enhancing interaction with human effect or cells. In addition to favourably altering binding affinities, customized mAbs that have enhanced effector function e.g., antibody dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are also gaining attraction.

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. Due to the species specificity of many products, selection of animal species in which the mAb binds and exhibit desired pharmacological effect is of prime importance. However, there are ohen fundamental differences between animal and human physiology, and consequently there are ohen still deficiencies in the translational phase. The overall goal of nonclinical studies for mAbs is to define the toxicological properties of the mAb and provide information for product development. The studies can be categorized to allow determination of the optimum timing of nonclinical studies required prior to the submission for US Biologics License Application (BLA) or European Marketing Authorization Application (MAA)



NON-CLINICAL DEVELOPMENT PROGRAM

Non-clinical safety evaluation plays an essential part in the overall development of mAbs. During the nonclinical and clinical development stage, strategic planning of the toxicity testing program for a given mAb is a key for developers and producers to achieve clinical trials or marketing approval in a timely manner. 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.

Based on the sponsor's drah product label and development strategy, Vimta's team develops 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 studies. Typically, nonclinical safety studies based on disease indication are:

Life-threatening	Non-life threatening	
Tissue cross-reactivity	Tissue cross-reactivity	Immunotoxicity
General toxicity	General toxicity	Reproductive & developmental toxicity
Safety Pharmacology	Safety Pharmacology	Carcinogenicity
Chronic toxicity	Chronic toxicity	

All nonclinical safety studies intended to support human clinical trials are conducted in compliance with Good Laboratory Practice (GLP). This includes bioanalytical assay for quantification of therapeutic antibody concentrations in serum for pharmacokinetic (PK) purposes or anti-therapeutic antibodies for immunogenicity assessment. In many cases, stand-alone safety pharmacology, local tolerance studies are not necessary. These types of studies can be incorporated into single-dose or repeat-dose toxicity studies, in order to reduce animal use. The decision to conduct a reproduction toxicity study in animal models is made on a case-by-case basis and depends on the target population and the intended clinical use of the product. Developmental toxicity studies are usually conducted when the product is indicated for a population that includes women of child bearing potential.

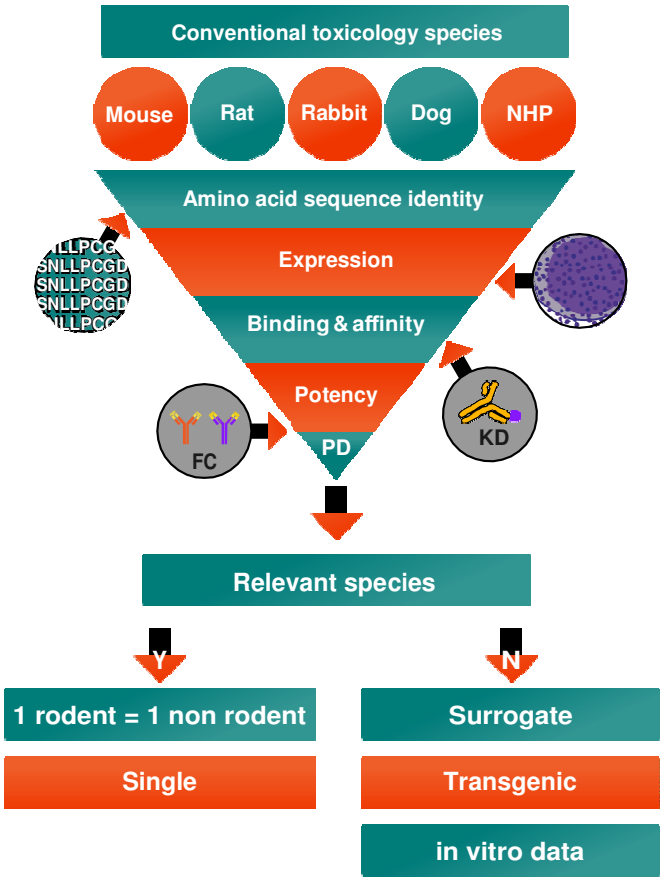
PRACTICAL CONSIDERATIONS FOR NON-CLINICAL STUDIES

The nonclinical evaluations of mAbs are designed using in vitro and in vivo test system to determine and understand the pharmacological properties of the antibody and achieve the goals for identification of target organs of toxicity, evaluation of potential reversibility of toxicity, determination of a safe starting dose for human Phase I clinical trials and subsequent dose escalation schemes along with providing information to monitor safety parameters in the clinical trials and support claims on the product label using safety data. The important factors for designing nonclinical studies include:

- 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

The studies and their timing are influenced by the chemistry, manufacturing and controls (CMC) development or clinical trial strategy, and any change in developmental aspects may lead to repetition of toxicity studies i.e., 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 and would attract unnecessary duplication of effort and resources. Similarly, process change in the manufacture of the antibody ohen necessitates comparability using invitro analytical methods followed by a bridging toxicity study or an entirely new toxicity study. A sufficient supply of the early phase material is considered essential for the success of the bridging study.

Using a 'non-relevant' species can confound or potentially delay translation into the clinic by providing information which can be scientifically misleading or offer no value in assessing risk to humans. Hence the primary consideration in designing safety studies for mAbs is the selection of a pharmacologically relevant species. Species differences in target affinity, expression pattern, mechanism of action, pharmacological activity and immunogenicity, support translation of preclinical findings to human. A thorough understanding of species differences at the outset is essential for designing appropriate studies using a relevant species and subsequent interpretation of results. In order to determine species relevance, a tiered approach is recommended, as illustrated by the species selection 'funnel' in Figure 1.



Conventional toxicology species such as the mouse, rat, rabbit and dog, are ohen unsuitable for non-clinical studies with mAbs for two main reasons: the mAb may not be pharmacologically active in these species; and immunogenicity may limit exposure aher repeated dosing. While there is a significant amount of data to show that immunogenicity in the nonclinical species does not predict for immunogenicity in humans, antidrug antibodies can mediate enhanced clearance or neutralisation of the therapeutic mAb, or even lead to adverse effects due to immune complex deposition and consequent inflammatory responses. Therefore, the validity of the non-clinical safety assessment may be compromised if a mAb is immunogenic in the nonclinical species. A single species may be sufficient, if it can be shown by means of kinetic, pharmacological and toxicological data that the species selected is a relevant model for the human. In the event of relevance of rodent, use of non rodent species can be reduced by using rodents for some aspects of hazard detection or for making go/no go decisions. However, in the event of a lack of relevant animal models, two acceptable options are recommended.

In some cases, despite considerable effort to identify pharmacologically relevant species, none are identified due to insufficient homology of the target across species, lack of expression of the target in naive, healthy animals, or complete absence of the target and/or the target pathway in species other than humans. When no pharmacologically relevant species can be identified, three options remain for non-clinical safety assessment:

- Testing the mAb in a transgenic mouse that expresses the human target

- Testing a surrogate mAb or homologue directed against the non-clinical orthologue; unless there is a robust scientific justification for using a non-rodent species, a rodent species should be used.

- Testing the mAb in an array of in vitro assays relevant for safety evaluation, for example using human primary cells and/or immortalised cell lines. In vitro dose-response data can then be integrated with projected clinical exposures to set first time in human doses

The alternative path taken should be based on the target, the mechanism of action of the mAb, and technical feasibility. It is important to note that all of the above alternatives for determining non-clinical safety have their own drawbacks i.e., a surrogate mAb must be extensively characterised and compared to the clinical candidate (binding epitope, isotype, potency in vitro etc.) and produced to similarly high manufacturing standards as the clinical candidate. At the same time, from a regulatory perspective, non-clinical safety studies with surrogates are considered for hazard identification only, as the surrogate is not the clinical candidate. Similarly, a transgenic mouse model must be extensively characterised to demonstrate functionality of the transgene. Furthermore, transgenic models are associated with a considerable immunogenicity risk, and may not be suitable for targets that interact with multiple ligands or accessory proteins (as this could potentially require multiple transgenes and become too challenging technically). A solely in vitro assay-based approach to safety testing precludes evaluation of risk due to chronic exposure or reproductive toxicity and thus may not be a viable option for non-life-threatening indications. In addition, there will be little data to support a starting dose other than that based on the minimal anticipated biological effect level (MABEL) which can greatly extend the duration of the first-time-in-human clinical trial. The value of the target and the risk:benefit ratio in the proposed patient population should therefore be carefully considered embarking on an alternative route, and early consultation with regulatory agencies on the path chosen is essential for any of the alternative approaches to be successful.

Toxicity testing is performed in both male and female animals and the number of animals per group may vary depending on the species being tested. Typically, 10-15 rodents per sex group in the main study plus an additional 5-10 animal 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. For TK evaluations, the number of time points desired determines the total number of animals. Normally a satellite group is included for rodent study. 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. In the absence of a PK study, collection of blood samples aher 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 aher each dose will, at the least, provide maximum (Cmax) and minimum (Cmin) serum concentration values. An increase in the Cmin values over time will indicate dose accumulation.

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. The duration of the toxicity study should equal or exceed the duration of the clinical trial and use at least the same route and number of antibody doses that will be administered to humans. A general toxicity study incorporates various measurements that include multiple end points such as clinical signs,body weight and changes in food consumption which could serve as general indicators, if the animal is experiencing some type of toxicity. Clinical pathology measures such as hematology, serum chemistry and urinalysis parameters offers 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. Anatomic pathology assessments, which include macroscopic and microscopic examination of tissues and organs, are used to identify the target organs of toxicity. If evidence for immunotoxicity is uncovered in the initial toxicity studies, then specific endpoints are 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. Since immunophenotyping is not a functional assay, other parameters such as the T cell-dependent antibody response are measured to assess mAb immunotoxicity.

The pharmacological effects of the antibody may last well beyond initial dosing and may result in the exposure response relationship rather than the dose-response relationship therefore design and interpretation of toxicity studies and a recovery period require special consideration. Despite cessation of antibody administration during the recovery period, five half-lives must elapse before almost the entire 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.

Summary

A relevant species is one in which the antibody is pharmacologically active, the target antigen is present or expressed and tissue cross-reactivity profile is 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 immuno-histochemical 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 immune-histochemical 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.

Over a period time Vimta team has built considerable understanding on 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 in accordance to global guidance documents describing preclinical safety testing. Vimta's non-clinical safety studies program includes design to identify potential toxicities covering anticipated dose, concentration, schedule, route and duration to be used in clinical studies. The methodology can not only help to minimise risk when it comes to the regulatory scrutiny of new candidate, but can contribute considerable savings in both time and cost for the developer.

REFERENCE

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- ICH S7A: Safety pharmacology studies for human pharmaceuticals
- ICH S5a: Note for guidance on the detection of toxicity to reproduction for medicinal products including toxicity to male fertility (CPMP/ICH/386/95)
- ICH M3: Nonclinical Safety studies for the conduct of human clinical trial for pharmaceuticals
- ICH M4: Organisation of the common technical document for the registration of Pharmaceuticals for human use

About Vimta

Vimta is a proficient preclinical partner facilitating integration of multiple scientific disciplines and evaluating relationships of dose, exposure, safety, and efficacy. Vimta provides critical preclinical research data and innovative solutions through conduct of right studies at the right time by experienced professionals using proven experimental design, and state-of-the-art facilities with advanced equipment. All work is at its core scientific and operational excellence. Our end-to-end connected infrastructure, multidisciplinary team and global quality compliance enables complete value-chain integration, remains a key driver of our growth in the diverse and differentiated arenas for medical devices, pharmaceuticals, vaccines, biologics, and combination products, encompassing the entire pharmaceutical spectrum. We are an organization focused on research activities to help bring new technologies/procedures to help clients for developing molecules.

Partnering with Vimta will position you better for success, allowing timely delivery of your products to the marketplace.