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# GENERIC PRECLINICAL DEVELOPMENT PLAN FOR HUMAN MONOCLONAL ANTIBODIES


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# SUMMARY

This document is an update to the Generic Monoclonal Antibody Product Development Plan first prepared by SRI in July of 2010. The general process for development of small molecule drugs and biologics (i.e., therapeutic proteins and mAbs) is similar among most agents across a broad range of therapeutic uses. While specific differences exist, and results of preliminary testing may trigger additional investigations, it is useful to understand the general process for drug development. This Preclinical Development Plan (PDP) outlines the steps required to move a new monoclonal antibody (mAb) through the steps needed to file an Investigational New Drug (IND) application and begin human clinical trials. Although this PDP provides a path to file an IND application, it is intended to be a generic general plan and each mAb candidate will have its own special challenges that require the need for individual evaluation to meet regulatory requirements for initiation of clinical trials. This plan is intended to guide investigators, but cannot replace the development of a comprehensive, product-specific plan, combined with pre-IND or other meetings with the U.S. Food and Drug Administration (FDA).

Even when a mAb candidate product is still in the early research stages, the recommended strategy is to develop a Target Product Profile (TPP) for it and to outline the key studies to enable submission of an IND application, along with appropriate go/no go decision points. Key issues for anti-infective products must be considered separately. The development process for anti-infective drug and biological products is covered by several guidelines provided by the FDA and the International Conference on Harmonisation (ICH). One of the first monoclonal guidance documents, *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use,* was issued by the FDA in 1994 and updated in 1997 for consistency with ICH documents dealing with this category of products. Processes for engineering human monoclonal antibodies have greatly improved over time, but many aspects of the older guidance documents are still applicable and should be considered during the development process for a mAb product.

The terms “antibody”, “monoclonal antibody” and “biologics” in the Points to Consider refer to intact immunoglobulins as those produced by hybridomas, and, as appropriate, immunoconjugates, immunoglobulin fragments and recombinant proteins derived from immunoglobulins, such as chimeric and humanized immunoglobulins, $F(ab')$ and $F(ab')_2$ fragments, recombinant immunoglobulin variable regions (Fvs), single-chain antibodies (also called nanobodies), and so on. Two or more mAbs administered at a fixed ratio are defined as “cocktails,” and their relevant targets may include multiple antigens of infectious pathogens or multiple binding sides (epitopes) on the same target. The guidance document states that the rationale for combining the products should be clear, the lack of interference among the mAbs in combination should be demonstrated, and the synergistic or additive effects should be characterized. Presentation of results of dose range-finding studies for each mAb component of the cocktail is desirable, but not necessarily required by the FDA.

Nonclinical studies that SRI recommends for inclusion in the IND are described below and are included as part of the IND submission of a mAb product before the initiation of Phase I clinical studies. These proceed in several stages with some progression overlaps for lead mAb(s) that had been identified and selected for development based on the results of *in-vitro* and *in-vivo* studies.

**STAGE 1**

- Establishment of a well-characterized Master Cell Bank for production of the mAb
- Manufacturing and control development of bulk mAb (active pharmaceutical ingredient) pilot lots
- Performance of pre-formulation / formulation studies to identify a probable clinical formulation
- Performance of efficacy studies to confirm pharmacological activity

**STAGE 2**

- Completion of pharmacokinetic (PK), immunogenicity, and range-finding toxicity studies using the pilot batch material produced with the manufacturing procedure established in Stage 1
- Pharmacokinetic/pharmacodynamic (PK/PD) modeling, if appropriate
- Performance of a tissue cross-reactivity study in appropriate species, including human tissues
- Performance of Mechanism of Action (MOA) studies
- Develop criteria for release (specifications)
- Development and validation of analytical methods
- Preparation of a pre-IND meeting request and information for pre-IND submission to FDA
- Address FDA feedback from the pre-IND meeting

**STAGE 3**

- Good Manufacturing Practices (GMP) production of bulk mAb and final drug product for Phase I clinical trial
- Completion of single- or repeat-dose toxicity studies (consistent with proposed clinical use) in one or two species, in compliance with FDA Good Laboratory Practices (GLP)
- Shelf stability studies of bulk mAb and drug product supplies to match the duration of ongoing clinical studies and to establish expiration dating
- Preparation and submission of an IND to the FDA.

SRI generally recommends that work proceed in the above three stages, with a go/no-go decision at the end of each stage. There will naturally be some overlap between stages as one study completes and another is in planning, but the breakdown above provides a general approach to evaluating the different portions of the development program and how they are arranged. The first stage consists of studies to demonstrate animal efficacy, the second consists of PK/range-finding studies, and the third consists of the full set of GLP and GMP studies. required to move the product to a successful IND application. Product development beyond Stage 3 is not included in this development plan. Typically, Stage 4 includes human clinical trials and advanced product manufacturing leading to registration of the product. Stage 4 includes activities geared towards further developing and finalizing the process, formulation, and analytical methods for both the pivotal clinical study stage and commercial launch of the final drug product. At this stage, the project teams initiate efforts to define the strategies for process validation and BLA submission. The BLA submission is a request for permission to market a biologic product and includes product/manufacturing information, pre-clinical studies, clinical studies, and proposed labeling. This is the final regulatory approval step before the biologic product can be marketed in the US (commercial launch).

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# INTRODUCTION

Drug and biological candidates are typically in the research stage of the drug discovery and development process when the National Institute of Allergy and Infectious Disease Division of Microbiology and Infectious Diseases (NIAID-DMID) requests a Product Development Plan (PDP) and a Target Product Profile, along with an outline of the key Investigational New Drug (IND)-enabling studies with go/no-go decision points. This PDP outlines the IND-enabling studies for the development of a single mAb or mAb cocktail (two or more antibodies) used either for treatment or postexposure prophylaxis.

The sections that follow outline the key steps required to bring a lead candidate to Phase I clinical trials. These sections include an outline of the specific steps required for completion of an IND application to the FDA. The approach and studies described for completion of an IND are recommendations and there may be other potential alternatives to a successful IND. The approach should be revisited as results become available.

## OVERVIEW OF THE TRANSITION FROM DISCOVERY TO DEVELOPMENT

Each drug candidate follows its own discovery and development path, yet every path has certain common characteristics, particularly as the candidate progresses through clearly prescribed regulatory steps for entry into and successful completion of human clinical testing. The major steps in this drug development process, and the requirements to successfully move a new therapeutic biologic through each stage of discovery and development are depicted in Figure 1.

The drug development process is typically divided into three major stages: discovery and lead optimization, nonclinical/preclinical development, and clinical development. In practice, there is significant overlap between these stages, with development work being initiated during lead optimization, and significant nonclinical work continuing after initiation of clinical trials. The IND is required for initiation of the first-in-human (FIH) clinical studies, but it is also the first step in the clinical development process, and the IND document will evolve over time as clinical research progresses, eventually leading to a Biologics License Application (BLA). Completion of these studies to demonstrate safety and efficacy in both animal models and humans and filing of the BLA are the final steps before market introduction. Although the details involved in each stage depend on the type of pharmaceutical/biological product, the general development process is similar for all drugs, including biologics.

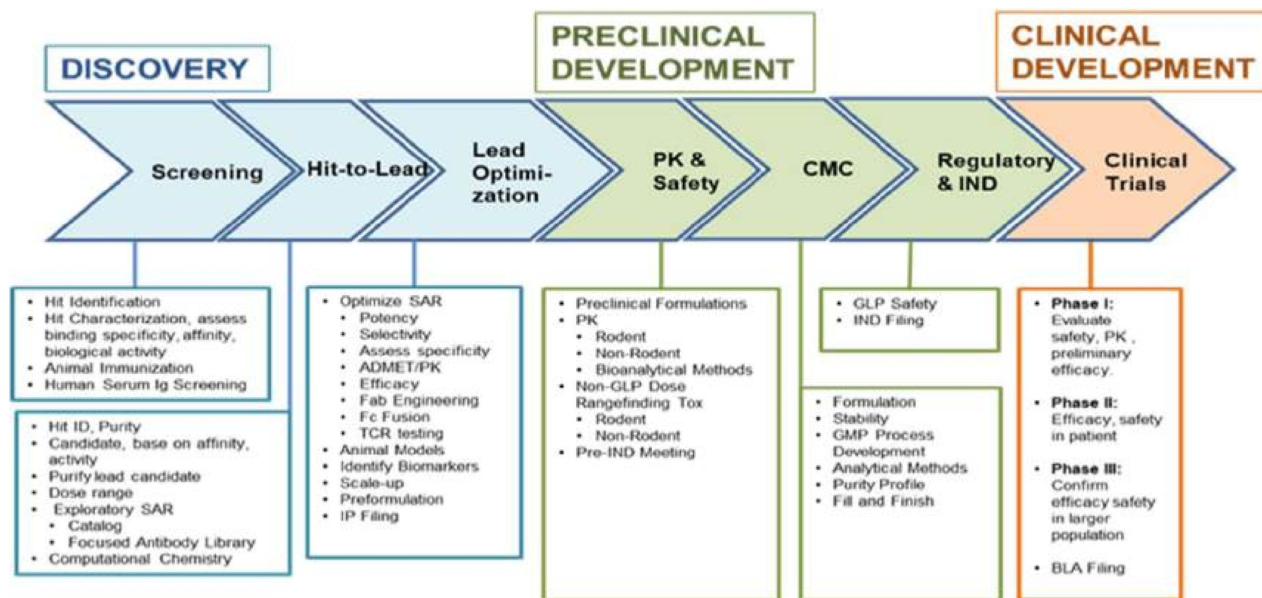



**Figure 1. The drug discovery and development process.**

The FDA provides guidance for designing and conducting both preclinical and clinical development of various classes of agents. These documents are available on the FDA web site, at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

The link above contains a large number of guidance documents (1,053 entries just under "drugs" alone) that apply to all pharmaceutical products, but there are additional documents related specifically to development of anti-microbial and anti-viral products. It is best to search for specific topics relevant to the indication of interest, but a small example list of available documents is presented in Table 1.

| TABLE 1.<br>EXAMPLES OF INFECTIOUS DISEASE RELATED, PRODUCT SPECIFIC<br>FDA GUIDANCE DOCUMENTS |
|---|
| Acute Bacterial Sinusitis — Developing Antimicrobial Drugs for Treatment |
| Anthrax: Developing Drugs for Prophylaxis of Inhalational Anthrax Guidance for Industry |
| Antiviral Product Development--Conducting and Submitting Virology Studies to the Agency |
| Antiviral Product Development--Conducting and Submitting Virology Studies to the Agency : Guidance for Submitting Influenza Resistance Data |
| Antiviral Product Development--Conducting and Submitting Virology Studies to the Agency: Guidance for Submitting HCV Resistance Data |
| Bacterial Vaginosis: Developing Drugs for Treatment Guidance for Industry |
| Chronic Hepatitis B Virus Infection: Developing Drugs for Treatment |
| Chronic Hepatitis C Virus Infection: Developing Direct-Acting Antiviral Drugs for Treatment Guidance for Industry |
| Chronic Hepatitis D Virus Infection: Developing Drugs for Treatment Guidance for Industry |
| Complicated Urinary Tract Infections: Developing Drugs for Treatment |

| **TABLE 1.<br>EXAMPLES OF INFECTIOUS DISEASE RELATED, PRODUCT SPECIFIC FDA GUIDANCE DOCUMENTS** |
|---|
| Cytomegalovirus in Transplantation: Developing Drugs to Treat or Prevent Disease |
| Guidance for Industry Acute Bacterial Sinusitis: Developing Drugs for Treatment |
| Hospital-Acquired Bacterial Pneumonia and Ventilator-Associated Bacterial Pneumonia: Developing Drugs for Treatment |
| Influenza: Developing Drugs for Treatment and/or Prophylaxis |
| Microbiological Data for Systemic Antibacterial Drug Products — Development, Analysis, and Presentation |
| Respiratory Syncytial Virus Infection: Developing Antiviral Drugs for Prophylaxis and Treatment Guidance for Industry |
| Smallpox (Variola Virus) Infection: Developing Drugs for Treatment or Prevention Guidance for Industry |
| Uncomplicated Urinary Tract Infections: Developing Drugs for Treatment Guidance for Industry |
| Vaginal Microbicides: Development for the Prevention of HIV Infection PDF |

In addition to these infectious disease specific guidance documents, FDA, European Medicines Agency (EMA), the World Health Organization (WHO) and the International Conferences on Harmonization (ICH) have released a number of guidance documents specifically related to mAbs, and some are specifically for mAbs directed at SARS-CoV-2 (COVID). Table 2 provides a summary of these documents.

| **TABLE 2.<br>INFECTIOUS DISEASE RELATED, MONOCLONAL ANTIBODY SPECIFIC REGULATORY GUIDANCE DOCUMENTS** |
|---|
| FDA: Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (February, 1997) |
| FDA: Development of Monoclonal Antibody Products Targeting SARS-CoV-2 for Emergency Use Authorization (December, 2023) |
| FDA: Monoclonal Antibodies Used as Reagents in Drug Manufacturing (March, 2001) |
| FDA: Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens (March, 2023) |
| FDA: Bispecific Antibody Development Programs (May 2021) |
| ICH: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1) (June, 2011) |
| EMA: Guideline on Development, Production, Characterization and Specification for Monoclonal Antibodies and Related Products (July, 2016) |
| WHO: Guidelines for the Production and Quality Control of Monoclonal Antibodies and Related Products Intended for Medicinal Use (April, 2022) |
| FDA: Product Development Under the Animal Rule (October 2015) |
| FDA: Rabies: Developing Monoclonal Antibody Cocktails for the Passive Immunization Component of Post-Exposure Prophylaxis (July 2021) |
| FDA: Nonclinical Safety Evaluation of Drug or Biologic Combinations (March 2006) |

The research stage of identifying potential new therapeutics or therapeutic classes typically includes basic biological research into disease mechanisms, with the goal of identifying suitable drug targets (e.g., SARS-CoV-2 spike protein, Gram+ bacterial cell wall, viral reverse transcriptase). Once a target has been identified, a high throughput screen is often developed to speed the process of identifying potential modulators of target activity. Random screening of large libraries of molecules derived from combinatorial chemistry or natural products can identify "hits," which are then further optimized by an iterative process of compound synthesis and *in vitro* and *in vivo* testing until a lead candidate is selected. Alternatively, if a natural ligand for the target is identified through basic research, confirmatory efficacy studies in a variety of animal model systems are often used at this stage of the process to delineate the potential applications and risk factors of the lead compound. These efficacy studies are usually reported as part of the IND application. A decision point is often established to determine whether a lead candidate meets predetermined criteria (typically demonstration of efficacy) and the program progresses into the preclinical development phase.

The next steps for developing the drug will be further discussed in this document. Repurposing of approved drugs for a different indication/disease may be able to skip particular steps such as safety testing and pharmacokinetics if the dosing regimen and route are unchanged from that of the approved drug.

## TECHNOLOGY READINESS LEVELS

Technology Readiness Level (TRL) categorization is a mechanism for assessing the stage of maturity of various technologies. Originally conceived by NASA in 1974 for space-related engineering programs, its use has expanded to other areas, including the development of medical products. There are a variety of different presentation of TRL levels related to biomedical product development that can be found at these links:

https://acqnotes.com/acqnote/tasks/technology-readiness-level
https://www.medicalcountermeasures.gov/trl/integrated-trls/.

While TRLs are popular within some Department of Defense programs and BARDA, they are not widely used by either the FDA or the pharmaceutical industry, and therefore may be unfamiliar to small companies, academic researchers, and other private organizations engaged in the drug development process.

Table 3 is provided as a summary of TRL levels because this may be useful for Investigators pursuing funding opportunities with organizations other than the NIH.

| TABLE 3.<br>TECHNOLOGY READINESS LEVELS[1] | | |
|---|---|---|
| **TRL #** | **Description** | **Development Stage[2]** |
| **TRL 1** | **Review of Scientific Knowledge Base**<br>Active monitoring of scientific knowledge base. Scientific findings are reviewed and assessed as a foundation for characterizing new technologies. | **Target Discovery** |
| **TRL 2** | **Development of Hypotheses and Experimental Designs**<br>Scientific "paper studies" to generate research ideas, hypotheses, and experimental designs for addressing the related scientific issues. Focus on practical applications based on basic principles observed. Use of computer simulation or other virtual platforms to test hypotheses. | **Assay Development** |
| **TRL 3** | **Target/Candidate Identification and Characterization of Preliminary Candidate(s)**<br>Begin research, data collection, and analysis in order to test hypotheses. Explore alternative concepts, identify and evaluate critical technologies and components, and begin characterization of candidate(s). Preliminary efficacy demonstrated *in vivo*.<br>**3A** Identify target and/or candidate.<br>**3B** Demonstrate *in vitro* activity of candidate(s) to counteract the effects of the threat agent.<br>**3C** Generate preliminary *in vivo* proof-of-concept efficacy data (non-GLP). | **Screening Hit Confirmation** |
| **TRL 4** | **Candidate Optimization and Non-GLP *In Vivo* Demonstration of Activity and Efficacy**<br>Integration of critical technologies for candidate development. Initiation of animal model development. Non-GLP *in vivo* toxicity and efficacy demonstration in accordance with the product's intended use. Initiation of experiments to identify markers, correlates of protection, assays, and endpoints for further non-clinical and clinical studies. Determination of immunogenicity.<br>**Animal Models:** Initiate development of appropriate and relevant animal model(s) for the desired indications.<br>**Assays**: Initiate development of appropriate and relevant assays and associated reagents for the desired indications, including development of a Master Cell Bank.<br>**Manufacturing:** Manufacture laboratory-scale (i.e., non-GMP quantities of bulk product and proposed formulated product.<br>**4A** Demonstrate non-GLP *in vivo* activity and potential for efficacy consistent with the product's intended use (i.e., dose, schedule, duration, route of administration, and route of threat agent challenge). | **Lead Optimization** |

| TABLE 3. TECHNOLOGY READINESS LEVELS[1] | | |
|---|---|---|
| **TRL #** | **Description** | **Development Stage[2]** |
| | **4B** Conduct initial non-GLP toxicity studies and determine pharmacodynamics (PD) and pharmacokinetics (PK) and/or immune response in appropriate animal models (as applicable), including a tissue cross reactivity assay in human and other relevant species.<br>**4C** Initiate experiments to determine assays, parameters, surrogate markers, correlates of protection, and endpoints to be used during non-clinical and clinical studies to further evaluate and characterize candidate(s). | |
| **TRL 5** | **Advanced Characterization of Candidate and Initiation of GMP Process Development**<br>Continue non-GLP *in vivo* studies, and animal model and assay development. Establish draft Target Product Profiles (TPPs). Develop a scalable and reproducible manufacturing process amenable to GMP.<br>**Animal Models:** Continue development of animal models for efficacy and dose-ranging studies.<br>**Assays:** Initiate development of in-process assays and analytical methods for product characterization and release, including assessments of potency, purity, identity, strength, sterility, and quality as appropriate.<br>**Manufacturing:** Initiate process development for small-scale manufacturing amenable to GMP.<br>**Target Product Profile:** Draft preliminary TPP. Questions of shelf life, storage conditions, and packaging should be considered to ensure that anticipated use of the product is consistent with the intended use for which approval will be sought from FDA.<br>**5A** Demonstrate acceptable Absorption, Distribution, Metabolism and Excretion (ADME) characteristics and/or immune responses in non-GLP animal studies as necessary for Investigational New Drug (IND) filing.<br>**5B** Continue establishing correlates of protection, endpoints, and/or surrogate markers for efficacy for use in future GLP studies in animal models. Identify minimally effective dose to facilitate determination of "humanized" dose once clinical data are obtained. | **IND Enabling Studies**<br>**CMC** |
| **TRL 6** | **GMP Pilot Lot Production, IND Submission, and Phase 1 Clinical Trial(s)**<br>Manufacture GMP-compliant pilot lots. Prepare and submit IND package to FDA and conduct Phase 1 clinical trial(s) to determine the safety and PK of the clinical test article.<br>**Animal Models:** Continue animal model development via toxicology, pharmacology, and immunogenicity studies. | **CMC**<br>**Regulatory & IND Submission** |

| TABLE 3. TECHNOLOGY READINESS LEVELS[1] | | |
|---|---|---|
| **TRL #** | **Description** | **Development Stage[2]** |
| | **Assays:** Qualify assays for manufacturing quality control and immunogenicity, if applicable.<br>**Manufacturing:** Manufacture, release, and conduct stability testing of GMP-compliant bulk and formulated product in support of the IND and clinical trial(s).<br>**Target Product Profile:** Update TPP as appropriate.<br>**6A** Conduct GLP non-clinical studies for toxicology, pharmacology, and immunogenicity as appropriate.<br>**6B** Prepare and submit full IND package to FDA to support initial clinical trial(s).<br>**6C** Complete Phase 1 clinical trial(s) to establish an initial safety, PK, and immunogenicity assessment as appropriate. | |
| **TRL 7** | **Scale-up, Initiation of GMP Process Validation, and Phase 2 Clinical Trial(s)**<br>Scale-up and initiate validation of GMP manufacturing process. Conduct animal efficacy studies as appropriate. Conduct Phase 2 clinical trial(s).[3]<br>**Animal Models:** Refine animal model development in preparation for pivotal GLP animal efficacy studies.<br>**Assays:** Validate assays for manufacturing quality control and immunogenicity if applicable.<br>**Manufacturing:** Scale-up and validate GMP manufacturing process at a scale compatible with U.S. government requirements. Begin stability studies of the GMP product in a formulation, dosage form, and container consistent with the TPP. Initiate manufacturing process validation and consistency lot production.<br>**Target Product Profile:** Update TPP as appropriate.<br>**7A** Conduct GLP animal efficacy studies as appropriate for the product at this stage.<br>**7B** Complete expanded clinical safety trials as appropriate for the product (e.g., Phase 2).[2] | **Clinical Research** |
| **TRL 8** | **Completion of GMP Validation and Consistency Lot Manufacturing, Pivotal Animal Efficacy Studies or Clinical Trials[3], and FDA Approval or Licensure**<br>Finalize GMP manufacturing process. Complete pivotal animal efficacy studies or clinical trials (e.g., Phase 3), and/or expanded clinical safety trials as appropriate. Prepare and submit BLA.<br>**Manufacturing:** Complete validation and manufacturing of consistency lots at a scale compatible with U.S. government | **NDA/BLA Market Approval** |

**TABLE 3.**
**TECHNOLOGY READINESS LEVELS[1]**

| TRL # | Description | Development Stage[2] |
|---|---|---|
| | requirements. Complete stability studies in support of label expiry dating.<br>**Target Product Profile:** Finalize TPP in preparation for FDA approval.<br>**8A** Complete pivotal GLP animal efficacy studies or pivotal clinical trials (e.g., Phase 3), and any additional expanded clinical safety trials as appropriate for the product.<br>**8B** Prepare and submit BLA to the FDA.<br>**8C** Obtain FDA approval or licensure. | |
| **TRL 9** | **Post-Licensure and Post-Approval Activities**<br>**9A** Commence post-licensure/post-approval and Phase 4 studies (post-marketing commitments), such as safety surveillance, studies to support use in special populations, and clinical trials to confirm safety and efficacy as feasible and appropriate.<br>**9B** Maintain manufacturing capability as appropriate. | **Post-Market Surveillance** |

1 This table does not serve as official FDA Guidance, nor does it represent FDA's current thinking on this topic. For the purposes of a regulatory application seeking licensure or approval for a specific medical product, additional data may be required by FDA.
2 See Figure 1 above for relevant Development Stage.
3 Identification of later regulatory stages of clinical development in this table (e.g., Phase 2, Phase 3) may not apply to some products being developed under the "Animal Rule." In this situation, other than human safety studies, no additional clinical data may be feasible or ethical to obtain. See Animal Rule discussion later in this document.

## TARGET PRODUCT PROFILES

A Target Product Profile (TPP) is a planning tool for drug candidates that provides an organized list of key components of a potential product profile with agreed-on criteria of acceptance. The FDA released a draft Guidance for Industry in 2007, discussing the use of TPPs as tools for planning, development, and communication with the FDA. This Guidance document was apparently never issued as a final, but in 2017 FDA posted a notice requesting further comments:

https://www.federalregister.gov/documents/2017/11/08/2017-24335/agency-information-collection-activities-submission-for-office-of-management-and-budget-review.

The document was withdrawn by the FDA in 2023 as being "inadequate." It is unclear whether FDA intends this document to be reissued.

The TPP should be a living document that is created early in the discovery process, and reviewed and updated throughout the various discovery, preclinical and clinical development stages of the drug candidate. Table 4 presents an example of an abbreviated TPP that would be appropriate for a parenteral therapeutic to treat or prevent an infectious disease.

**TABLE 4.
EXAMPLE OF A TARGET PRODUCT PROFILE**

| Product Targets | Minimum Acceptable Criteria | Preferred Acceptance Criteria |
|---|---|---|
| Product Indication | Prevention or treatment of [pathogens and infection type] | Prevention and treatment of [pathogens and infection type] |
| Patient Population | Adults | Adults and children |
| Route of Administration | Intravenous | Intravenous |
| Dosage Form | Sterile injectable | Sterile injectable |
| Regimen | 1x/day<br>OR<br>Weekly | 1x/week<br>OR<br>Monthly |
| Efficacy | 90% pathogen inhibition/survival | 100% pathogen inhibition/survival |
| Safety | No observed adverse effect level (NOAEL) 10-fold human dose<br>OR<br>Safety and tolerability comparable to currently marketed biologics | NOAEL 100-fold human dose<br>OR<br>Safety and tolerability improved relative to currently marketed biologics (reduced frequency and/or severity of adverse events) |
| Storage Conditions | Frozen | Refrigerated |
| Shelf-life Stability | 1 yr | 3 yr |

### Other Considerations in TPP Development

In the early phases of a drug discovery program, it is of vital importance to consider both the target indication and the intended patient population for eventual marketed use. It is often helpful to visualize what the final marketed drug product will look like when sitting on a pharmacy shelf, or when used in a hospital or clinic. It is therefore important to consider all of the following points early in the discovery process.

**Eventual Clinical Use.** The eventual target patient population and use may influence early discovery programs. A drug intended for a geriatric population will have very different safety considerations than a drug intended for children or pregnant women. Likewise, pediatric formulations given as a syrup may have issues related to taste that would not be of concern in a capsule intended for adults.

**Route.** It is critical to consider the final clinical route of exposure early in the drug discovery process. The majority of mAbs will be administered by intravenous (iv) injection, but other routes may be considered for specific indications including intramuscular (im), dermal, intraocular, intranasal, subcutaneous injection (sc) or inhaled aerosol.

**Formulation.** Development of an appropriate formulation to fit the intended route is critical and should be a consideration at the earliest stages of drug discovery, and continuing through preclinical and clinical development. Multiple formulations may need to be developed during the lifetime of the program in order to support dosing requirements in preclinical animal models in addition to the eventual formulation for clinical use. As noted above, the physicochemical characteristics of drugs will dictate the feasibility of specific formulations that may be required to achieve the desired routes of exposure.

**Scale-Up feasibility.** A successful lead candidate that advances into Phase II or III clinical trials and eventually to market will require manufacturing on at least a kilogram scale, and for market, potentially kiloton scale. It is therefore important to consider early in discovery the reagents and starting materials used in the synthesis process for the drug substance. Highly toxic, explosive or flammable materials are of minimal concern at the milligram synthesis level, but become problematic or totally impractical at the time of scale-up. Likewise, Cost of Goods (COG) is an important factor in the viability of a molecule. As with the practical considerations related to scale-up mentioned above, the cost of reagents or starting materials can become prohibitive when considering manufacturing for later stage clinical trials or market approval. A drug intended for treatment of malaria in sub-Saharan African populations that costs $2,000/gram to make will not be a financially viable product. Likewise, reagents that are available only in limited milligram (mg) supplies may not be practical if later-stage manufacturing requires kilogram (kg)-level manufacturing. Challenges for mAb manufacturing focus less on chemical ingredients than on the challenges of scaling cell culture systems from mg to kg production levels.

**Market Analysis.** To develop a best-in-class compound, a product-specific PDP would typically outline the specific competitor products for a particular therapeutic class or disease indication. For this generic plan, no specific alternatives can be listed. For anyone developing a new therapeutic for treatment of a medical condition for which other treatments exist (e.g., a broad-spectrum small molecule antiviral vs a mAb specific to a viral spike protein), it is important to assess other products or targets, including their mechanism of action, potential commercial value, cost of production or development, patent life, and other scientific, regulatory, or business factors that might enhance or limit the practical adoption of a new product.

For many anti-infectives, it will be important to compare results against marketed drugs and be able to demonstrate either enhanced potency against drug-resistant strains, fewer adverse effects, decreased costs, or other clear rationale for investing in a research program for a particular product. When considering clinical trials, outcomes should be geared towards improved performance (i.e., a superiority trial) versus simply being no worse than other drugs (i.e., a non-inferiority trial). Minimally, a new product should be differentiated from existing products (e.g., broader spectrum, better therapeutic index, activity against resistant organisms, lower manufacturing costs, etc.) to make a convincing case that a drug provides improved therapeutic benefit over the current standard-of-care.

## CATEGORIES OF ANTIBODY PRODUCTS

Monoclonal antibodies and mAb cocktails being developed to target viral pathogens, including newly emerging viruses, generally use mechanisms of action that may include virus neutralization, Fc-mediated effector functions, or both. Monoclonal antibodies may neutralize the virus by binding the viral attachment protein or the host cell receptor (e.g., angiotensin-converting enzyme ACE2 for coronaviruses, or sialic acid for influenza), thereby blocking attachment to host cell receptors; may prevent the fusion of the viral and host cell membranes (e.g., respiratory syncytial virus F protein); and/or may inhibit other viral or host factors necessary for entry. These products are referred to as neutralizing mAbs. Some mAbs directed against viruses mediate Fc-effector functions in addition to or instead of neutralizing virus entry. Other mAbs may target alternative cellular receptors or cellular proteins that facilitate virus infection.

Though over 150 mAb products have been approved by various regulatory agencies across the globe, a relatively small number of anti-infectives have received approvals. Table 5 lists all currently approved anti-infective mAbs (as of May 2024; adapted and updated from Lyu et al., 2022).

The different types of therapeutic antibodies are briefly described below.

**Natural Occurring Monoclonal Antibodies (mAbs):** Monoclonal antibodies (mAbs) are immunoglobulins produced by identical immune cells, all recognizing the same epitope. They are fundamental components of the adaptive immune system, contributing to the recognition and neutralization of pathogens.

**Engineered Antibodies:**

**Bispecific Antibodies:** Bispecific antibodies are engineered to simultaneously bind two different epitopes, enabling unique therapeutic mechanisms. This class of antibodies is designed to harness the specificity of immune responses for targeted therapeutic interventions. For example:

> **Blincyto (Blinatumomab)** is approved for the treatment of acute lymphoblastic leukemia (ALL). It engages CD19, a surface antigen expressed on B cells, and CD3, a component of the T-cell receptor complex. This engagement facilitates the formation of a cytolytic synapse between T cells and leukemic B cells, resulting in T-cell-mediated killing of the malignant cells.
>
> **Emicizumab (Hemlibra)** is utilized in the management of hemophilia A. It functions by bridging activated factor IX and factor X, thereby restoring the blood coagulation cascade in patients with deficient or dysfunctional factor VIII. This novel mechanism reduces the frequency of bleeding episodes in individuals with hemophilia A.

**Multi-specific Antibodies:** Multi-specific antibodies expand upon the concept of bispecific antibodies, enabling the simultaneous binding to more than two targets. This versatility holds promise for addressing complex diseases with multiple pathological pathways. Currently, there are no commercially approved multi-specific antibodies; however, several candidates are under active research and development for various therapeutic indications.

**Nanobodies:** Nanobodies, also known as single-domain antibodies, are derived from the variable domain of heavy-chain antibodies found in camelids. Their small size (~15 kDa) and unique structure make them attractive candidates for therapeutic intervention. Currently, nanobodies are in preclinical and clinical development stages for a range of targets and indications.

**Fragment Fab and Fc Fusions:** Fragment Fab and Fc fusions involve the fusion of antibody fragments (Fab) or antibody constant regions (Fc) with other molecules to impart specific therapeutic functionalities. Various fragment Fab and Fc fusion proteins are in preclinical and clinical development, with applications spanning from targeted drug delivery to modulation of immune responses.

**F(ab')2 Fragment-Based Drugs:** F(ab')2 fragments are antibody fragments consisting of two antigen-binding Fab regions connected by disulfide bonds. These fragments retain the antigen-binding capacity of the parent antibody while offering advantages such as improved tissue penetration and reduced immunogenicity. For example:

**Raxibacumab** is approved for the treatment and prevention of inhalational anthrax. It functions by neutralizing the protective antigen component of anthrax toxin, thereby preventing the toxin from entering and disrupting host cells, ultimately mitigating the effects of anthrax infection.

**Digoxin Immune Fab (Digifab)** is utilized for the reversal of digoxin toxicity by binding to digoxin molecules in the bloodstream. This binding prevents digoxin from exerting its pharmacological effects, effectively neutralizing its toxicity, and restoring normal physiological function.

**Engineering Approaches**

**Genetic Engineering:** Genetic engineering techniques, such as recombinant DNA technology, enable the insertion of antibody genes into host cells for the large-scale production of therapeutic antibodies.

**Bispecific Antibodies:** Various engineering platforms, including CrossMab and DVD-Ig, facilitate the generation of bispecific antibodies with dual specificities, enhancing their therapeutic potential.

**Fragmentation:** Enzymatic digestion techniques, such as pepsin digestion, yield F(ab')2 fragments from whole antibodies, offering improved tissue penetration and altered pharmacokinetic profiles.

**Purification Techniques:** State-of-the-art purification techniques, such as chromatography, enable the isolation and purification of desired antibodies or fragments, ensuring the production of high-quality therapeutic products.

There are several different categories of antibody products as shown in Figure 2. These can include whole antibodies, antigen-binding fragments (Fab), variable fragments (including single-chain and dimeric single-chain fragments), and bispecific antibodies, including trifunctional antibodies and bi-specific T-cell engagers (BiTEs). BiTEs are most often developed for cancer therapy, where one of the antibody chains bind to a tumor marker, and the other to T-cells via the CD3 receptor.

**TABLE 5.
LIST OF APPROVED ANTI-INFECTIVE MONOCLONAL ANTIBODIES**

| Antibody | Brand name | Approved By | First Approval | Type | Target | Indication |
|---|---|---|---|---|---|---|
| Nebacumab | Centoxin | EMA | 1991 | Human | Lipid A region of endotoxin | Sepsis |
| Palivizumab | Synagis | FDA | 1998 | Humanized | F protein of RSV | Respiratory syncytial virus |
| Raxibacumab | Raxibacumab | FDA | 2012 | Human | Protective antigen of Bacillus anthracis | Inhalational anthrax |
| Bezlotoxumab | Zinplava | FDA, EMA, Japan | 2016 | Human | Clostridium difficile toxin B | Prevent recurrence of Clostridium difficile infection |
| Obiltoxaximab | Anthem | FDA, EMA | 2016 | Chimeric | Protective antigen of the Anthrax toxin | Inhalational anthrax |
| SII rmab | Rabishield | India | 2016 | Human | Rabies virus glycoprotein | Rabies |
| Ibalizumab-uiyk | Trogarzo | FDA | 2018 | Humanized | CD4 | HIV |
| Rabimabs | Twinrab | India | 2019 | Human | Rabies virus glycoprotein | Rabies |
| Atoltivimab+ Odesivimab + Maftivimab | Inmazeb | FDA | 2020 | Human | Ebola virus glycoprotein | Ebola |
| Levilimab | Ilsira | Russia | 2020 | Human | IL-6 receptor | COVID |
| Ansuvimab | Ebanga | FDA | 2020 | Human | Ebola virus glycoprotein | Ebola |
| Olokizumab | Artlegia | Russia | 2020 | Humanized | IL-6 receptor | COVID |
| Regdanvimab | Regkirona | Brazil, EMA | 2021 | Human | SARS-CoV-2 spike protein | COVID |
| Sotrovimab | Xevudy | EMA, PMDA | 2021 | Human | SARS-CoV-2 spike protein | COVID |
| Ormutivimab | Xunke | China | 2022 | Human | Rabies virus glycoprotein | Rabies |
| Tixagevimab + Cilgavimab | Evusheld | EMA | 2022 | Human | SARS-CoV-2 spike protein | COVID |
| Nirsevimab | Beyfortus | FDA, EMA, China | 2023 | Human | F protein of RSV | RSV |

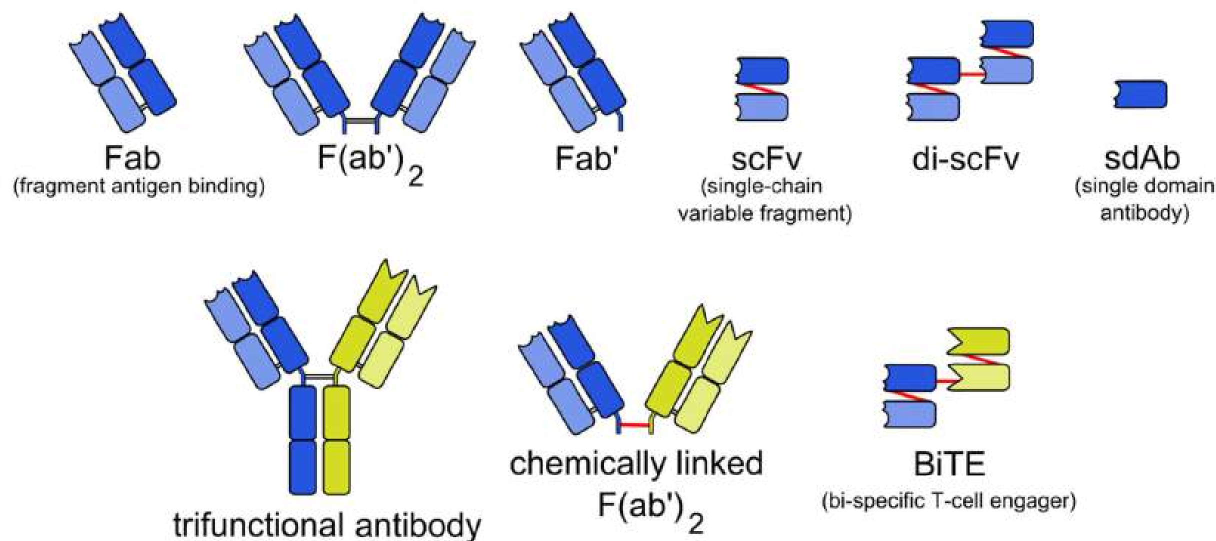



**Figure. 2. Different type of therapeutics antibodies. (Courtesy of Anypodetos https://commons.wikimedia.org/w/index.php?curid=8999674)**

At this time, the only antibodies approved for anti-infective indications are full human, humanized or chimeric mAbs, though a few products are in development including the $F(ab')_2$ afelimomab, targeting TNF-α for treatment of sepsis; and the scFv efungumab, targeting Hsp90 for treatment of candida infection.

### Discovery of Monoclonal Antibodies

The methods and assays associated with mAb discovery fall outside the scope of this document. There are excellent review articles and book chapters covering this material (e.g., see Li and Zhu, 2010; Buss et al., 2012; Castelli et al., 2019; Xu et al., 2019; Hoover et al., 2021; Zhang, 2023) and readers are referred to these sources for further information.

## PRECLINICAL DEVELOPMENT PLAN

### Overview of the Preclinical Development Process

The immediate goal of any drug discovery project is to obtain evidence of the safety and efficacy, in humans, of a new biological or chemical entity (NCE). Successful completion of the project ultimately leads to an IND application to the FDA for approval to initiate human clinical trials. The early stage of development for a biological drug product is defined by multiple activities with the goal of submitting an Investigational New Drug (IND) application to the regulatory authorities and gaining approval to initiate Phase I clinical trials. These activities include development of a manufacturing process for the drug substance, development of a formulation and design of the drug product, analytical development, pre-clinical toxicology and safety studies in animals, GMP manufacturing of the Drug Substance (DS) and Drug Product (DP), writing of the common technical document and submission of the IND application to the regulatory authorities for review and approval to initiate the clinical trials.

A typical preclinical development plan will consist of six major efforts (Figure 3): (1) manufacture of drug substance (DS; in this case, the purified, unformulated mAb), both in pilot efforts not conducted under FDA GMP regulations; (2) pre-formulation and formulation (dosage design); (3) analytical and bioanalytical methods development and validation; (4) metabolism and pharmacokinetics testing; (5) toxicology testing (both safety and safety pharmacology conducted under non-GLP and GLP conditions); and (6) GMP manufacture of drug product (DP; final formulated mAb) for clinical trials. The IND application is essentially a description of the results of all these activities. These are not isolated activities but are interconnected. The timing of the various tasks involved in the broader effort is critical to completing a timely and cost-effective development program with effective go/no-go decision points.

No acceptable preclinical development plan can be drafted without reasonable knowledge of the anticipated clinical plan. It is standard practice to work backwards from the product label indications intended for FDA approval to design an appropriate Phase III trial; the nature of that trial will determine the appropriate Phase II clinical design, which will help to define the appropriate Phase I safety design study. To draft an appropriate preclinical plan, the design of the first-in-human Phase I trial is critical because the GLP toxicology studies must mimic, to some degree, the initial intended human use (dose and route).

Without exception, we recommend planning for a pre-IND meeting with the FDA to obtain some assurance from the agency that it considers the intended GLP toxicology studies acceptable in relation to the intended initial clinical use. This meeting should ideally take place after the first toxicology dose range-finding studies and before the initiation of the definitive GLP studies. The details of the pre-IND meeting will be discussed later in this document.

Figure 3 outlines the interconnected steps in the preclinical development process, including the manufacturing, safety, and regulatory activities required for IND approval and initiation of a Phase I clinical trial.

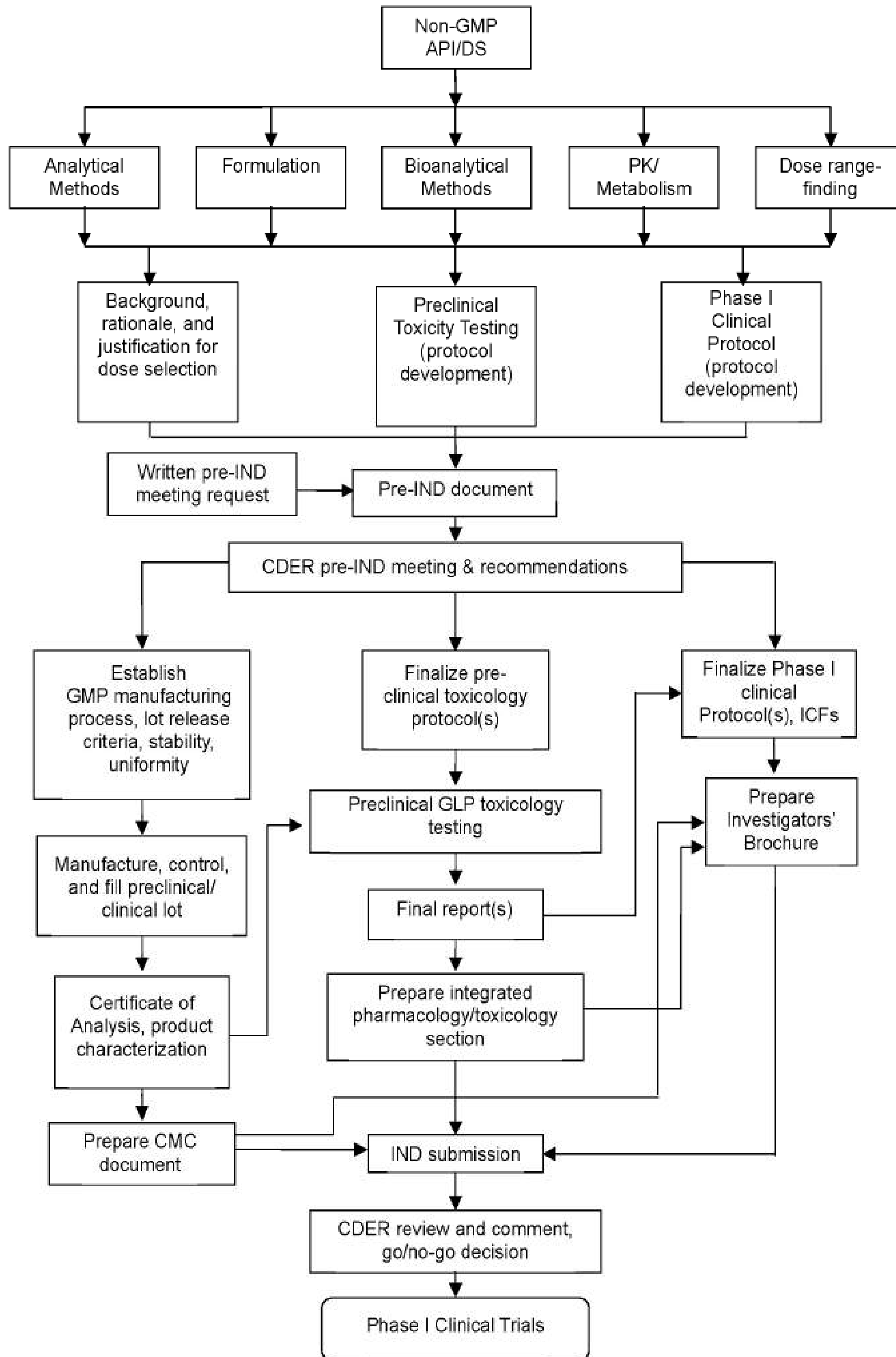



**Figure 3. Flow chart of preclinical development steps to Phase I trial.**

## CHEMISTRY, MANUFACTURING AND CONTROLS (STAGES 1-3)

### Overview

Manufacturing processes for newly developed biological or chemical entities require ongoing evaluation, and usually modification of manufacturing steps and procedures over time. However, once the decision is made to produce a product for clinical use, the steps in manufacturing should be directed toward compliance with current GMP requirements and conformance with recommendations for implementing manufacturing controls that are appropriate for the investigational clinical phase. Identification of a safety concern or a lack of sufficient data to evaluate safety is the only reason the FDA has ever given for a clinical hold based on the Chemistry, Manufacturing and Controls (CMC) section of the IND. The goals of a well-designed development plan must include a reliable cell bank preparation method for producing a safe and effective product.

In production of mAbs, manufacturing steps may vary for particular expression systems. In all cases, however, the manufacturer must:

- Establish a reliable and continuous source from which the antibody can be produced (e.g., a Master Cell Bank).
- Conduct appropriate in-process testing to address safety concerns for each expression system and demonstrate manufacturing control.
- Develop robust and efficient fermentation and purification techniques.

From the beginning of the manufacturing process and throughout development, complete documentation must be maintained. Names and sources of all production constituents (reagents and materials), including the constituents of each culture medium, must be recorded. Since product contamination can cause serious clinical consequences, the risk of viral contamination is a significant concern for all biotechnology products, including mAbs derived from cell lines. Contamination has been known to arise from a number of sources, including the cell line itself (cell substrates) as well as adventitious agents introduced during production. Therefore, documentation is critical to the success of the IND application, and it is essential during both antibody development and manufacturing stages.

### Establishing a Master Cell Bank (Stage 1)

A reliable and continuous source of antibody is established by production of a GMP Master Cell Bank (MCB), which undergoes characterization and stability evaluation. A sufficient number of vials (usually at least 100) are prepared to allow for adequate testing (characterization), antibody product production, and retention. The MCB is analyzed for identity, purity, genetic integrity, sterility, endogenous and adventitious agents (as needed), strength or concentration, and stability. Extensive testing for endogenous and nonendogenous viral contamination is performed (per Guidance for Industry—ICH Q5A, Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin). Manufacture of cell banks is performed according to GMP regulations, with adequate documentation and testing. A summary of the different production systems is provided in Table 6.

**TABLE 6.**
**SUMMARY OF THE CHARACTERISTICS OF DIFFERENT MONOCLONAL ANTIBODY PRODUCTION SYSTEMS**

| Production System | Characteristics | Pros | Cons |
|---|---|---|---|
| Mammalian Cells | Capable of post-translational modifications, proper protein folding, high-quality antibody production | Ability to produce complex proteins, suitable for therapeutic antibodies | Expensive and time-consuming, limited scalability, risk of viral contamination |
| Bacterial Cells | Rapid growth, cost-effective, well-established systems | High yield, simple downstream processing | Lack of post-translational modifications, inability to produce complex proteins |
| Yeast Cells | Efficient protein folding, scalable production, simple genetic manipulation | High yield, cost-effective | Limited post-translational modifications, not suitable for all antibodies |
| Insect Cells | High protein yield, capable of post-translational modifications, well-suited for complex proteins | Efficient protein secretion, suitable for large-scale production | Expensive culture medium, time-consuming process |

The choice of cells for making the MCB depends on their expected attributes, including plasmid identity and stability, stable growth and yield, absence of contaminants, and monoclonal antibody (mAb) quality. Regardless of the production system, GMP conditions and release assays for the MCB are conducted to ensure consistency and quality control.

The differences between End of Production Cells (EPC) and Working Cell Bank (WCB), both involve cells at the limit of *in vitro* age with limited passages. The EPC evaluation is conducted at the end of production to assess for introduced contaminants due to growth conditions, while WCB preparation involves fewer passages and abbreviated testing for detecting newly introduced contaminants. MCB is prepared first, allowing for sufficient characterization and pilot batch development to resolve any manufacturing issues before proceeding to GMP production batches. This approach helps mitigate costs and risks associated with potential failures to meet criteria. Additionally, manufacturing facilities typically require thorough testing to confirm cell lines are free of potential contaminants before entering their facility, ensuring compliance with regulatory standards.

**Drug Substance (Stage 2-3)**

Production of the purified drug substance (DS), in this case the purified mAb, usually requires preparation of one or two non-GMP pilot or development batches prior to the initial production of the batch records for GMP production.  Adequate testing to ensure reasonable safety allows this material to be used for the following studies:

- Developing the final product formulation: Formulation development involves a systematic process to optimize the composition of the final product, ensuring stability, efficacy, and patient safety. Assays, Tests, and Limits: This stage involves conducting compatibility studies with excipients, assessing physicochemical properties (pH, osmolality, viscosity), and performing accelerated stability studies to establish shelf-life. Limits are set based on regulatory guidelines and product specifications.
- Performing initial toxicology studies such as dose range-finding studies, pharmacokinetic and immunogenicity studies: Toxicology studies are performed for assessing the safety profile of the mAb, encompassing various parameters such as dose-response relationships, absorption, distribution, metabolism, and excretion (ADME), and potential immunogenicity. Assays, Tests, and Limits: Dose range-finding studies will involve administering varying doses of the mAb to evaluate toxicity levels. Pharmacokinetic studies assess the mAb's ADME properties, while immunogenicity assays detect immune responses. Limits are established based on acceptable toxicity levels and immunogenicity risks.
- Developing analytical methods: Analytical techniques are developed for characterizing the mAb's attributes, including purity, potency, and stability, throughout its lifecycle. Analytical methods include high-performance liquid chromatography (HPLC), mass spectrometry (MS), and electrophoresis. Assays are validated for specificity, accuracy, precision, linearity, and robustness. Limits are defined based on method validation criteria and regulatory requirements.
- Obtaining initial stability data: Testing is conducted to assess the mAb's stability under various storage conditions, providing critical insights into shelf-life and storage recommendations. Accelerated stability studies are performed under elevated temperature and humidity conditions, while long-term stability studies assess degradation over extended periods. Assays measure degradation products, potency, and other critical attributes. Limits are established based on predefined degradation profiles and regulatory guidelines.

Once the mAb is declared a candidate for drug development, a series of activities begin in parallel. One of these is the development of a stability-indicating or potency assay. The assay must be robust enough to separate the DS from its degradation products in a variety of sample matrices. This assay forms the cornerstone for all evaluations of the mAb during the preformulation and formulation development stages, ensuring product quality and efficacy.

In the development of preclinical analytical methods for generic monoclonal antibodies (mAbs), a comprehensive set of assays is established to evaluate the quality, potency, and purity of the product. The assays typically required are included in Table 7.

**TABLE 7.
ANALYTICAL ASSAYS COMMONLY USED FOR
TESTING OF MONOCLONAL ANTIBODIES**

| Analytical Test | Test Parameters | Acceptance Criteria |
|---|---|---|
| Reverse Phase High-Performance Liquid Chromatography | Impurities | Impurities below specified thresholds |
| (RP-HPLC) | | |
| **Potency Assays** | | |
| Cell-Based Assays | Biological activity | Potency within specified range |
| Enzyme-Linked Immunosorbent Assay (ELISA) | Binding affinity | Affinity within specified range |
| Biological Activity Assays | Desired biological effect | Activity consistent with reference product |
| **Identity and Structure Assays** | | |
| Peptide Mapping | Primary structure, post-translational modifications | Consistency with reference product |
| Circular Dichroism (CD) and Fourier Transform Infrared | Secondary and tertiary structure | Structural integrity within specified range |
| **Spectroscopy (FTIR)** | | |
| Mass Spectrometry | Molecular weight, glycosylation | Consistency with reference product |
| **Stability Indicating Assays** | | |
| Forced Degradation Studies | Stability, degradation pathways | Stability under stress conditions |
| Accelerated Stability Studies | Long-term stability prediction | Stability under accelerated conditions |
| **Method Validation and Optimization** | | |
| Validation Parameters | Specificity, accuracy, precision, linearity, robustness | Methods validated according to regulatory guidelines |
| Optimization Parameters | Buffer composition, pH, temperature, detection methods | |
| **Reference Standards and Controls** | | |
| Test Parameters | Consistency and comparability | Results consistent with reference product |
| **Documentation and Reporting** | | |
| Documentation | Detailed procedures, parameters, results | |
| Reporting | Comprehensive reports for regulatory submission | |

**Manufacturing Process Development**

**Preclinical Development Plan:**

- Identification of Target Antigen: A suitable target antigen is identified, considering factors such as antigen specificity and relevance to therapeutic applications. A cell line, typically utilizing CHO cells for their capability to mimic human-like glycosylation patterns, is used for mAb production.
- Cell Line Development and Culture Optimization: The chosen cell line undergoes rigorous optimization of culture conditions to maximize mAb yield and quality. Parameters such as media composition, pH, temperature, and agitation are fine-tuned to enhance cell growth and productivity.
- Purification Strategies: Protein A affinity chromatography serves as the initial capture step, selectively binding the mAb. Subsequent chromatography steps, including ion exchange chromatography and hydrophobic interaction chromatography, are optimized for enhanced purification and impurity removal. Furthermore, additional purification methods such as precipitation or extraction may be deployed based on the specific characteristics of the mAb and impurities.
- Analytical Testing: A battery of analytical tests is conducted to comprehensively evaluate the stability, potency, and safety profile of the mAb. This encompasses assays for protein concentration, potency assessments, stability testing under diverse conditions, and safety evaluations.

**Fermentation and Purification Process Development**

**Fermentation Optimization:**

- Parameters critical to fermentation, including temperature, pH, agitation, and dissolved oxygen levels, are carefully optimized using Design of Experiments (DoE) methodologies. This optimization aims to maximize cell growth and mAb production rates.
- Media formulation and feed strategies are systematically optimized to provide essential nutrients and sustain cell viability throughout the fermentation process, ensuring optimal productivity.

**Purification Techniques:**

- The purification process usually starts with protein A affinity chromatography, selectively capturing the mAb from the cell culture supernatant.
- Subsequent chromatography steps, such as ion exchange chromatography and hydrophobic interaction chromatography, are optimized to further purify the mAb and remove impurities.
- Ultrafiltration and diafiltration steps are employed for concentration and buffer exchange, facilitating the attainment of the desired purity level.

**Impurity Reduction:**

- Process parameters are fine-tuned to minimize impurities such as host cell proteins (HCP), DNA, and other process-related impurities, ensuring the integrity and safety of the final mAb product.
- Robust virus clearance studies are conducted to validate the efficacy of purification processes in eliminating viral contaminants, thus ensuring the safety of the purified mAb product.

**Testing Parameters:**

- Critical parameters, including cell viability, cell density, specific growth rate, and product titer, are continuously monitored throughout the fermentation process to maintain optimal conditions for mAb production.
- Purification efficiency is assessed through a comprehensive array of analytical techniques such as high-performance liquid chromatography (HPLC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), measuring mAb concentration, purity, and aggregate levels.
- Additional tests, including assessments of endotoxin levels, host cell DNA content, and protein concentration, are conducted to ensure the product's quality and compliance with stringent regulatory standards.

**Manufacturing**

**Fermentation Process:**

To cultivate genetically modified microorganisms for the production of monoclonal antibodies (mAbs). Fermentation is conducted in large-scale bioreactors under controlled conditions including temperature, pH, agitation, and dissolved oxygen levels. Genetically engineered microorganisms are introduced into the bioreactor to initiate growth and antibody expression. Nutrients are supplied to support cell growth and antibody production.

**In-process Testing:**

- **Cell Density Measurement:** Utilizing methods such as optical density or cell counting to quantify the concentration of microorganisms (cells/mL).
- **Viability Assessment:** Determining the percentage of viable cells using viability stains or flow cytometry.
- **Antibody Titer Analysis:** Enzyme-linked immunosorbent assay (ELISA) or other quantitative assays to measure antibody concentration (g/L).
- **Metabolic Activity Monitoring:** Monitoring parameters such as substrate consumption and metabolite production rates.

**Parameters and Limits:**

- **Cell Density:** ±10% deviation from target density.
- **Viability:** Maintained above 90% throughout fermentation.
- **Antibody Titer:** Target concentrations based on process optimization.
- **Metabolic Activity:** Fluctuations beyond predetermined thresholds prompt investigation.

**Purification Process:**

To isolate and purify monoclonal antibodies from the fermentation broth. Purification involves multiple steps including chromatography, filtration, and centrifugation to separate antibodies from impurities and other cellular components. Chromatographic techniques exploit differences in size, charge, and affinity to achieve purification.

**In-process Testing:**

- **Protein Concentration Determination:** Spectrophotometric methods or protein assays to quantify antibody concentration.
- **Purity Analysis:** Analytical techniques such as SDS-PAGE or capillary electrophoresis to assess purity.
- **Aggregate Level Measurement:** Size exclusion chromatography or analytical ultracentrifugation to detect antibody aggregates.
- **Endotoxin Testing:** Limulus amebocyte lysate (LAL) assay to quantify endotoxin levels (EU/mL).

**Parameters and Limits:**

- **Protein Concentration:** Consistency maintained throughout purification.
- **Purity:** Typically, >95% pure based on total protein content.
- **Aggregate Levels:** Below 2–5% of total protein content.
- **Endotoxin Levels:** Compliance with regulatory standards (≤0.5 EU/mL for injectable products).

**Quality Control and Documentation:** Standard Operating Procedures (SOPs) are adhered to throughout both processes. Any deviations from expected parameters are thoroughly investigated and documented to ensure product quality and regulatory compliance.

**Viral Clearance**

The following methods are employed for viral clearance:

1. **Filtration**: Employing filters with a nominal pore size smaller than the size of viruses. Typically, filters with a pore size of 20 nanometers or smaller are used.
2. **Chromatography**: Utilizing techniques such as ion exchange chromatography to selectively remove viral particles based on their charge characteristics.
3. **Chemical Inactivation**: Treatment with agents like solvent/detergent or low pH to inactivate viruses by disrupting their structure or nucleic acids.
4. **Precipitation**: Employing methods like polyethylene glycol precipitation to precipitate and remove viral particles from the solution.
5. **Affinity Chromatography**: Utilizing ligands specific to viral particles to capture and remove them selectively.
6. **Depth Filtration**: Employing depth filters with varying pore sizes to remove viral particles through a combination of size exclusion and adsorption.

**Evaluating Efficacy:**

- **Validation Studies**: Comprehensive studies are conducted to validate the efficacy of each viral clearance method.
- **Virus Spike Studies**: Viral particles are intentionally spiked into the process stream at various stages to evaluate the clearance efficiency.
- **Quantitative PCR (qPCR)**: Quantitative PCR assays are used to quantify the reduction of viral load at each step of the process.
- **Validation Criteria**: Clearance methods must demonstrate a log reduction value (LRV) sufficient to ensure the safety of the final product, typically achieving at least 4 to 6 logs reduction for enveloped viruses.

**Unprocessed Bulk Analysis:**

**Appearance:**

- Visual inspection for clarity, color, and particulate matter. Acceptance criteria: Appearance consistent with the reference standard.

**Microbial Purity (Bioburden):**

- Microbial enumeration tests such as plate count method or membrane filtration method. Acceptance criteria: Bioburden below specified limits per unit volume.

**Adventitious Agents:**

- Testing for adventitious agents including viruses, mycoplasma, and other potential contaminants. Acceptance criteria: Absence of detectable levels of adventitious agents.

**Mycoplasma:**

- Detection of mycoplasma contamination using specific assays such as polymerase chain reaction (PCR) or indicator cell culture methods. Acceptance criteria: Absence of mycoplasma contamination.

**Protein Content:**

- Quantification of protein concentration using methods such as Bradford assay or UV spectroscopy. Acceptance criteria: Protein content within specified range based on the expected yield from the manufacturing process.

**Purification Analysis:**

**Appearance and Color:**

- Visual inspection for clarity, color, and particulate matter. Acceptance criteria: Appearance consistent with the reference standard.

**pH:**

- Measurement of pH. Acceptance criteria: pH within specified range suitable for stability and efficacy of the product.

**Potency:**

- Assessment of biological activity using validated bioassays such as cell-based assays or binding assays. Acceptance criteria: Potency within specified range based on the reference standard.

**Sterility:**

- Testing for bacterial and fungal contamination using validated methods such as membrane filtration or direct inoculation. Acceptance criteria: Absence of microbial growth.

**Endotoxin:**

- Quantification of endotoxin levels using the Limulus Amebocyte Lysate (LAL) assay. Acceptance criteria: Endotoxin levels below specified limits per dose.

**Residual Host Cell Proteins:**

- Quantification of residual host cell proteins using immunoassays such as ELISA. Acceptance criteria: Residual host cell proteins below specified limits per dose.

**Identity:**

- Verification of product identity using methods such as mass spectrometry or peptide mapping. Acceptance criteria: Consistent identity with the reference standard.

**Integrity and Purity:**

- Evaluation of protein integrity and purity using SDS-PAGE under reduced and non-reduced conditions. Acceptance criteria: Presence of expected bands with minimal impurities.

**Impurities:**

- Detection and quantification of impurities using analytical techniques such as HPLC or capillary electrophoresis. Acceptance criteria: Impurity levels below specified limits.

**Sequence Fidelity:**

- Verification of amino acid sequence using peptide mapping or sequencing methods. Acceptance criteria: Sequence fidelity consistent with the reference standard.

**Quality Assurance:**

The Quality Assurance Unit (QAU) oversees and audits all aspects of GMP production:

- **Facility Inspection**: Inspection of manufacturing facilities to ensure compliance with GMP regulations.
- **Batch Record Review**: Review of preliminary and completed batch records to ensure adherence to established procedures.
- **Manufacturing Program**: Assessment of the manufacturing program to ensure consistency and compliance with regulatory requirements.
- **Certificate of Analysis (CoA)**: Preparation of a final CoA summarizing product specifications and test results.
- **Product Release**: Release of the product by the QAU upon meeting all specified criteria.
- **Storage**: Storage of the labeled drug substance (DS) under controlled conditions until further processing.

### Drug Product (Stage 3)

Prior to initiating development activities, it is critical to define the QTPP (Quality Target Product Profile). The QTPP is focused squarely on the CMC attributes including the intended use in clinical setting, route of administration, dosage form, delivery systems, dosage strength(s), formulation, storage conditions, container closure system, and drug product quality criteria (e.g., sterility, purity, stability and drug release) appropriate for the intended marketed product. QTPP typically has Indication, Molecule Type, Route of administration, dosage form, dose, protein content per container, biocompatibility and drug product presentation (final concentration in the drug product).

#### Analytical Development

Establishment of the analytical assays for defining the degradation and impurity profile of the DP is often done in parallel with both establishment of the manufacturing process and development of the formulation. The analytical methods themselves are typically defined as those for quality, safety, activity, quantity, and purity including process and product related, and can be subdivided into both product specific and compendial assays. The product specific assays are, as the name suggests, specific to each product, and generally are concerned with drug substance product quality, activity or potency.

Depending on the proposed mechanism of action, assays should be developed to support the control strategy for confirming the biologics product properties and potency. Examples of assays are:

**Properties**

- SDS-PAGE
- IEX-chromatography
- Size exclusion chromatography
- Capillary isofocusing electrophoresis
- Force degradation
- Peptide mapping
- Glycan analysis

**Activity/Potency**

**1. Binding Assays**

These essays are established during product development in the form of a direct binding essay such as enzyme linked immunosorbent assay (ELISA) or a surface plasmon resonance (SPR) assay. FDA recommends a potency assay that is a better reflection of the intended mechanism of action then direct binding assay.

**2. Viral neutralization Assays**

The FDA recommends establishing an *in vitro* viral neutralization assay early in development. This type of assay can be useful for advancing development, quality control, and characterization of neutralizing clonal antibodies targeting viral attachment and entry.

### 3. Fc-effector Function Assays

For monoclonal antibodies demonstrating Fc effector functions, appropriate method should be included as part of the specifications to ensure consistent mAb potency and functions. The mAbs engineered to alter binding to Fc receptors and complement components characterization studies should be conducted on a one-time basis to demonstrate the engineered mAbs function as designed.

### In-use compatibility studies

Depending on the intended target organs and delivery route the Phase I clinical trials may be initiated with IV, intramuscular, intrathecal, subcutaneous, intraocular or other appropriate route if feasible at this stage and as defined by the QTPP. Prior to using DP in the clinic, in-use compatibility, and stability studies with the appropriate delivery system such as IV diluents and delivery systems, closed system transfer devices (CSTD), and syringe components are necessary to ensure the safety and delivery of the active pharmaceutical ingredient. For IV delivery, the steps necessary for preparation of the diluted DP in the IV bag, types of IV bags, acceptable storage time and temperature, priming of the IV line, types of in-line filters and delivery time are described in the Pharmacy Manual. At the early stage of development IV infusion compatibility studies should be minimal and demonstrate compatibility with either saline and/or dextrose with a single representative IV bag/infusion set.

### GMP DP Manufacturing and Stability Monitoring

At this stage the DP should be designed so that no special process requirements are needed to fill the DS into the primary packaging material. The easiest pathway here is to make formulated bulk DS directly into formulated bulk DP by the following process: formulated bulk DS, mixing, bioburden filtration, transfer hold in a different vessel, sterilizing filtration to produce formulated bulk DP, filling, stoppering, capping, and visual inspection.

An ongoing formal stability evaluation is needed for the drug product. The stability protocol generally includes both long-term storage conditions and accelerated temperature storage conditions. Long-term stability is to evaluate product going into a human clinical study and to generate data for product labeling. Accelerated temperature storage is used to evaluate conditions under which the product's instability and sensitivity to temperature excursions can be anticipated.

### DP Small Scale and Engineering Runs

A DP manufacturing run including process development for thawing, transfer and filtering, dilution, mixing homogeneity, sterilizing filtration, container filling, freeze-drying cycle where applicable, stoppering, sealing and inspection should be carried out using the final process, or similar, pivotal material or a suitable placebo depending on what is available either at small scale or closer to manufacturing scale through engineering or demonstration batches. These studies are primarily to qualify the unit operations and validate that the expected quality metrics can be met. The engineering batch can also be used to determine the mixing parameters range including mixing speed range corresponding to different batch volumes to proceed to pivotal batches and later for PPQ batches. Additionally, the engineering batch can be used to determine the acceptable parameters for freeze drying cycle for freeze dried products, nitrogen, or inert gas overlay to control the % oxygen overlay in vials for oxidation sensitive products, the maximum hold period post bioburden in the transfer vessel prior to sterile filtration and whether filter and line flushes/rejects are appropriate. The engineering batch to set up the filling line and capping

parameters prior to proceeding to the GMP batch. It is suggested that the drug product release testing and if needed, stability of the engineering batch are evaluated, so that a process control strategy can be fine-tuned based on the product quality results before locking the process for the GMP batch.

#### GMP DP Batch for Pivotal Clinical Studies

After the DP team evaluates the results of the process control and product quality results from the engineering run DP batch, the team will be ready to initiate the GMP DP batch using the pivotal DS material produced under GMP practices. All analytical testing including in-process controls, batch inspection, DP release and DP stability should be done using qualified methods that are intended for process validation (PPQ) and commercial production. It is ideal to complete analytical method validation prior to pivotal material release to ensure that the methods do not vary much between release of pivotal material to PPQ material. If changes in analytical methods and/or new methods are introduced post pivotal material lot release, then appropriate bridging studies need to be performed using pivotal lots to demonstrate that such methods are indeed useful for monitoring CQAs and are stability indicating prior to method validation.

### Preliminary Preclinical Safety

The amount of monoclonal bulk DS required for safety testing depends on the toxicity of the DS and the species selected for animal studies. Toxicology studies typically use “GMP-like” material that is similar to the purity of the ultimate GMP clinical mAb bulk DS but is not made under formal GMP compliance, though the final GMP DP may also be used. This initial material is typically used for the preliminary toxicology studies and for analytical methods development. For a mAb, initial dose range-finding and pharmacokinetic (PK) studies can typically be completed with only a few grams of material, depending on the species selected for testing (see section on Safety Testing later in this document).

## PREFORMULATION STUDIES AND FORMULATION DEVELOPMENT (STAGE 2)

### Overview and Preformulation

The specific physicochemical properties of the mAb will dictate which formulation options are available. If the molecule presents physicochemical or biological challenges, the formulation approach chosen will depend on the required or desired degree of dosing flexibility. One of the more challenging aspects of developing mAb pharmaceuticals is dealing with and overcoming their inherent physical and chemical instabilities. A successful process has three stages: preformulation, which includes stabilization of the active substance in bulk form, formulation in the designated dosage forms (drug delivery), and fill and finish using aseptic manufacturing procedures. The following sections describe typical approaches.

#### Preformulation

Preformulation includes physical and chemical testing using a variety of characterizing instruments. Additionally, preformulation studies are essential to determine the compatibility of initial excipients with the active substance for biopharmaceutical, physicochemical, and analytical stability. Excipients are chosen to enhance or maintain stability of the active compound. Following are some of the characterizations performed during this phase.

**pH Effects on mAb.** A mAb solution of desired pH (5–7) and buffer system (acetate and phosphate) can be prepared by diafiltration. Effects of pH on the mAb as a function of time should be monitored.

**Thermal Effects.** Thermal denaturation experiments are performed to evaluate the change in mAb structure by heating samples from 15ºC to 90ºC at the rate of 1°C per minute and cooling back to 15ºC (thermal cycle). Antibody structure is analyzed using far ultraviolet-circular dichroism [UV-CD (FUV-CD)] spectroscopy, aromatic residue fluorescence spectroscopy, and differential scanning calorimetry (DSC). Surface hydrophobicity of the mAb in these samples is analyzed using an extrinsic fluorescence probe, 8 anilinenapthalene-1-sulfonate (ANS).

**Monoclonal Antibody Aggregation.** Antibody aggregation is studied using light scattering and size exclusion chromatography (SEC) analyses.

**Excipient Compatibility.** Different excipients to be included in the formulations will need to be tested for compatibility. These include lyophilization aids, bulking sugars, isotonicity aids, preservatives, and perhaps others. Mixtures of the active mAbs and an excipient will be incubated at 5°C and 45°C for 1 week, and will then be analyzed with SEC, fluorescence spectroscopy, ultraviolet (UV) spectroscopy, and a potency testing assay.

The monomer content, soluble aggregates, and clips due to hydrolysis are monitored by SEC. Fluorescence Spectroscopy is used to monitor changes in the mAb tertiary structure. A decrease in the denaturation temperature shown in a DSC reflects a destabilizing effect of the preservative on formulation stability. The biological activity (potency) of the mAb will also need to be measured.

**Preservative Potency Tests.** Since the mAb is particularly likely to be degraded in the absence of preservatives, a test to identify the best preservative for the specific mAb may be needed. The compatibility of 2-6 parenteral preservatives (benzyl alcohol, chlorobutanol, methylparaben, propylparaben, phenol, and m-cresol) with the formulated mAb should be screened. The preservatives will be added to the formulated antibody based on their commonly used concentration ranges in marketed products. In addition to the excipient tests, these samples should be challenged, and their resistance to bacterial growth will be analyzed.

### Formulation Development

An early decision to be made is whether the final mAb formulation will be a frozen liquid, refrigerated liquid, or lyophilized, although sometimes all the strategies are explored in parallel. In any case, a placebo formulation may also be required, depending on the design of the Phase I trial. Monoclonal antibodies are typically formulated into single dose vials, as single dose formulations are easier to protect against contamination.

#### Developing a Frozen Liquid Formulation

A frozen formulation typically includes the active mAb, a stabilizing excipient, a tonicity adjuster, a preservative, an aggregation preventative, and miscellaneous excipients that help to maintain stability of the formulations. Four important stress tests for developing frozen liquid formulations are: shake test (agitation), surfactant test, freeze-thaw test, and heating experiments. Each formulation configuration is shaken in a vial to determine whether it forms aggregates. Then a surfactant (usually a polysorbate detergent) is added to prevent formation of precipitates to make it harder for the mAb to form aggregates. Formulations are checked through multiple freeze-thaw cycles (which can take about a week) to check the effects of temperature and freezing process stresses. Most mAbs are stable around 2–8°C, but few are stable at room temperature or above.

The following is an example of a typical freeze thaw cycle. Various mAb solution formulations are filled into the chosen containers. The test samples are frozen (at either –70°C or –20°C) and thawed 3 times over 2 weeks. Samples stored at 5°C will be used as controls. Particulate formation is monitored at each time point by visual inspection, and insoluble aggregates in the samples are quantified using dynamic light scattering (DLS). For each formulation, the glass transition (Tg) and denaturation (Tm) temperatures are determined using modulated DSC; soluble aggregate levels are assessed using SEC and SDS-PAGE.

Container closure compatibility with the vessel and closure must be determined. mAbs can adhere to the surface of conventional glass, and delaminated glass causes lower adsorption. Certain kinds of plastics may be better than glass. Stoppers are tested for reactivity with the liquid formulation by inverting vials to give the stopper complete contact with the formulation, and then containers are stored horizontally to create more surface area and provide greater opportunity for the mAbs to degrade.

The filling process should remove as much oxygen as possible from the vial's headspace because oxygen can cause degradation. Potential product extractables and leachables must be characterized and listed in regulatory submissions.

**Developing a Liquid Formulation**

Liquid formulations (stored at 4ºC or above) are generally discouraged because of the practical considerations of long-term storage and stability. Stability can be increased by freezing the formulation at –20°C or lower. If proteins are easily reconstituted, lyophilized formulations may be developed, but doing so requires additional processing and can add to the cost of the final product. For preclinical and early clinical work, refrigerated liquid formulations are frequently used as an alternative to development of a frozen or lyophilized formulation. The steps involved and evaluations required are essentially identical to those for a frozen liquid formulation except that freeze-thaw evaluations are not required.

**Developing a Lyophilized Formulation**

A lyophilized product should have the following characteristics: (1) long-term stability, (2) short reconstitution time, (3) elegant cake appearance, (4) maintenance of characteristics of the original dosage form after reconstitution, and (5) isotonicity after reconstitution. The lyophilization process includes three stages: freezing, primary drying, and secondary drying. The relatively small amount of bound water is removed during the secondary drying, but most of the fluid is removed during primary drying. Lyophilization cycles are developed to minimize or avoid any destabilization of the mAb. Excipients are essential for effective lyophilization, and these include buffers, bulking agents, stabilizers, and tonicity adjusters.

Buffers are essential for maintaining pH of the formulation. Bulking agents are especially important in cases in which low levels of active ingredients are used. Crystalline bulking agents help form an elegant cake structure. In addition to being bulking agents, disaccharides form an amorphous sugar glass and have proven to be most effective in stabilizing mAbs during lyophilization. The need for tonicity adjusters will depend on the stability requirements of the bulk solution or those for the route of administration.

### Additional Studies Supporting Formulation Development

A variety of other studies may also be needed in addition to the above formulation activities including a transportation study, a photostability study, an in-use stability study, container/closure system studies, and possibly a device compatibility study. Scaled-down photostability studies based on DP manufacturing conditions and expected in-use exposure should be carried out at the beginning of the commercial formulation development to understand if any residues are particularly susceptible to photodegradation and if excipient such as peroxide scavengers, hydroxyl radical scavengers, or metal chelators are necessary to reduce photodegradation of the DP. An extractable and leachable risk assessment is conducted identifying all equipment, single-use parts and container-closure components which may lead to changes in the impurities profile of the drug product. For this, it is important to identify an appropriate container-closure system.

## DEVELOPMENT OF STABILITY DATA (STAGE 2-3)

### Stability of Pilot Batches

Once the process of making a mAb formulation is standardized, pilot batches of the material are made and placed on a stability testing schedule to determine the storage stability of the drug product. The storage conditions will depend on the dosage form, but typically include both the anticipated long-term as well as accelerated or stressed storage conditions.

Observations are made at the end of every evaluation period. Tests to be performed for pilot stability of the dosage form typically include: (1) appearance, (2) assay, (3) impurities, and (4) potency.

### Stability of GMP Batches

Stability of GMP batches will be extensive, typically performed every 3 months for the first year and once every six months for years 2 and 3. If the stability of the product is expected to be less than 1 year, then additional time points at 1 and 2 months should be tested. Early stability time points may also be necessary if the stability program will run concurrent with a human trial, to ensure stability throughout the trial. The design of a typical stability program is shown in Table 8.

**TABLE 8.
TYPICAL DESIGN FOR ACCELERATED STABILITY TESTS OF PRELIMINARY FORMULATIONS**

| Storage Condition (ICH) | Time Point (months) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 3 mo. | 6 mo. | 9 mo. | 12 mo. | 18 mo. | 24 mo. | 36 mo. |
| -20°C | X | X | X | X | X | X | X | X |
| 4°C | | X | X | X | X | X | X | X |
| 25°C/60%RH | | X | X | X | X | X | X | X |

Observations will be taken at the end of every time point, and changes (if any) in appearance, color, odor and precipitation will be noted. Important assays will include content, stability defining HPLC/PAGE, efficacy assays etc.

## ANALYTICAL AND IMMUNOLOGICAL METHODS (STAGE 1-3)

### Analytical and Bioanalytical Methods Development

Starting from the initial drug discovery phase, analytical methods are established throughout the drug development process. These applications can be categorized into two major subdivisions: pharmaceutical analysis (Drug substance and drug product) and bioanalysis. Pharmaceutical analysis involves the measurement of an analyte in a neat sample or formulation, and bioanalysis is the quantification of an analyte in a biological matrix.

Reliable analytical methods are required to test and qualify incoming components, in-process materials, bench formulations, DS, drug product, and stability samples. They must be accurate, precise, and specific to be suitable for quality control under GLP and GMP conditions. In addition, FDA and International Conference on Harmonisation (ICH) guidelines require stability testing on each lot of DS and DP. Therefore, analytical methods may need to be developed for a variety of materials and circumstances, each with a different intended purpose. For example, a screening analytical method needed for formulation development may not require the same performance characteristics as a stability-indicating method for drug product.

Analytical support consists of two phases: research and development (R&D) and regulated studies (GLP/GMP). The R&D phase includes analytical method development and analytical support for preformulation and formulation process development. The rest of the analytical work is conducted according to GLP and GMP guidelines and is performed with well-documented methodology and tighter performance characteristics and specifications. The specifications of the tested materials are made more stringent as the drug development process progresses, and therefore the performance characteristics and reproducibility of the analytical methods must improve as well.

It is essential to use methodology validated to test the DS to be used in clinical manufacturing. The method must satisfy two requirements: (1) it must be accurate, requiring high specificity, precision, and reproducibility; (2) it must be practical, with the necessary ruggedness, sensitivity, and linearity. Assay methods are verified under the ICH guidelines for reproducibility, specificity, selectivity, accuracy, linearity, precision, applicable concentration range, limit of detection, limit of quantitation, ruggedness, and robustness. Several test methods, such as the United States Pharmacopeia (USP) <61> MLT, are well established, and the FDA expects to see a justification for the failure to use them. The release specifications for a GMP DS typically include a combination of tests outlined above in Table 7.

Similar methods are also employed to evaluate formulations developed as part of the preformulation activity and may look like the following.

Appearance (color, clarity)

- Particulate Matter (both visible and subvisible)
- pH
- osmolality
- protein concentration
- Chromatographic purity (generally HPLC)
- Water content (if lyophilized)
- Container closure integrity testing (needed only at the GMP stages)

- Sterility
- Bacterial endotoxin
- Extractable volume

Consideration should be given to establishing a particle profile early in development and continue to characterize the profile through to commercialization due to their potential impact on immunogenicity.

SEC, DLS, AUC, MS, peptide mapping, CE, IEX, IEF, and CE-HPLC, forced degradation and stability studies are used to assess stability and storage-induced modifications. The above studies provide evidence that the mAb will be stable and homogeneous in the proposed formulation for the period of time required to complete dosing after preparation of the material. For example, if stability studies demonstrate that a mAb remains in solution with stirring and is stable for 10 days after preparation when stored refrigerated, it is feasible to prepare dosing solutions once weekly. In contrast, compounds with poor stability may require fresh daily preparation. Finally, concentration analysis of dose solutions is required to ensure that they have been prepared properly. Analysis of every preparation is not required; however, regular, representative samples are necessary. This effort might typically include analysis of the Week 1 and Week 4 samples of a 28-day GLP rat toxicity study.

From a practical standpoint, GLP studies of mAbs are typically conducted using GMP manufactured drug product or GMP pilot batches. Additional analyses may therefore be unnecessary to support GLP studies because the material has already been fully characterized and stability determined prior to initiation of the GLP studies. There may be exceptions if the GLP studies require dilution of the GMP lot (e.g., to achieve lower dose levels).

The suitability of a final compound for pharmaceutical use requires establishment of its identity and purity, as well as knowledge of its chemical and physical properties. The purpose of analyzing a formulation is to verify the active component; assess its potency; determine its shelf-life stability and the uniformity and dissolution properties of its dosage units; and determine whether the formulation process has resulted in degradation products or impurities. It is important to ensure that materials of known purity and defined quality are used in all studies and that they conform to applicable FDA regulatory requirements.

**Bioanalytical Methods**

Physiologic fluids such as blood, serum, or urine are analyzed to determine the fate and disposition of a DS administered to a test animal or patient. Aliquots of blood may be sampled over time to determine therapeutic drug concentration ranges. Often the goal is to assess the overall absorption, disposition, metabolism, and excretion (ADME) of the drug substance. The concentration of the drug in the biological matrix changes with time, typically over a broad range, and necessitates quantitation limits at levels much lower than those required for formulated or bulk drugs. An appropriate bioanalytical method is required to detect drug at these low levels, as well as linearly over an appropriate range. Matrix effects and stability issues can also make accurate analysis of the analyte difficult; these include endogenous materials extracted from the biological matrix that may interfere in the analysis, enzymes in the biological fluid that are capable of metabolizing or interacting with the analyte, plasma proteins to which the analyte can bind, concomitant drugs that might interfere in the analysis, and so on. All these factors must be considered when planning an analysis.

To determine blood levels of a mAb for PK and toxicokinetic studies, ligand binding assays need to be developed. Frequently the assay of choice is an ELISA specific for the mAb. If the test article is a mixture of multiple mAbs, an ELISA specific for each component is developed, and the blood samples (either plasma or sera) are tested in independent assays. We expect that the analytical method conditions selected will undergo further optimization and validation, depending on the conditions of use and regulatory phase of development.

Similarly, antidrug antibody screening assays need to be developed to assess the potential for loss of efficacy and serious side effects of the mAbs. These ELISA assays are designed to detect antibody responses against the mAbs. The assay configuration can include double antigen bridges and mAb capture and immunoglobulin detection.

**Assay Validation**

Validation of an analytical method identifies the sources of potential error and quantifies the performance characteristics of an assay. Regulatory requirements for assay validation are summarized in Guidance for Industry: Bioanalytical Method Validation, FDA CDER, May 2001 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf); USP <1225> Validation of Compendial Procedures; and Validation of Analytical Procedures: Text and Methodology, Q2(R1), ICH Harmonized Tripartite Guideline, November 2005. These apply to samples from both GLP animal studies and human clinical subjects.

The validation process should address the following key parameters:

a. Standards: Calibration standards are used in spiking strategies to validate the assay and provide a positive control in routine analysis.

b. Accuracy: Existing analytical procedures, when available, independently measure the analyte and confirm the values reported by the assay undergoing validation.

c. Precision: Repeated sampling of a defined, homogeneous sample determines the degree of agreement among individual test results, expressed as the variance, standard deviation, or coefficient of variation. Elements of precision testing include:

   i. Repeatability—Intra-assay variability under the same operating conditions (same operator and day)

   ii. Intermediate Precision—Intra-assay variability by different analysts or assays on different days

   iii. Reproducibility—Variability of assays performed in different laboratories.

d. Specificity: The analyte of interest (i.e., the mAb) will be present in samples from a wide variety of sources and with excipients, degradants, impurities, and matrixes with the potential for interfering backgrounds. Additionally, analytes are frequently measured in biological fluids (e.g., serum, saliva, urine, bronchioalveolar lavage) that can vary among animals or human subjects or in an individual subject over time, and these variations can affect assay performance. Therefore, the performance of an assay must be validated in multiple samples of the actual biological matrix that will be collected and analyzed. In addition, potential interference from other substances (e.g., aspirin, antibiotics, prescription medications associated with the patient population tested) must be tested.

e. Detection Limit: The limit of detection (LOD) must be quantitated by spiking a control with a known quantity of analyte standard.
f. Quantification Limits: These are defined as the lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) of the analyte that can be measured with an acceptable level of accuracy and precision; one must also define the acceptable signal-to-noise ratio of the analysis.
g. Linearity and Range: The range in which the assay result is proportional to the biomarker concentration defines the functional limits of the assay.
h. Robustness: The effects of environmental variations (e.g., temperature, humidity, electrical surge, concussion) and performance variations (e.g., incubation times, longevity of reagents, variability within or between patients) define the limits on reliability in normal use.

Acceptance criteria for each parameter listed above should be established in advance.

## FDA GUIDANCE FOR PHARMACEUTICAL GMP

The FDA's efforts to modernize regulations for biologics manufacturing, including monoclonal antibodies (mAbs), have been significant. Beginning with the initiative "Pharmaceutical cGMPs for the 21st Century – A Risk-Based Approach" in September 2004, the FDA has aimed to enhance the quality standards for veterinary and human biological products. The full report is accessible here. Subsequent progress reports, such as the one published in May 2007, provide updates on this initiative, available here:

https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/pharmaceutical-quality-21st-century-risk-based-approach-progress-report

Collaborating closely with the International Conference on Harmonisation (ICH), the FDA has contributed to the development of guidelines specifically tailored to biologics manufacturing. Notable among these is the Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity to a Reference Product, emphasizing comparisons in manufacturing processes, analytical methods, and product characterization. This Guidance document can be found here:

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/quality-systems-approach-pharmaceutical-current-good-manufacturing-practice-regulations

Furthermore, the Guidance for Industry: Development of Therapeutic Protein Biosimilars provides recommendations on the development and approval pathways for biosimilar products, emphasizing considerations such as cell line selection, expression system optimization, purification processes, and comparability assessments.

Another essential document, the Guidance for Industry: Biosimilars and Interchangeable Biosimilars: Licensure for Fewer Than All Conditions of Use for Which the Reference Product Has Been Licensed, offers recommendations on demonstrating interchangeability of a biosimilar with its reference product. It includes considerations for manufacturing comparability studies, immunogenicity assessments, and post-market monitoring.

In addition to FDA guidance, the ICH has contributed significant guidelines relevant to biologics manufacturing. These include:

- ICH Topic - Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process, published in 2004. Access it here.
- ICH Topic - Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products, from November 1995. Found here.
- ICH Topic - Q1B Photostability Testing of New Active Substances and Medicinal Products, released in 1996. Available here.
- ICH Topic - Q1A (R2) Stability Testing of New Drug Substances and Products, from 2003. Access it here.

The guidelines above provide a robust framework for ensuring the quality, safety, and efficacy of biologic products, including mAbs, throughout their development, manufacturing, and post-market phases.

## EFFICACY

### Overview

Pharmacology proof-of-principle studies are used in early stage preclinical development to help determine test article efficacy for a specific indication. Good evidence of efficacy in an animal model can provide confidence in candidate selection. *In vitro* and *in vivo* models are used to assess whether the drug can produce the desired effect. The objective of the efficacy studies is to determine if the mAb will act as a therapeutic in an animal model of infection.

Both *in vitro* and *in vivo* efficacy data are needed to support a development program. Development should not proceed until efficacy against targeted infectious agent is confirmed in at least one animal model. If no animal model exists, a suitable assay may need to be established. Establishing a new animal model for efficacy can be challenging and time consuming but minimizes the risk of failure at a later stage of development which can be costly. In cases of extremely rare diseases, in the absence of a feasible animal model (e.g., smallpox), it may be possible to advance drug candidates, even to the clinical stage, using *in vitro* efficacy only, or even surrogate agents (e.g., vaccinia or monkey pox as a model for smallpox).

### Animal Rule

Of relevance to anti-infective agents for biodefense is the "Animal Rule." This approach to drug approvals is a regulatory approach to the development of medical countermeasures, officially known as "Approval of Biological Products/New Drugs when Human Efficacy Studies are not Ethical or Feasible," put in effect in 2002. It is defined in 21 CFR 314.610 and 601, subpart H. Additional information can be found at:

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.610

The Animal Rule allows the FDA to approve certain biologics and drugs used to reduce or prevent toxicity of chemical, biological, radiological, or nuclear substances based on evidence of effectiveness from appropriate animal studies when adequate and well-controlled efficacy in humans cannot be ethically conducted. Since the PK and efficacy animal studies are surrogates for humans, they must be conducted in GLP compliance, with validated assays, for most Animal Rule applications.

The FDA guidance document can be found at:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078923.pdf.

Safety of these products can be studied in human volunteers in subsequent clinical studies unless adequate human safety has already been established through previous clinical trials.

Data from animal studies must be sufficient to establish effectiveness in humans. Such effectiveness can be established when:

- The biological agent's mechanism of efficacy is well understood.
- Endpoints in the animal trials are clearly related to benefit in humans.
- The product's effects are demonstrated in a species expected to react similarly to humans.
- Data allow selection of an effective human dose.

Using the Animal Rule, 16 products have been approved to date. A complete list of current Animal Rule approvals is available on the FDA website at:

https://www.fda.gov/media/150191/download

Products approved for infectious diseases include levofloxacin, ciprofloxacin and moxifloxacin for plague; tecovirimat monohydrate and brincidofovir for smallpox; and obiltoxaximab, raxibacumab, ANTHRASIL and BioThrax® for anthrax.

## TOXICOLOGY AND PHARMACOKINETICS STUDIES (PRELIMINARY STAGE 2 & DEFINITIVE STAGE 3)

### Overview

Despite numerous technical advances in the science of toxicology and attempts to develop *in silico* screening, the primary methods used to assess safety remain single- and repeat-dose toxicology studies conducted in rodent and nonrodent species. However, protein products such as mAbs present unique regulatory concerns. SRI highly recommends communication with the FDA to obtain some assurance from the agency that it considers the intended toxicology studies acceptable in relation to the intended therapeutic because the uniqueness of each product and the rapidly evolving technologies used in antibody development require a flexible case-by-case approach for biologics review that is based on a strong scientific understanding of relative risks and benefits. Although pilot and range-finding studies need not conform to GLP regulations, definitive pivotal studies must be conducted under GLPs and performed to meet the testing requirements of the FDA as codified in title 21 of the Code of Federal Regulations (21 CFR) and the ICH guidance.

### Considerations for Species Selection

Toxicology studies supporting drug development are typically conducted in two species, one rodent and one nonrodent. Rabbits or nonhuman primates (NHPs) are primarily used for testing vaccines or mAbs, whereas dogs are most often used for small molecule drugs. For most biologics, rabbit is the preferred species owing to the significant cost savings; however, based on antibody cross-reactivity or other studies, a decision may be made to use NHPs such as cynomolgus macaques.

Note that FDA has recently weakened the strict requirement for two species, provided a strong scientific justification was provided. There are several mAb projects that have successfully reached IND with a single species (rabbit), or with a comprehensive assessment in one species (rat) plus a small confirmatory assay in a non-human primate study. Therefore, while the FDA's official position is that two species are always required, in practice this is not always the case. The Draft Consensus Guideline Addendum to ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1) elaborates on this topic by stating if there are two pharmacologically relevant species for the clinical candidate (one rodent and non-rodent) then both species should be used for short-term toxicology studies of up to one month duration. The use of one species is justified when the biological activity of the biopharmaceutical is well-understood or the clinical candidate is active in only one species.

In addition, even when two species may be needed to characterize short-term toxicity, the use of only one species in long-term toxicity studies may be justified if the short-term toxicity profile is similar between the two nonclinical species. In these situations, the rodent species should be considered for long-term studies unless there is a scientific justification for using non-rodents. Furthermore, the Guideline discourages the use of two nonrodent species in the same nonclinical program.

For antibody products against foreign targets, such as bacterial or viral targets, the ICH S6(R1) Addendum recommends a limited scope of toxicology testing: one short-term safety study in one species (with adequate justification of species selection); additional toxicity studies, including reproductive toxicity, are not considered appropriate in this situation. An alternate strategy proposed in the guidance is to incorporate safety assessment endpoints in proof-of-principle studies using animal models of disease. This approach is likely to provide information on potential safety endpoints associated with the target.

In cases where no appropriate nonclinical species can be identified, the use of relevant transgenic models expressing the human receptor, or of homologous proteins ("tool molecules") developed to demonstrate species-specificity may be acceptable alternatives. The information provided by studies employing homologous molecules will be limited to hazard characterization only – that is, identification of adverse effects due to exaggerated pharmacology, and may not allow for quantitative risk assessment - evaluation of dose response and identification of a NOAEL or similar limit dose useful for establishing first-in-human dose.

A recent FDA Guidance document discourages the use of NHPs due to recent COVID-19 pandemic-induced shortages of NHPs, and they indicate that NHPs should be used only when "the sponsor can provide a scientifically compelling reason why NHPs must be used" (FDA, 2022). While this guidance has since been withdrawn as the public health emergency status expired, the supply constraints on NHPs remains a persistent issue, and the current recommendation from FDA is that other models be given preference, where scientifically justified, in order to limit use of NHPs to those situations where no other species is relevant (Brown and Wange, 2023). The November 2023 FDA Guidance on "COVID-19: Developing Drugs and Biological Products for Treatment or Prevention" specifically recommends conducting COVID-treatment related preclinical proof-of-principle studies in small animal models due to limited availability of NHPs.

For purposes of this PDP, we assume that rat and rabbit are the two species that will be evaluated, and that full safety and PK assessments will be required in both species. The PDP also includes proposed designs for PK and safety studies using NHPs, for situations where use of other species has been ruled out as non-relevant due to the biology of the target and the characteristics of the therapeutic product. A strong scientific justification for the use of NHPs is likely to be required by the FDA in these situations.

**Bispecific Antibodies**

Evaluation of the expression profile and individual target specificity is a required step in the selection of nonclinical species for antibodies with dual specificity, and *in vitro* pharmacology data may provide data to support a scientific rationale; however, comparative studies between the bispecific antibody and its constituent monospecific products are not likely to be required.

**Immunogenicity**

Monoclonal antibodies and other biologic products intended for administration to humans may lead to an immune response following administration to animal species used in preclinical safety studies. In practical terms, this is evident as detection of anti-drug antibodies (ADA) in the course of toxicology studies. The development of ADA in nonclinical species is not predictive of the immunogenicity potential of a drug in humans, and are not considered to be adverse events by the FDA. Nevertheless, ADAs are typically measured in repeat-dose toxicity studies to aid with interpretation of results from these studies. This is because ADAs may impact pharmacokinetic and/or pharmacodynamic parameters by removing the drug from circulation or may alter the incidence and/or severity of adverse effects via complement activation or immune complex formation and/or deposition. The formation of ADAs may also impact design of subsequent nonclinical studies, for example a significant incidence of neutralizing antibodies in a short-term study could make a longer-term study unfeasible due to loss of pharmacological effect upon extended dosing.

**Nonclinical Study Duration in Relation to Proposed Clinical Studies**

Preliminary toxicity studies are often performed as part of the lead compound selection process. For IND-directed safety studies, only one complete GLP-compliant safety study for each relevant nonclinical species is generally required for an IND application. The route of administration of these studies must be the same as the proposed clinical route. For example, if the proposed route is intravenous (iv), the drug is administered iv to rats and rabbits. The duration of administration and dose regimen must at a minimum conform to the proposed clinical protocol. For example, to support a 14-day daily dose administration human clinical trial typically requires a toxicity study of 14–28 days duration with a post-treatment recovery phase. The frequency of dosing (e.g., three times a week for 4 weeks) in the animal studies should also reflect the clinical dosing schedule, though more frequent administration in animal studies may be employed to present a "worst case scenario" model of toxicity. Because mAbs frequently have a long half-life, once weekly dosing may be proposed for the clinic, and therefore once weekly doses should be proposed for the preclinical safety studies, as well.

Safety tests should be performed with GMP or "GMP-like" DP whenever possible. While this is not a formal FDA requirement, sponsors are required to demonstrate that the clinical drug is essentially the same as that used in animal safety studies. If a significant difference is observed between GMP and non-GMP batches, the safety studies could be considered invalid and the Sponsor may be asked to repeat them, with significant negative impact on program timeline and budget. Treatment groups should include a recovery group to evaluate whether adverse effects are transient or irreversible. Because most repeat-dose toxicity studies of therapeutics reveal some adverse effects at high doses, it is essential to test for reversibility of adverse effects.

**Reproductive and Developmental Toxicity Studies**

To support clinical trials that will include large populations of patients of reproductive age, reproductive toxicity testing is required for later phase clinical trials. If required for a specific program, these tests should be conducted in a pharmacologically relevant species. Reproductive toxicity studies are generally not required for Phase I trials unless the therapeutics are specifically intended for the treatment of pregnant women. They are unlikely to be required for most mAbs.

**Genotoxicity/Carcinogenicity Studies**

Genotoxicity studies conducted for small molecules are not applicable to monoclonal antibodies since these are not expected to interact directly with DNA or other chromosomal material. Therefore, these studies are not recommended for the safety assessment of monoclonal antibodies. Similarly, standard carcinogenicity assays are not appropriate for monoclonal antibodies. A product-specific assessment may be needed depending on a specific cause for concern, e.g., use in immunocompromised populations, products with potential to induce clonal expansion, cell proliferation, or other mechanisms relevant for carcinogenicity.

There may be exceptions, in particular when a monoclonal antibody is targeting a component in the bone marrow. FDA may request conduct of an *in vivo* micronucleus assay, to be conducted as part of a rodent toxicity study. There are unlikely to be many cases like this in mAbs targeting infectious disease pathogens.

## PHARMACOKINETICS OVERVIEW

Pharmacokinetics, the study of the time course of drug concentration, distribution, and elimination of a drug in the body, is a key determinant in the selection of a viable drug candidate. Results of these tests provide an early evaluation of the properties of potential pharmaceuticals and allow concentration of additional efforts on only the most promising compounds.

PK parameters are derived from the measurement of drug concentrations in the serum or blood, providing information that can guide future animal and clinical studies for the selection of the dose levels and timing of administration. The IND package requires PK data generated in two species (one rodent and one nonrodent), preferably using the same two species used for the safety studies. These studies usually include multiple dose levels so that dose dependency can be evaluated. In many mAb development programs, ELISA assays are utilized to detect each individual mAb antibodies in rabbit and rat serum, and will be developed and validated for the purpose of use for PK and TK analysis. The ELISA assay will detect the individual antibodies in the PK and TK in toxicology study. It may be possible to combine PK studies with toxicology studies if there is information indicating that the mAb's half life is short enough to evaluate all PK parameters within the timeframe of the toxicology study. It is not uncommon for detectable level of mAbs to be present 60 days (or longer) after administration which is longer than most IND enabling toxicology studies so combining PK and toxicology studies should only be done when appropriate. Alternatively, longer recovery periods in toxicology studies can accommodate the long half-life seen with many mAbs (e.g., increasing a typical 14-day recovery period to 60 days).

## RECOMMENDED TOXICITY AND PHARMACOKINETICS STUDIES

### Pharmacokinetics and Dose Range-Finding Studies (Stage 2)

Initial animal studies will focus on establishing a reasonable dose range for testing in two species, and in establishing the basic PK parameters, including bioavailability, half-life ($t_{1/2}$), maximum concentration ($C_{max}$), and time to maximum concentration ($T_{max}$). These studies are typically not conducted in compliance with FDA GLP regulations unless the therapeutic is being developed under the Animal Rule (described above). If a therapeutic is likely to be developed under the Animal Rule, the definitive PK studies (but not dose range-finding or pilot studies) should be conducted in full GLP compliance.

The specific studies and phases involved in this activity are outlined below.

#### Bioanalytical Method Development and Validation (Two Species Validation)

Bioanalytical methods must be developed and validated for biological samples to support preclinical PK and toxicokinetic (TK) studies. The bioanalytical method for detecting and measuring the mAbs is an ELISA specific to mAb, or in the case of a cocktail each mAb in the cocktail. To validate the method, linearity, accuracy, precision, specificity, range, limit of detection, and system suitability are established. The following steps will be taken for this effort:

- Develop method in rat serum.
- Validate method in rat serum.
- Cross-validate method in rabbit serum.
- Prepare Method Development and Validation Report.

Prior to performing any studies involving administration of the lead candidate to animals, a pilot stability analysis in serum should be performed to determine whether additives should be included in the blood collection tubes to prevent breakdown of the test antibodies during the processing steps before the bioanalytical assay. This stability study will entail development of an assay for each mAb in serum first, and then application of the assay to the pilot stability samples.

#### ELISA Method Development and Validation for the Immunogenicity Analysis

An ELISA assay to detect host antibodies produced in response to treatment with the drug/test article antibody must be developed. The assay will not detect antibody titers against individual test antibodies, but rather the total amount of antibodies against the antibody test articles. The ELISA assay should be developed and validated for detection in serum from each nonclinical species used in development. Briefly, plates will be coated with an equal amount of each antibody, and after several washes, serial dilutions of the sera from the treated (and untreated control) animals will be added to the plates. After incubation and washes, a directly labeled reporter antibody (species-specific) will be added to the plates followed by a substrate. The optical density will be read in an ELISA plate reader.

### Tissue Cross-Reactivity Study (Stage 1-2)

Tissue cross-reactivity (TCR) studies are typically required for mAbs to confirm that they are not binding to a nontarget tissue and producing an adverse effect. Assays are routinely conducted in human tissues and in tissues from the proposed preclinical species (e.g., rat and rabbit), though recent FDA discussions have questioned the value of assessment of species other than human. The Draft Consensus Guideline Addendum to ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceutical S6(R1) states that TCR should not be used for selection

of relevant species for safety evaluation but data with human tissues can provide useful information on potential human off-target binding. The S6(R1) Guideline Addendum limits the circumstances when TCR studies are conducted on animal tissues: only those tissues that are positive in humans may be evaluated in animal species to aid in interpretation of preclinical safety findings, and conducting TCR assays on full panels of animal tissues is not recommended. While the TCR requirement may be reduced to human tissues only at some future date, the current FDA guidance is for multiple preclinical species plus human tissues. Limiting TCR evaluation to just human tissues should be discussed with the FDA at the Pre-IND meeting.

Appropriate cell lines may be required for the assays. For example, positive control cell lines using cells transfected with binding epitopes of the two different mAbs may be used. Likewise, a negative control cell line transfected with empty or nonfunctional vector may be appropriate. Studies may be conducted as a screening (non-GLP) study, but GLP compliance may be recommended, especially if the Animal Rule will be used for the IND application.

The first step in the TCR studies is development and validation of an assay to measure TCR of the test article (either a single mAb or a mixture of multiple mAbs). This effort includes:

- Determination of optimum fixation and detection method
- Conjugation of the test article to enable detection.
- Verification of conjugation
- Verification that conjugation does not affect mAb avidity for its target.
- Titration of test article and secondary/tertiary antibodies.

Once methods are developed and validated, the TCR assay is performed on a set of tissues from the desired species. Tissues are collected from three unrelated donors and tested as described below. The tissue list varies slightly based on the species being evaluated. The list for humans is shown below. Tissues used for rat, rabbit, or other species may vary slightly from those listed.

- Adrenal
- Bladder
- Blood cells (smear)
- Bone marrow
- Breast
- Cerebellum
- Cerebral Cortex
- Colon
- Endothelium (Aorta)
- Eye
- Fallopian Tube
- Gastrointestinal tract
- Heart
- Kidney
- Liver
- Lung
- Lymph node
- Ovary
- Pancreas
- Parathyroid
- Pituitary
- Placenta
- Prostate
- Skin
- Spinal cord
- Spleen
- Striated muscle
- Testis
- Thymus
- Thyroid
- Tonsil
- Ureter
- Uterus (cervix)
- Uterus (endometrium)

Studies may be conducted either as all species in a single protocol/report, or as three separate studies. If a mAb cocktail is used, it may be wise to test each mAb individually; with this approach, a mAb with binding can be abandoned, while leaving a “clean” report of a remaining mAb for further preclinical work. The studies consist of the following steps:

- Prepare fresh frozen sections 5–8 µm thick
- Confirm tissue integrity by H&E stain and immunostain using appropriate antibody
- Evaluate slides with at least two dilutions of conjugated test article and appropriate negative control
- Include positive and negative control cell lines in each assay run
- Score (by pathologist) to indicate relative staining intensity

**Range-Finding Study in Rats (Stage 2)**

- Five males and five females (5M/5F) per dose group; control and three dose levels.
- Two weekly dose administration via iv injection on Days 1 and 8. Animals sacrificed on Day 9.
- Daily clinical observations Days 1-9.
- Body weights on Days 1, 5,8 and 9 before necropsy.
- Clinical pathology (hematology, clinical chemistry) on Day 9.
- Macroscopic evaluation at necropsy; 5/sex/group will be necropsied on Day 9.
- Organ weights determined at necropsy.
- Histopathologic evaluation of high dose and control tissues; target organs evaluated in mid- and low-dose groups.

**Single Dose PK Study in Rats (Stage 1-2)**

- Three groups: low, mid, and high dose levels
- Groups of 15M/15F animals (five sets of 3M/3F, each evaluated at time points following a single administration for a total of ~15 time points; e.g., predose, 5 min, 1, 3, 6, 24, and 48 hours postdose administration, and once on Days 4, 6, 8, 11, 15, 29, 57, and 72. Half-life of mAbs can be long, and times will be adjusted accordingly. If the half-life is known to be shorter based on other studies, the time period will be set to approximately four times the anticipated half-life.
- Serum samples are collected for TK analysis; additional samples are collected on Day 72 for immunogenicity evaluation.
- Evaluation of mAb levels in serum using validated bioanalytical method (ELISA).
- Calculation of PK parameters: $C_{max}$, $T_{max}$, area under the plasma concentration time curve to the last timepoint and to infinity ($AUC_{last}$, $AUC_{inf}$), terminal elimination phase half-life ($t_{½}$), volume of distribution (Vd), and clearance (Cl) are standard pharmacokinetic parameters that are calculated using noncompartmental methods. Other parameters may be determined depending on the study design and the objectives of the study. The impact of immunogenicity on serum concentrations will be considered in the determination of pharmacokinetic parameters.

**Range-Finding Toxicity Study in Rabbits (Stage 2)**

- 3M/3F per dose group; control and 3 dose levels
- Two weekly dose administrations via iv injection on Days 1 and 8. Animals sacrificed on Day 9.

- Daily clinical observations Days 1-9.
- Body weights on Days 1, 5, 8, and 9 before necropsy.
- Clinical pathology (clinical chemistry, hematology, and coagulation) before necropsy on Day 9.
- Macroscopic evaluation at necropsy; 3/sex/group will be necropsied on Day 9.
- Organ weights determined at necropsy.
- Histopathologic evaluation of high dose and control tissues; target organs evaluated in mid and low dose groups.

**Single Dose PK Study in Rabbits (Stage 2)**

- Two groups, low and high dose levels. 3M/3F animals per group.
- All rabbits evaluated at time points following a single administration for a total of ~15 time points; e.g., predose, 5 min, 1, 3, 6, 24 and 48 hours postdose administration, and once on Days 4, 6, 8, 11, 15, 29, 57, and 72. Serum samples are collected.
- Serum samples are collected for TK analysis; additional samples are collected on Day 72 for immunogenicity evaluation.
- Evaluation of mAb levels in serum using validated bioanalytical method (ELISA).
- Calculation of PK parameters: $C_{max}$, $T_{max}$, area under the plasma concentration time curve to the last timepoint and to infinity ($AUC_{last}$, $AUC_{inf}$), terminal elimination phase half-life ($t_{½}$), volume of distribution (Vd), and clearance (Cl) are standard pharmacokinetic parameters that are calculated using noncompartmental methods. Other parameters may be determined depending on the study design and the objectives of the study. The impact of immunogenicity on serum concentrations will be considered in the determination of pharmacokinetic parameters.

**Single Dose Range Finding and PK Study in NHP (Stage 2)**

- 2M/2F per dose group; control and two dose levels (low and high dose)
- Single dose administration via iv injection on Day 1.
- Daily clinical observations Days 1-9; weekly thereafter.
- Body weights on Days 1, 5, 8, and 9.
- Clinical pathology (clinical chemistry, hematology, and coagulation) on Day 9.
- Serum samples are collected for TK analysis from all animals at ~15 time points following a single administration e.g., predose, 5 min, 1, 3, 6, 24 and 48 hours postdose administration, and once on Days 4, 6, 8, 11, 15, 29, 57, and 72.
- Additional serum samples are collected on Day 72 for immunogenicity evaluation.
- Evaluation of mAb levels in serum using validated bioanalytical method (ELISA).
- Calculation of PK parameters, including $C_{max}$, $T_{max}$, area under the plasma concentration time curve to the last timepoint and to infinity ($AUC_{last}$, $AUC_{inf}$), and terminal elimination half-life ($t_{½}$). The impact of immunogenicity on serum concentrations will be considered in the determination of pharmacokinetic parameters.
- This is a survival study; animals are returned to the colony upon study completion.

**Safety Studies (Stage 3)**

Definitive, IND-directed toxicology studies are required to demonstrate safety of a drug before initiation of human trials. All studies are required to be conducted under full GLP compliance, as outlined below. These GLP studies (as well as any GLP studies conducted under the PK section described above) must include analytical chemistry support to confirm purity, identity, stability, homogeneity, and concentration of dose formulations.

**Five Week Repeat Dose Toxicity Study in Rats (GLP)**

| Group | Article | Dose Level (mg/kg) | Route | Dose Volume (ml/kg) | Number of Animals | | |
|---|---|---|---|---|---|---|---|
| | | | | | Main Group (Day 29) | Recovery Group (Day 57) | TK Satellite Group |
| 1 | Vehicle Control | 0 | IV | TBD | 10M + 10F | 5M + 5F | 9M + 9F |
| 2 | Test | Low | IV | TBD | 10M + 10F | 5M + 5F | 9M + 9F |
| 3 | Test | Mid | IV | TBD | 10M + 10F | 5M + 5F | 9M + 9F |
| 4 | Test | High | IV | TBD | 10M + 10F | 5M + 5F | 9M + 9F |
| Total Animals | | | | | 40M + 40F | 20M + 20F | 36M + 36F |

- 15M/15F per dose group; control and 3 dose levels.
- Five weekly dose administrations via iv injection on Days 1, 8, 15, 22, and 29.
- Daily clinical observations.
- Body weights on Days 1, 8, 15, 22, 29 and at necropsy on Days 30 (1 day after last dose administration) and 57 (4 weeks after dose administration).
- Food consumption weekly.
- Clinical pathology (clinical chemistry, hematology, urinalysis) before necropsy (10/sex/group on Day 30 and 5/sex/group on Day 57, which are 1 and 28 days, respectively, after the end of weekly dose administration).
- Satellite TK groups; serum drug levels on 3M/3F per group at a total of 6 time points: i.e., predose, 5 min, 2, 4, 8 and 24 hours on Days 1, 29, and 57. No other toxicological evaluations for the TK animals.
- TK samples will be analyzed by the validated ELISA method.
- Immunogenicity analysis; samples taken predose (5/sex on extra animals not included in the study design) and on Days 30 and 57.
- Ophthalmology prestudy and the week before each necropsy.
- Urinalysis in the week before each necropsy.
- Necropsy 10/sex/group on Day 30 and 5/sex/group on Day 57.
- Organ weights determined at necropsy.
- Histopathologic evaluation of high dose and control tissues; target organs evaluated in mid and lowdose groups.

**Five Week Repeat Dose Toxicity Study in Rabbits (GLP)**

| Group | Dose (mg/kg) | Route | Dose Volume (ml/kg) | Number of Animals | |
|---|---|---|---|---|---|
| | | | | Main Group (Day 29) | Recovery Group (Day 57) |
| 1 | 0 | IV | TBD | 5M + 5F | 5M + 5F |
| 2 | Low | IV | TBD | 5M + 5F | 5M + 5F |
| 3 | Mid | IV | TBD | 5M + 5F | 5M + 5F |
| 4 | High | IV | TBD | 5M + 5F | 5M + 5F |
| Total Animals | | | | 20M + 20F | 20M + 20F |

- 5M/5F per dose group; control and 3 dose levels.
- Five weekly dose administrations via iv injection on Days 1, 8, 15, 22, and 29.
- Daily clinical observations.
- Body weights on Days 1, 8, 15, 22, 29 and at necropsy on Days 30 and 57.
- Food consumption weekly.
- Clinical pathology (clinical chemistry, hematology, and coagulation) before necropsy on Days 30 and 57, which are 1 day and 4 weeks after the last dose administration.
- TK analysis; serum collected on 5M/5F per group, at predose, 5 min, 2, 4, 8 and 24 hours on Days 1, 29, and 57.
- Immunogenicity analysis; samples taken predose and on Days 30 and 57.
- Ophthalmology prestudy and the week before each necropsy.
- Macroscopic evaluation; 5/sex/group will be necropsied on Days 30 and 57.
- Urinalysis: urine collected by cystocentesis if available at necropsy.
- Organ weights determined at necropsy.
- Histopathologic evaluation of high dose and control tissues, including the injection site; target organs evaluated in mid and low dose groups.

**Five Week Repeat Dose Toxicity Study in NHP (GLP)**

| Group | Dose (mg/kg) | Route | Dose Volume (ml/kg) | Number of Animals | |
|---|---|---|---|---|---|
| | | | | Main Group (Day 29) | Recovery Group (Day 57) |
| 1 | 0 | IV | TBD | 3M + 3F | 2M + 2F |
| 2 | Low | IV | TBD | 3M + 3F | 2M + 2F |
| 3 | Mid | IV | TBD | 3M + 3F | 2M + 2F |
| 4 | High | IV | TBD | 3M + 3F | 2M + 2F |
| Total Animals | | | | 12M + 12F | 8M + 8F |

- 5M/5F per dose group; control and 3 dose levels.
- Five weekly dose administrations via iv injection on Days 1, 8, 15, 22, and 29.
- Daily clinical observations.
- Body weights on Days 1, 8, 15, 22, 29 and at necropsy on Days 30 and 57.
- Food consumption weekly.
- Clinical pathology (clinical chemistry, hematology, and coagulation) predose and before necropsy on Days 30 and 57.
- TK analysis; serum collected on 3M/3F per group, at predose, 5 min, 2, 4, 8 and 24 hours on Days 1, 29, and 57.
- Immunogenicity analysis; samples taken predose and on Days 30 and 57.
- Ophthalmology prestudy and the week before each necropsy.
- Safety pharmacology endpoints (cardiovascular and respiratory measurements) can be included as an optional endpoint; animals will be fitted with telemetry transmitters for data collection.
- Macroscopic evaluation; 3/sex/group will be necropsied on Day 30 and 2/sex/group will be necropsied on Day 57.
- Urinalysis: urine will be collected prestudy and before necropsy.
- Organ weights determined at necropsy.
- Histopathologic evaluation of high dose and control tissues, including the injection site; target organs evaluated in mid and low dose groups.

**Compliance Considerations for Animal Research**

Much of the preclinical development phase involves work using laboratory animals. This work frequently falls under a variety of federal laws, accreditations and guidance documents including the Office of Laboratory Animal Welfare (OLAW), the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and for all non-rodent species, the U.S. Department of Agriculture (USDA). NIH's Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals can be found on the Office of Laboratory Animal Welfare (OLAW):

https://olaw.nih.gov/sites/default/files/PHSPolicyLabAnimals.pdf

This document presents the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, which include minimization of pain and distress, ensuring that the appropriate number of animals is used, and other key considerations for the humane care and use of laboratory animals.

Prior to engaging in studies that use laboratory animals, animal welfare compliance must be ensured.

# PROJECT MANAGEMENT AND RISK MITIGATION

## Project Management

Project management in a preclinical development program provides oversight and control of the various disciplines contributing to project completion on time and within the project budget. Project Managers (PMs) are responsible for coordinating technical resources and facilities between multiple projects and contracts. The Project Management group is responsible for working with staff to develop an overall plan for the path to IND, BLA, and market introduction, and for implementing the entire development plan, from discovery through preclinical and clinical development phases. The plan needs to address the interactions among the groups or companies that will perform the studies. A decision matrix identifying the person or function responsible for critical go/no-go decisions is helpful. A lead PM is typically assigned to each program, with the responsibility for communicating issues and status to the project team; managing resources; identifying, coordinating, and tracking project activities; and communicating project status at regularly scheduled meetings or by direct communication within the development team or with outside service providers.

An integrated schedule is necessary in order to be certain that materials or information needed by each functional group is available in a timeframe that fits with their internal schedules and with the overall schedule. Project management tools such as Microsoft Project Gantt charts, used to identify tasks, relationships, and timelines, are helpful for tracking progress. Tracking is crucial to ensuring that the target IND filing date is met. Potential risks and roadblocks along the way must be identified to minimize their impact on schedule, timeline, or resources. This is discussed in further detail in the next section.

## Risk Mitigation

Discovery and preclinical development of anti-infective therapeutics is a complicated process involving multiple scientific fields, regulatory constraints, GLP/GMP compliance, and testing and reporting requirements, all of which entail some risk of failure or unanticipated events that can affect plans, change strategy, or even eliminate a lead candidate. Identifying, assessing, and managing risks (real and potential) are integral to the entire development lifecycle. A risk management plan should be created for each stage of product development, with key personnel participating to identify, assess, and provide input on resolving issues or addressing unexpected findings. Some risks are controllable, but others are not; for instance, the results of an efficacy study may be uncontrollable, whereas the costs of the same study may be somewhat controllable. Uncontrollable risk can be evaluated and possibly resolved before more resources are expended on development; alternatively, it may have the potential to stop the program or eliminate the lead candidate. The evaluation of such instances is known as a go/no-go decision point and is sometimes referred to as an "early exit strategy." Although early abandonment of a program is viewed by some as program failure, acknowledging the necessity of exit could conserve funds and resources for other potential candidates. Identifying risks that could trigger an early exit strategy is thus encouraged, and one tool that can be used for doing so is the TPP, as previously discussed. A TPP can establish acceptable and preferred standards for assessing the key results at each stage of development. If acceptable criteria are not met, early-stage activities should be evaluated for further optimization, or all work on the drug candidate may need to be halted.

Contingency plans should be developed to address controllable risks. Drug development involves several technical groups with varying responsibilities, with each group identifying critical tasks that, if not properly executed, will adversely affect the cost and timeline of development. One approach is to determine the tasks that are key to successfully meeting program objectives and compile a list of contingency plans should one of the tasks fail to meet expectations. Contingency planning could include alternative sources for materials (i.e., approval of multiple vendors to supply the same production materials) or assembling a list of available consultants for troubleshooting or providing alternative solutions. Contingency planning may be limited by the resources available, but at a minimum, key tasks should be monitored with input from management or senior scientific staff in the appropriate technical group.

At some point during development, certain tasks or studies may be outsourced. Selecting, qualifying, and managing the activities of vendors, subcontractors, or outside service providers are important, yet time-consuming; however, a risk management overview should always be included at some level for any outsourcing component of the development program.

Interaction with a regulatory agency (e.g., FDA, European authorities) and submission of specific documents are required when advancing a drug candidate from the preclinical development phase into the clinical trial phases. There is no guarantee that the submitted information will allow for initiation of the desired human clinical trials; however, such risk can be minimized when a clear regulatory strategy is developed early in the product development process or in the late discovery stage. Identifying a regulatory path, planning for a pre-IND meeting with the FDA, and engaging experienced regulatory personnel to develop and interact with the FDA are all ways to minimize regulatory risk and to avoid a clinical hold. The route of dose administration, dosing regimen, target population, characterization specifications, potency, and stability of the potential DP are all important elements that will be subject to regulatory considerations. These early-stage regulatory activities and interactions with regulatory agencies, such as the FDA or their overseas counterparts, take time and entail associated costs, yet the potential savings far outweigh the cost of a clinical hold or having to repeat a nonclinical or clinical study.

Technical and regulatory considerations are important, but two items that are equally important to the successful execution of a development program are the budget and schedule. Although these two items are only somewhat controllable, they often constitute important metrics for measuring program progress and success. A schedule with a list of tasks and key milestones, with a go/no-go decision to be made at each milestone, is a valuable tool for managing progress. For a conservative and risk-adverse approach, tasks or studies could be initiated in sequence rather than in parallel. Doing so will lengthen the overall duration of the preclinical development program, but will allow adequate time for data review and interpretation before initiating the next set of task(s) and slow the rate of financial expenditures ("burn rate"). If the schedule or timing of a key milestone is critical, tasks can be initiated in parallel; doing so will accelerate the program (and burn rate), but any unanticipated event could deplete funds that could have been used on other activities or programs. Although initiating predevelopment tasks in parallel can be riskier, this approach is frequently used to accelerate the development process for preclinical programs. To minimize risk, reviews with go/no-go decisions and an exit strategy should be in place for this approach. Budget is particularly critical for small start-up companies, where a finite amount of funding is raised with the expectation that this will be sufficient to reach a key development milestone (e.g., initiation of Phase I trials). Not preparing for unexpected events can literally lead to the end of a company.

In conclusion, a risk management plan should be developed, to include strategies for addressing unexpected results and issues with vendors or subcontractors, and covering each of the various stages of the development program. A regulatory strategy developed early in the program along with a TPP will help guide decision making. The schedule and budget should incorporate key milestones with go/no-go decisions and define the allowable level of risk. The key to a successful risk management plan is to expect the unexpected and to be prepared to respond to surprises that arise during the development process.

**Summary of Recommended Tasks and Approximate Costs**

Tables 9 and 10 summarize the anticipated costs of CMC and Safety/PK components, respectively, at a typical contract research organization (CRO) for the preclinical development tasks recommended for a typical development program up through IND completion. Estimates in this table exclude the cost of efficacy studies, which are outside the scope of the NIAID-DMID preclinical resource program.

The development program outlined below assumes that efficacy and mechanism of action studies have been completed and that the lead candidate has been shown to have efficacy in an appropriate animal model. These estimates are also based on a single antibody. If a cocktail of two antibodies is proposed, the costs will approximately double, because each antibody will require its own cell bank, validation, and manufacture. There may be some savings in the formulation phase, but these savings would be relatively small (15-20%). Development costs (toxicology, etc.) would not increase appreciably for a cocktail because these studies generally are conducted on the final drug product, regardless of the number of mAbs present. Pharmacokinetics costs would increase somewhat for a cocktail, as separate analysis of each mAb is nearly always required.

**TABLE 9.**
**ESTIMATED COSTS OF CMC PHASE OF DEVELOPMENT**

| Task/Study | Estimated Internal Costs | Estimated External Costs | Estimated costs for Materials & Supplies |
|---|---|---|---|
| **Stable Cell Line Development** | $1,955,800-$2,306,800 | NA | $154,000-$175,600 |
| Cell line Development (human or humanized antibody), Selection and Stability | | | |
| **Production of a Master Cell Bank and Testing** | $191,200-$205,200 | $183,400-$196,600 | $56,200-$81,600 |
| **Pilot Operations** | 181,200-$2,544,400 | $116,200-$121,600 | $208,000-$251,200 |
| 1L/7L | | | |
| Fermentation/Purification Process Transfer | | | |
| BR Doc Prep Approval | | | |
| Non-GMP BR Approval | | | |
| Scale up Lot (130L) | | | |

| TABLE 9. ESTIMATED COSTS OF CMC PHASE OF DEVELOPMENT | | | |
|---|---|---|---|
| **Task/Study** | **Estimated Internal Costs** | **Estimated External Costs** | **Estimated costs for Materials & Supplies** |
| **GMP Lot Production** | $2,458,000-$2,992,600 | $132,400-$148,600 | $502,400-$610,400 |
| GMP BR Approval | | | |
| GMP Lot (130L) | | | |
| **Lot Release** | | | |
| GMP Testing | | | |
| Extended Characterization | | | |
| **Viral Clearance** | $154,000-$175,600 | $184,000-$195,600 | NA |
| MuL V Virus with process lot | | | |
| **Test Method Development and Qualification** | $432,000-$594,000 | NA | $43,200-$59,400 |
| A280 | | | |
| SDS-PAGE Assay | | | |
| Ion Exchange | | | |
| RP HPLC | | | |
| SEC HPLC | | | |
| DNA Detection | | | |
| Host Cell Protein | | | |
| Peptide Map | | | |
| **Formulation Development and Qualification** | $297,000-$405,000 | NA | $27,000-$37,800 |
| 5 mg/mL Formulation | | | |
| **Stability (BDS)** | $340,200-$464,400 | NA | $16,200-$21,600 |
| 6 core release tests, IND program (3 months) | | | |
| **Preparation of CMC Materials and Shipping** | $108,000-$129,600 | NA | NA |
| **Project Management** | $637,200-$864,000 | NA | NA |
| **Total CMC Costs: $8,377,600 - $12,581,600** | | | |

| TABLE 10.<br>ESTIMATED COSTS OF PK/TOXICOLOGY/IND PHASE OF DEVELOPMENT | |
|---|---|
| **Task/Study** | **Estimated Total Costs** |
| **Pharmacokinetics and Range-Finding** | |
| Bioanalytical (ELISA) Method Development and Validation (one species) | $450,000–$550,000 |
| ELISA Method Development and Validation for the Immunogenicity Analysis (one species) | $500,000–$550,000 |
| Tissue Cross-Reactivity (one species, non-GLP) | $300,000-$350,000 |
| 2-Weekly Dose Range-Finding Study in Rats | $125,000-$175,000 |
| 2-Weekly Dose Range-Finding Study in Rabbits | $200,000-$250,000 |
| Single Dose PK Study in Rats with Serum ELISA and Immunogenicity Analysis | $450,000-$550,000 |
| Single Dose PK Study in Rabbits with Serum ELISA and Immunogenicity Analysis | $550,000-$650,000 |
| Single Dose Range-Finding and PK Study in NHP with Serum ELISA and Immunogenicity Analysis | $1,500,000-$2,000,000 |
| Preformulation/Formulation Development | $450,000–$1,000,000 |
| **Pre-IND Meeting, GLP Studies and IND** | |
| Preparation of pre-IND Package/pre-IND Meeting (similar cost for Type C meeting) | $50,000-80,000 |
| 5-Weekly Repeat Dose Toxicity Study in Rats with Recovery (GLP) | $1,000,000-$1,500,000 |
| 5-Weekly Repeat Dose Toxicity Study in Rabbit with Recovery (GLP) | $1,250,000-1,750,000 |
| 5-Weekly Repeat Dose Toxicity Study in NHP with Recovery (GLP) | $4,000,000-$4,500,000 |
| IND Preparation | $150,000-250,000 |
| **Total Safety/PK/IND Costs:10,975,000-14,155,000** | |

## Pre-IND Meeting

Prior to the conduct of IND-directed clinical trials or GMP manufacturing, a pre-IND meeting (also referred to as a Type B meeting) with the appropriate FDA Division representatives is recommended to discuss the proposed preclinical and clinical trial protocols as well as the manufacturing and controls of the DS and DP. The main purpose of this meeting is to ask FDA representatives specific questions concerning the drug's development process involving the preclinical, manufacturing, and clinical approach to ensure that the proposed clinical trial can go forward following the IND submission. The request for the meeting is typically submitted ~60 days ahead of a proposed meeting date for meetings with CDER or CBER. The request should include a background document, including a proposed agenda, the list of specific questions

requiring FDA input or guidance, and the specific objectives of the meeting. Specifically referring to the pre-IND, the information provided to FDA should contain background and rationale for the proposed investigation of the drug candidate, as well as summaries of the manufacturing and controls process (including flowcharts), the proposed preclinical safety testing, and the proposed Phase 1 clinical trial(s). The FDA responds within 21 days after it receives a meeting request and, if the FDA agrees to the meeting, it occurs within a specified timeframe, depending on the type of meeting requested. With FDA input during the pre-IND meeting, the PDP and proposed clinical study concept are revised if necessary, and the preclinical studies are initiated as soon as the manufactured product is available. Table 11 summarizes the key components included in a pre-IND meeting package.

**TABLE 11.**
**INFORMATION INCLUDED IN PRE IND MEETING PACKAGE**

| Information needed | Description |
| --- | --- |
| Cover letter | Request for a pre-IND meeting |
| Information for meeting request | Agenda, list of objectives, list of specific questions, list of attendees, proposed Agency participants, suggested dates and time for the meeting |
| Pre-IND Briefing Document | |
| Product and meeting information | Information from the Meeting Request, providing updates to the original information as needed. on Meeting Grant details. |
| Final list of questions | Questions presented in the original meeting request are considered “draft” and can be modified when submitting the pre-IND briefing document. |
| Introduction | Background and rationale |
| Chemistry, Manufacturing, and Controls | Development chemistry, manufacturing procedures for drug substance and final product (including placebo), proposed labeling, analytical control procedures, release criteria, stability and related product information |
| Non-clinical animal studies | Data on completed studies and proposed plans for toxicological and activity studies for inclusion in the IND |
| Clinical | Proposed clinical study protocol concept |
| Previous human experience | Any relevant information on the construct or a similar construct used in human studies |
| References | Relevant publication(s) |

### Other FDA Meetings

FDA face-to-face meetings are defined as Type A, B & C. Type A meeting is needed to help an otherwise stalled product development program proceed. Type B meetings include Pre-IND, end of phase-1, phase-2, and phase-3 meetings, pre-NDA/BLA meeting.

Any other meeting with FDA that does not fit Type A or Type B is known as a Type C meeting. Type C meetings are useful for discussing with FDA new indications for currently marketed drugs, or for novel therapeutics that have no clear precedent in currently marketed products.

### IND Submission

The IND application submitted to the FDA pulls together all of the components of the discovery, preclinical and clinical development phases of the candidate drug. The required content and format are described in detail in 21 CFR Section 312. In summary, the contents include:

- Form 1571: Investigational New Drug Application
- Introductory Statement: drug background, structure, scientific rationale, and all preliminary efficacy data
- General Investigational Plan: proposed Phase 1 safety study, risks and benefits, and an outline of a future investigational approach
- Investigator Brochure: guidance to the clinical investigator concerning essential facts regarding the investigational drug candidate for use in the clinical trial
- Clinical Study Protocol: designed based on the E6 Good Clinical Practice: Consolidated Guidance
- Chemistry, Manufacturing, and Controls (CMC): identifies the manufacturer, process description with flow diagram(s) of the active DS and final DPs (including placebo), control test procedures, release acceptance criteria and specifications, certificates of analysis, labeling/packaging description, and stability of the drug over time
- Pharmacology and Toxicology: reports on animal studies providing all available information concerning the drug's effects and mechanisms of action, ADME, and safety profile when given at the dose level and by the mode of administration proposed for the clinical trial
- Previous Human Experience: described for the drug candidate or any similar DP, class, or configuration; available references to studies, regulatory submissions, and publications are provided.

The IND information and data consist of approximately 6–10 volumes, identified as Serial Number 0000 and accompanied by Form FDA 1571, which indicates the drug Sponsor and provides the particulars of the IND submission. A Certificate of Compliance (Form 3674) must accompany the IND application as required under the Federal Food Drug and Cosmetic Act or under the Public Health Services Act. The certification requirement went into effect on December 26, 2007, with a guideline published January 2009 and revised March 2009 to correct an error, available at:

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/form-fda-3674-certifications-accompany-drug-biological-product-and-device-applicationssubmissions

Once the IND is submitted, the sponsor must wait 30 calendar days before initiating any clinical trial; however, within the 30-day time frame, the FDA may put the IND on hold or request additional data prior to the start of the clinical study. FDA does not typically contact the submitter to indicate that their IND has been approved. If the submitter does not receive a formal notice of clinical hold from the FDA within 30 days of receipt, they may start clinical dosing on Day 31.

At the time of IND filing or prior to the start of the study, a Form FDA 1572 (Statement of Investigator) must be sent to the FDA providing information concerning each clinical study site and the site's investigator(s) information. Form FDA 1572 must be completed and sent by the sponsor to the FDA before the study can be initiated at a clinical study site.

After the initial IND submission, maintenance of the IND is performed through protocol and information amendments, safety reports, general correspondence and annual reports. Each additional submission is accompanied by a completed Form FDA 1571, identified with a consecutive serial number and the assigned IND reference number. Form 3674 is provided if required.

**Common Technical Document (CTD)**

The agreement to assemble all the quality, safety, and efficacy information in a common format (called CTD, or Common Technical Document) has revolutionized the regulatory review processes and led to harmonized electronic submission that, in turn, enabled implementation of good review practices. For industries, it has eliminated the need to reformat the information for submission to the different regulatory authorities that follow ICH guidelines (i.e., U.S., Europe, Japan, Canada). When submitted electronically, the document is frequently referred to as the Electronic Common Technical Document (eCTD). The eCTD contains the same technical information as the CTD, but it is entered into a XML database as a series of specific PDF documents.

The CTD is a set of specifications for application dossiers for the registration of medicines designed to be used across Europe, Japan, and the United States (Figure 4). It was developed by the European Medicines Agency (EMA, Europe), the FDA (U.S.) and the Ministry of Health, Labor and Welfare (Japan). The CTD format is maintained by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

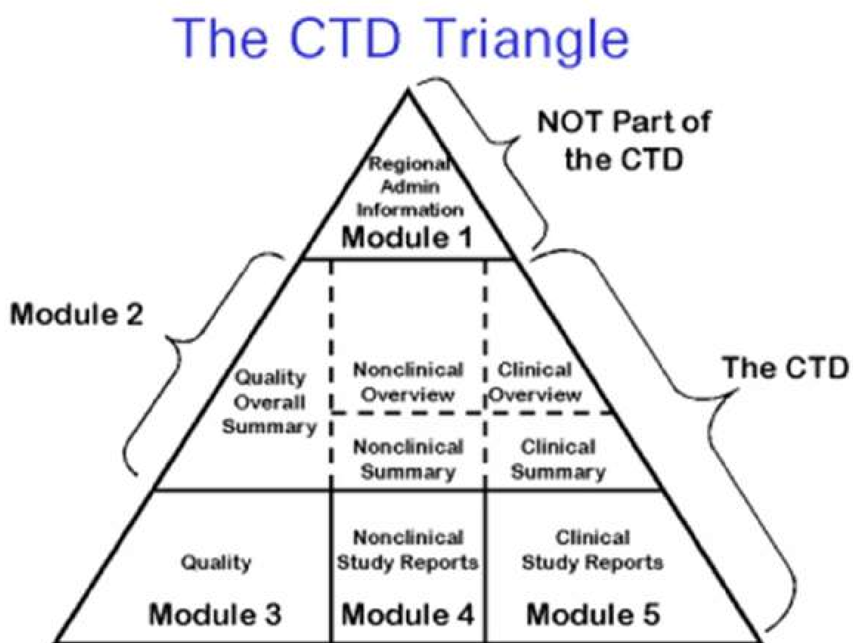



**Figure 4. The CTD triangle.**

The CTD is organized into five modules:

1. Administrative and prescribing information
2. Overview and summary of modules 3 to 5
3. Quality (pharmaceutical documentation)
4. Safety (toxicology studies)
5. Efficacy (clinical studies).

Detailed subheadings for each module are specified for all jurisdictions. The contents of Module 1 and certain subheadings of other modules will differ, based on national requirements. Module 1 is region specific and Modules 2, 3, 4 and 5 are intended to be common for all regions. In July 2003, the CTD became the mandatory format for new drug applications in the European Union (EU) and Japan, and the strongly recommended format of choice for BLAs submitted to the FDA. After the United States, EU and Japan, the CTD has been adopted by several other countries including Canada and Switzerland.

Although some divisions of the FDA previously accepted paper INDs during the first few years of transition to the eCTD format, the FDA has now largely moved to mandated electronic submissions. This allows the "building blocks and development of a future BLA" with minimal repetition of data during the different development phases. Submission of documents in an eCTD format typically requires both specialized software and regulatory know-how, and a variety of regulatory consulting organizations now provide this service. It is recommended to identify appropriate partners for eCTD compilation and submission about the time submitters begin preparing for a Pre-IND meeting.

**International Regulatory Considerations for Initiating Clinical Trials**

The requirements for independent review of medicinal products before they are allowed on the market grew in response to tragedies involving unregulated DPs. In the United States, the use of ethylene glycol as a vehicle for sulfanilamide caused acute renal failure and several deaths in the 1930s. This led to the passage in the U.S. of the Federal Food, Drug, and Cosmetic Act in 1938, which required drug makers to demonstrate the safety of a drug prior to introducing it into interstate commerce. In the late 1950s and early 1960s, severe birth defects produced by thalidomide in humans led to increased regulation of the drug development process. With the introduction of more stringent laws, regulations, and guidelines for safety, quality and efficacy of DP, the regulatory process became more complex. Moreover, each country had different requirements. As a result, drug makers were required to duplicate time-consuming and expensive test procedures in order to market a drug in different countries.

During the 1980s it was recognized that there was a need to align requirements between regions. European countries were working on harmonizing requirements among its member states in preparation for a single market in pharmaceuticals. In addition, there were discussions between the United States, Europe and Japan. Finally in April 1990, the International Conference on Harmonisation (ICH) was founded in Brussels.

The ICH is a consortium of regulators (US FDA; Japan Ministry of Health, Labor and Welfare; EU) and industry organizations (US Pharmaceutical Research and Manufacturers of America, Japanese Pharmaceutical Manufacturers Association and the European Federation of Pharmaceutical Industries and Associations). In addition, there are three observers (World Health Organization, Health Canada, and European Free Trade Association). The International Federation of Pharmaceutical Manufacturers and Associations (representing manufacturers in both the developed and developing world) is a nonvoting member.

Global interest in harmonizing clinical trial guidelines grew outside the original three ICH regions. As a result, the Global Cooperation Group (GCG) was formed as a subcommittee of the ICH Steering Committee in 1999. A few years later, recognizing the need to engage actively with other harmonization initiatives, representatives from five Regional Harmonisation Initiatives were invited to participate in GCG discussions, namely, Asia-Pacific Economic Cooperation, Association of Southeast Asian Nations, East African Community, Gulf Central Committee, Pan-American Network for Drug Regulatory Harmonization and Southern African Development Community. A further expansion of the GCG was agreed in 2007 and regulators were invited from countries with a history of ICH Guideline implementation and/or where major production and clinical research are done (Australia, Brazil, China, Chinese Taipei, India, Republic of Korea, Russia and Singapore). A summary of international regulatory requirements for FIH trials by country is included in Appendix A.

The ICH has developed a series of guidance documents (available at:

http://www.ich.org/products/guidelines.html)

which provide a consensus of requirements for:

- Quality: issues related to the chemistry, manufacturing, and stability of the DP
- Safety: issues related to the pharmacology, pharmacokinetics, and toxicology of the drug in laboratory animals (including isolated human cells)
- Efficacy: issues related to clinical studies
- Multidisciplinary: issues related to multiple areas, such as timing of nonclinical studies with respect to clinical trial phase and organization of drug submissions

These guidance documents are accepted as the most current regulatory positions related to each of these subject areas.

For FIH studies, The ICH M3(R2) Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (available at:

https://database.ich.org/sites/default/files/M3_R2__Guideline.pdf.

Provides the framework on the timing of nonclinical studies with respect to the clinical trial phase. A summary of nonclinical tests required to comply with the M3 guidance prior to a FIH study is provided in Table 12. Although there is substantial agreement on most issues, there are still differences between regions and investigators should check with their regulatory authorities to ensure compliance. A summary of regulatory requirements for FIH studies in select countries around the world (as of May 2012) is presented in Table 13, below. The reader is advised that regulatory science and requirements are frequently changing. Prior to submitting any clinical trial application, the Sponsor should review each country's specific requirements.

**TABLE 12.**
**LISTING OF NONCLINICAL STUDY TYPES REQUIRED PRIOR TO A FIRST IN HUMAN (FIH) STUDY**

| Study Type | GLP Compliant | Description |
|---|---|---|
| Acute toxicity study | No | Studies that investigate the adverse effects of short-term exposure to relatively high doses of a drug on animals. Primarily used to set dose levels for subsequent PK and safety pharmacology studies. |
| Pharmacokinetics | Typically No<br><br>Yes for Animal Rule | Studies that investigate the process of drug absorption, distribution and elimination.<br><br>GLP pharmacokinetic studies for animal rule approvals are performed typically using the same animal species used for efficacy testing. These studies may be stand-alone PK studies or part of the GLP pivotal efficacy study performed for product approval. Typically these are performed after IND submission.<br><br>Under the U.S. FDA Animal Rule, GLP pharmacokinetic studies are required to allow extrapolation of exposure levels in animal models that are efficacious to human patients. |
| Primary pharmacology | No | Studies that investigate the effects of a drug on the function of the target organ. |
| Tissue Cross-Reactivity | Yes | Studies that evaluate potential for binding to non-target tissues. |
| Repeat dose toxicity studies | Yes | Studies that investigate the adverse effects of repeated drug administration on the animal; in general two species (rodent and nonrodent) are used. |

**TABLE 12.
LISTING OF NONCLINICAL STUDY TYPES REQUIRED PRIOR TO A FIRST IN HUMAN (FIH) STUDY**

| Study Type | GLP Compliant | Description |
|---|---|---|
| Safety pharmacology | Yes | Studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions (neurological, respiratory, cardiovascular) in relation to exposure in and above the therapeutic range. Requirement is compound-dependent, if potential for these liabilities is present based on mechanism of action. Relevant safety pharmacology evaluations can be conducted as part of repeat-dose toxicity studies. |
| Toxicokinetic evaluations | Yes | The generation of pharmacokinetic data, either as an integral component in the conduct of nonclinical toxicity studies or in specially designed supportive studies, in order to assess systemic exposure. These data may be used in the interpretation of toxicology findings and their relevance to clinical safety issues. |

**TABLE 13.**
**SUMMARY OF FIH REQUIREMENTS BY COUNTRY**

| Country | ICH | Manufacture | | | Nonclinical | | | | | Clinical | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ICH Compliance | preGMP/Pilot Batch | GMP Batch | Import Drug for FIH Study? | Primary Pharmacology | Safety Pharmacology | Toxicokinetic Evaluations | Single-Dose Rangefinding Studies | Repeat Dose Toxicity Studies | Protocol | Investigators Brochure | Informed Consent | Women of Childbearing Potential* |
| Argentina | Yes | ND | ND | Yes | GLP | GLP | GLP | GLP | GLP | Yes | Yes | Yes | Allowed |
| Australia | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| Brazil | Yes | ND | ND | Yes | GLP | GLP | GLP | GLP | GLP | Yes | Yes | Yes | Allowed |
| Canada | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| Chile | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | ND |
| China | Yes | NC | C | No | GLP@ | GLP | GLP | GLP | GLP | Yes | Yes | Yes | Allowed |
| Colombia | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | ND |
| EMA | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| India | Mostly Yes | NC | NC/C | No | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| Japan | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| Mexico | Yes | NC | NC/C | ND | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| Peru | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | ND |
| Russia | Yes | NC | NC/C | No | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| South Africa | Yes | NC | NC/C | No | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |
| United States | Yes | NC | NC/C | Yes | Non GLP | GLP | GLP | Non GLP | GLP | Yes | Yes | Yes | Allowed |

Yes – Required for FIH study
No – Not required for FIH study
NC – Batch may be used for nonclinical studies
C – Batch may be used for clinical studies
ND – Not described in local regulation

NonGLP – Required nonclinical study, but not required to be GLP compliant
GLP – Required GLP compliant nonclinical study
*Women of childbearing potential allowed in FIH study without nonclinical embryo-fetal developmental toxicity studies in two species
@ In some special cases, core battery needs to be under GLP condition

**Clinical Development and Trial Design Considerations**

At a relatively early point in preclinical R&D, as preliminary animal efficacy, bioavailability, and non-GLP toxicology data become available, investigators should consider the ultimate goal of a clinical development program, and whether or not achievement of this goal is likely to address a specific unmet medical need that will exist at the time of eventual licensure, given the evolving competitive landscape. Preliminary clinical input on the target product profile is therefore essential, as early as the TLR 2 phase of development. Prior to embarking on GLP-compliant IND-enabling studies, it is recommended that a Phase 1 study synopsis and high level draft or working version clinical development plan through licensure be drafted to ensure that it is possible to envision a clinical program that is feasible, efficient, and not encumbered by excessive technical and regulatory risk.

Although the main clinical focus of the pre-IND and IND submission packages will be the Phase 1 FIH study, this should be placed within its proper context: a practical and scientifically sound development plan that culminates in approval of a product that is expected to address a specific unmet medical need. Attention to these broader development considerations in the clinical sections of early regulatory submissions, even if only in a tentative manner and at a high level, should also mitigate regulatory risk, because the FDA is ultimately interested in ensuring that the risk-benefit ratio for an investigational product supports licensure. That risk-benefit ratio is tied to the architecture and outcome of the clinical program, as well as the proposed clinical indication.

A detailed draft Phase 1 clinical study synopsis should be included in the pre-IND briefing document, including an outline of study design, eligibility criteria, safety, PK analyses, efficacy variables (if applicable), planned interventions/evaluations and other relevant study details. A full clinical protocol and informed consent form must be included in the IND submission. The FDA Guidance Document, “Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers” may be a useful reference:

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers

Clinical programs for antibacterial agents have advantages over trials in some other therapeutic areas because knowledge of pharmacokinetic–pharmacodynamic relationships for a particular class of antibiotics, or in suitable animal models, can serve to frame human dose selection, or at least identify a smaller range of dose levels that are likely to be associated with clinical benefit. For example, if it is known that time above minimum inhibitory concentration is a critical determinant of pharmacodynamic (i.e., antibacterial efficacy), this greatly assists dose selection for late-phase clinical studies. In addition, whole-blood bactericidal activity can be used as a biomarker of pharmacodynamic effects in Phase 1 studies of anti-infective agents in an effort to minimize the risk of efficacy failure in later clinical development, as in the case of a recent study of volunteers treated with escalating doses of an experimental oxazolidinone for tuberculosis (Wallis et al., 2010).

For antiviral programs, a PK parameter (e.g., ratio of $C_{trough}$ to serum-corrected ED50) can be helpful in choosing doses for later clinical studies. Often, patients are enrolled in early Phase 1b studies of antiviral agents in which treatment is administered as a highly abbreviated course of monotherapy to chronically infected patients, with the use of a surrogate marker (e.g., viral load) to assess efficacy. Biomarkers and adaptive designs have revolutionized clinical trials, but require careful use in any program in collaboration with an expert in strategic clinical drug development.

In general, FIH studies generally enroll small numbers (e.g., 25–35) of subjects in separate cohorts treated with progressively increasing doses until the maximum tolerated dose is reached. Several patients within each cohort are often randomized to a placebo control group. Dose escalation to the next cohort is contingent upon demonstration of safety in the prior cohort. Major objectives include the evaluation of safety, tolerability, and pharmacokinetics. A "single ascending dose" (SAD) Phase 1 study may be fused with the "multiple ascending dose" (MAD) study in a staggered cohort design. Dose refinement is typically accomplished in subsequent and larger Phase 2 studies that also evaluate efficacy. Confirmatory (i.e., pivotal) Phase 3 efficacy studies support licensure. The requisite size of a safety database depends on many factors, including the perceived risk-benefit ratio. Increasingly, adaptive trial designs are being leveraged to enhance the efficiency of drug development. These may take many forms, such as adaptive randomization, hypothesis generation, group-sequential design, sample size adjustment, and "seamless" Phase 2/3 studies with a "drop the loser" approach. The FDA Animal Rule poses special considerations in clinical development, with animal efficacy experiments playing a vital role in human dose selection. Considerations germane to animal models in this context are discussed in Section IV.F3 of this document.

**Pediatric Research Equity Act and Compliance**

Following a decade of legal and regulatory attempts to address lack of pediatric use information for drug products, on December 3, 2003, the Pediatrics Research Equity Act (PREA) was signed into law that took into account the suspended Pediatric Rule. PREA requires all applications (or supplements to an application) submitted under section 505 of the Act (21 U.S.C. 355) or section 351 of the Public Health Service Act (42 U.S.C. 262) for a new active ingredient, new indication, new dosage form, new dosing regimen, or new route of administration to contain a pediatric assessment unless the applicant has obtained a waiver or deferral. It also authorizes FDA to require holders of approved NDAs and BLAs for marketed drugs and biological products to conduct pediatric studies under certain circumstances.

The Food and Drug Administration Safety and Innovation Act, signed into law July 2012, requires manufacturers of drugs subject to PREA to submit a Pediatric Study Plan (PSP) early in the drug development process with the intent to identify and beginning planning early for the necessary pediatric studies. Draft guidelines, published July 2013, provide FDA's most current thinking at publication and are intended to assist in the submission of an initial PSP or any PSP amendment.

(http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM360507.pdf).

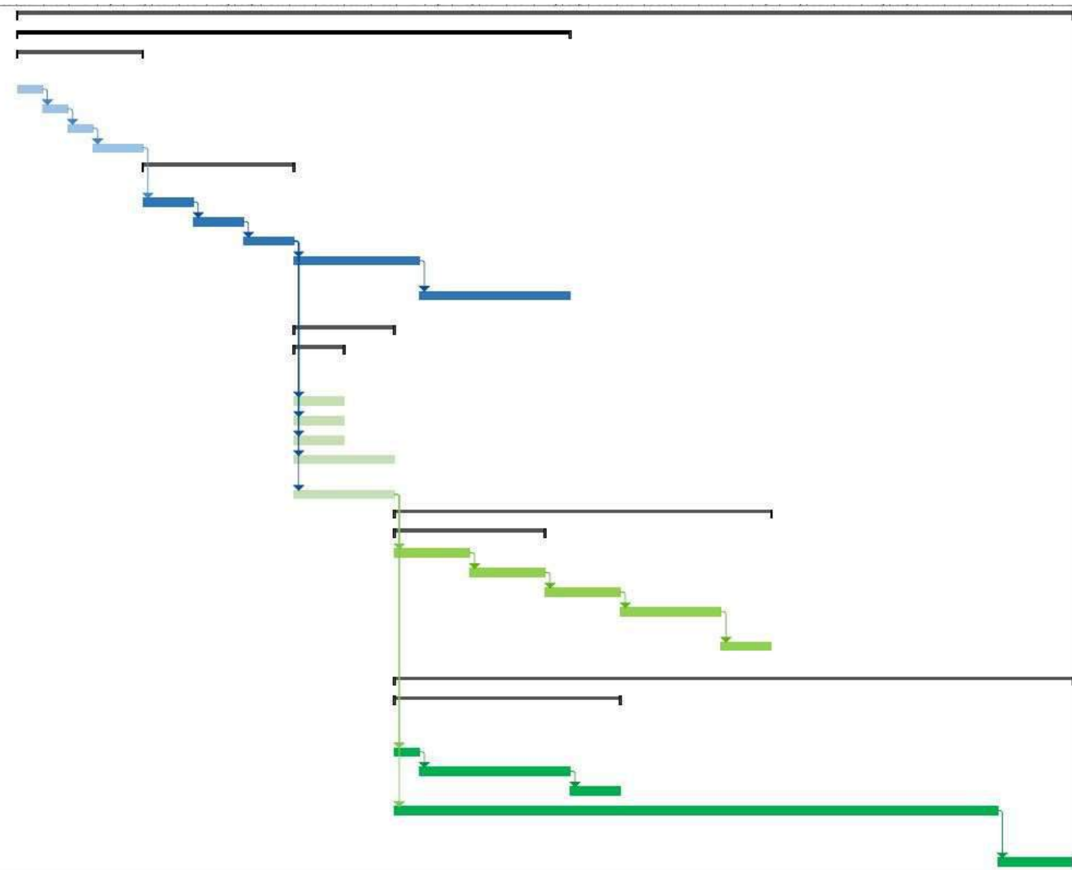
The guideline indicates the initial PSP should be provided no later than 60 calendar days after the date of the end-of-Phase 2 meeting or as early as practical before the initiation of any Phase 3 studies. Although a PSP is not required for the Phase 1 IND study, it is recommended that the need and requirements for pediatric studies be taken into consideration at the time the Phase 1 clinical study is being planned and developed.

## ESTIMATED SCHEDULE

A Gantt chart showing the estimated schedule of activities to prepare for IND submission is provided on the next page.

# ESTIMATED SCHEDULE

| **Generic mAb Preclinical Development Plan** | **840 days** | **Thu 1/2/25** | **Wed 3/22/28** | |
|---|---|---|---|---|
| **Stage 1** | **440 days** | **Thu 1/2/25** | **Wed 9/9/26** | |
| **Establishment of Well-Characterized Master Cell Bank (MCB) for Production of the mAb** | **100 days** | **Thu 1/2/25** | **Wed 5/21/25** | |
| Identify Suitable Cell Lines | 1 mon | Thu 1/2/25 | Wed 1/29/25 | |
| Establish Initial Cultures | 1 mon | Thu 1/30/25 | Wed 2/26/25 | 4 |
| Perform Cell Line Characterization | 1 mon | Thu 2/27/25 | Wed 3/26/25 | 5 |
| Scale-up Culture and Establish MCB | 2 mons | Thu 3/27/25 | Wed 5/21/25 | 6 |
| **Manufacturing and Control Development of Bulk mAb (Active Pharmaceutical Ingredient) Pilot Lots** | **120 days** | **Thu 5/22/25** | **Wed 11/5/25** | |
| Development of Manufacturing Process | 2 mons | Thu 5/22/25 | Wed 7/16/25 | 7 |
| Generation of Initial Pilot Lots | 2 mons | Thu 7/17/25 | Wed 9/10/25 | 9 |
| Quality Control Testing and Analysis | 2 mons | Thu 9/11/25 | Wed 11/5/25 | 10 |
| Performance of Preformulation/Formulation Studies to Identify a Probable Clinical Formulation | 5 mons | Thu 11/6/25 | Wed 3/25/26 | 11 |
| Performance of Efficacy Studies to Confirm Pharmacological Activity | 6 mons | Thu 3/26/26 | Wed 9/9/26 | 12 |
| **Stage 2** | **80 days** | **Thu 11/6/25** | **Wed 2/25/26** | |
| **Completion of Pharmacokinetic (PK), Immunogenicity, and Range-Finding Toxicity Studies with Established Manufacturing Procedure** | **40 days** | **Thu 11/6/25** | **Wed 12/31/25** | |
| Performance of PK Studies | 2 mons | Thu 11/6/25 | Wed 12/31/25 | 11 |
| Performance of Immunogenicity Studies | 2 mons | Thu 11/6/25 | Wed 12/31/25 | 11 |
| Conduct Range-Finding Toxicity Studies | 2 mons | Thu 11/6/25 | Wed 12/31/25 | 11 |
| Performance of TCR Study in Several Species, Including Human Tissue | 4 mons | Thu 11/6/25 | Wed 2/25/26 | 11 |
| Performance of Mechanism of Action (MOA) Studie | 4 mons | Thu 11/6/25 | Wed 2/25/26 | 11 |
| **Stage 2-3** | **300 days** | **Thu 2/26/26** | **Wed 4/21/27** | |
| **Develop Criteria for Release (Specifications)** | **120 days** | **Thu 2/26/26** | **Wed 8/12/26** | |
| Define Critical Quality Attributes | 3 mons | Thu 2/26/26 | Wed 5/20/26 | 20 |
| Set Acceptance Criteria | 3 mons | Thu 5/21/26 | Wed 8/12/26 | 23 |
| Development and Validation of Analytical Methods | 3 mons | Thu 8/13/26 | Wed 11/4/26 | 24 |
| GMP Production of Bulk mAb and Final Product for Phase I Clinical Trial | 4 mons | Thu 11/5/26 | Wed 2/24/27 | 25 |
| Preparation of a Pre-IND Meeting Request and Information for FDA Submission | 2 mons | Thu 2/25/27 | Wed 4/21/27 | 26 |
| **Stage 3** | **540 days** | **Thu 2/26/26** | **Wed 3/22/28** | |
| **Completion of Repeat-Dose Toxicity Studies in One or Two Species, in Compliance with FDA Good Laboratory Practices (GLP)** | **180 days** | **Thu 2/26/26** | **Wed 11/4/26** | |
| Study Design | 1 mon | Thu 2/26/26 | Wed 3/25/26 | 20 |
| Conduct Studies | 6 mons | Thu 3/26/26 | Wed 9/9/26 | 30 |
| Data Analysis | 2 mons | Thu 9/10/26 | Wed 11/4/26 | 31 |
| Shelf Stability Studies of Bulk mAb and Drug Product Supplies to Support Ongoing Clinical Studies and to Establish Expiration Dating | 24 mons | Thu 2/26/26 | Wed 12/29/27 | 20 |
| Prepare and Submit IND to FDA | 3 mons | Thu 12/30/27 | Wed 3/22/28 | 33 |



**REFERENCES**

Brown PC, Wange RL. Considerations regarding the use of nonhuman primates in assessing safety endpoints for pharmaceuticals. *Regul Toxicol Pharmacol*. 2023 Sep;143:105449. doi: 10.1016/j.yrtph.2023.105449. PMID: 37453554.

Buss, N.A.P.S., Henderson, S.J., McFarlane, M., Shenton, J.M., de Haan, L. Monoclonal antibody therapeutics: history and future, Curr. Opin. Pharmacol. 12:615-22 (2012) doi: 10.1016/j.coph.2012.08.001.

Castelli, M.S., McGonigle, P, Hornby, P. The pharmacology and therapeutic applications of monoclonal antibodies. Pharmacol. Res. Perspect. 7:e00535 (2019) doi: 10.1002/prp2.535.

FDA, Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use, February 28, 1997.

FDA, Draft Guidance for Industry and Review Staff Target Product Profile - A Strategic Development Process Tool.

Hoover, J.M., Prinslow, E.C., Teigler, J.E., Truppo, M.D., La Porte, S.L., Therapeutic antibody discovery. Chapter 23 of The Science and Practice of Pharmacy, ed. Remington, pp417-436 (2021) doi:10.1016/B978-0-12-820007-0.00023-4.

ICH, The International Conference on Harmonisation (ICH) Guidance, Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, (63 FR 51074; September 24, 1998).

FDA, Guidance for Industry: Bioanalytical Method Validation, FDA CDER, May 2001.

FDA, Nonclinical Considerations for Mitigating Nonhuman Primate Supply Constraints Arising from the COVID-19 Pandemic; Guidance for Industry. Issued 2/24/2022, withdrawn 5/11/2023

ICH, Draft Consensus Guideline Addendum to ICH S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceutical S6(R1), (October 29, 2009)

Lushniak, Boris, MD, MPH, FDA's Assistant Commissioner for Counterterrorism Policy.

Li, J., Zhu, Z. Research and development of next generation of antibody-based therapeutics, Acta Pharmacologica Sinica, 31:1198-1207 (2010) doi: 10.1038/aps.2010.120.

Lyu et al., The global landscape of approved antibody therapies. Antib. Ther. 5:233-257 (2022)

Xu, Y. et al., Structure, heterogeneity and developability assessment of therapeutic antibodies. MAbs, 11:239-264 (2019) doi: 10.1080/19420862.2018.1553476.

Zhang, Y. Evolution of phage display libraries for therapeutic antibody discovery. MAbs, 15:2213793 (2023) doi: 10.1080/19420862.2023.2213793.

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