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PRECLINICAL DEVELOPMENT PLAN: SMALL MOLECULE ANTI-INFECTIVES

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SUMMARY

This document is an update to the Small Molecule Generic Preclinical Development Plan first prepared by SRI in December 2007, with subsequent updates in 2012 (Revision 1) and 2015 (Revision 2). The general process for drug development is similar among most agents across a broad range of therapeutic uses. While specific differences between chemical structures exist, and results of preliminary testing may trigger additional studies for a given compound, it is useful to understand the general process for drug development. This Preclinical Development Plan (PDP) is therefore provided as a "generic" document that applies, in general, to the development of a typical small molecule anti-infective agent.

The recommended strategy for all drug candidates is to first develop a Target Product Profile (TPP) and to outline the key studies that lead to a regulatory filing for human clinical testing, which has been based on appropriate go/no-go risk assessment decisions. Once the TPP has been developed and data are accumulated, this then enables submission of an Investigational New Drug (IND) application in the mandated electronic Common Technical Document (eCTD) format.

Drug discovery is typically treated as a "research" phase in which basic research leads to new drug targets, and molecules against these targets are discovered and tested. This phase is usually considered to be separate from preclinical development because there are no specific regulatory requirements, and because it can be characterized as a highly iterative process that is unique to each molecule or scaffold. Drug discovery most commonly matures through several stages from hit identification, to hit-to-lead, to lead optimization. During this process, in addition to the obvious need for a compound to demonstrate efficacy in appropriate in vitro and/or in vivo models, there are a variety of aspects to be taken into consideration to assure that new molecules are viable potential therapeutics. These include preliminary in vitro studies for absorption, distribution, metabolism, elimination and toxicity (ADMET), preformulation, early rodent pharmacokinetics and toxicity studies, pharmacokinetic/pharmacodynamic (PK/PD) determinations, and other factors related to the eventual viability of the candidate compound.

Once promising lead compounds have been identified and optimized as part of the lead optimization phase, compounds may be moved into preclinical development. The minimum recommended requirements for initiation of preclinical development are as follows:

- One gram of relatively pure (>95%) test article synthesized to permit preliminary work to begin
- No intellectual property issues for synthesis, use, or treatment
- Confirmed in vitro potency assays, with more than one reproducible study
- Confirmed in vivo efficacy in an appropriate animal model

These steps only permit initiation of preclinical development, and many additional steps are required before advancing a drug candidate to human Phase 1 clinical trials. These steps include a broad range of activities including:

- Completion of pharmacokinetic (PK) and dose range finding toxicity studies

- Performance of preformulation/formulation studies to identify both preclinical animal and clinical formulations
- Synthesis of batches of bulk drug substance (DS) containing the active pharmaceutical ingredient (API) under non-GMP, GMP pilot batch, and finally under FDA current Good Manufacturing Practices (cGMP) conditions
- Analytical chemistry method development and validation
- Completion of repeat-dose toxicity studies in two species, in compliance with U.S. Food and Drug Administration (FDA) Good Laboratory Practices (GLP)
- Clinical manufacturing of Phase 1 drug product
- Shelf stability studies of bulk DS and drug product (DP) supplies to support ongoing clinical studies and to establish expiration dating
- Preparation and submission of pre-IND and IND documents to the FDA

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LIST OF ACRONYMS AND ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
ADME	Absorption, Distribution, Metabolism, and Excretion
ANMAT	Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (Argentina)
ANVISA	Agência Nacional de Vigilância Sanitária (Brazil)
API	Active Pharmaceutical Ingredient
AUC	Area Under the Plasma Concentration Versus Time Curve
BCRP	Breast Cancer Resistant Protein
BLA	Biologics License Application
CBER	Center for Biologics Evaluation and Research (U.S.)
CDE	Center for Drug Evaluation (China)
CDER	Center for Drug Evaluation and Research (U.S.)
cGMP	current Good Manufacturing Practices
C_{max}	Maximum plasma drug concentration
CMC	Chemistry, Manufacturing, and Controls
CNS	Central Nervous System
CoA	Certificate of Analysis
COFEPRIS	Comisión Federal Para La Protección Contra Riesgos Sanitarios (Mexico)
COG	Cost of Goods
CONEP	Comissão Nacional de Ética em Pesquisa (Brazil)
CRO	Contract Research Organization
CTA	Clinical Trial Application
CTD	Common Technical Document
CTN	Clinical Trial Notification
CTX	Clinical Trial Exemption
CYP	Cytochrome P450
DCGI	Drug Controller General of India
DMSO	Dimethylsulfoxide
DP	Drug Product
DRF	Dose range finding
DS	Drug Substance
EC	Ethics Committee
ECG	Electrocardiogram
eCTD	Electronic Common Technical Document
ED50	Dose Level Effective for 50% of Subject Population
EMA	European Medicines Agency
EU	European Union
EUA	Emergency Use Authorization
FDA	(U.S.) Food and Drug Administration
FIH	First in Human

FOB	Functional Observation Battery
GCG	Global Cooperation Group
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HPLC	High Performance Liquid Chromatography
HR	Heart Rate
HREC	Human Research Ethics Committee
ICF	Informed Consent Forms
ICH	International Conference on Harmonisation
IMPD	Investigational Medicinal Product Dossier
IND	Investigational New Drug
INS	Instituto Nacional de Salud (Peru)
INVIMA	Instituto Nacional de Vigilancia de Medicamentos y Alimentoa (Columbia)
IRB	Institutional Review Board
ISR	Incurred Sample Reanalysis
IV	Intravenous
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
MCC	Medicines Control Council (South Africa)
MIC	Minimum inhibitory concentration
MLA	Mouse Lymphoma Assay
MoH	Ministry of Health (Chile)
MS	Mass Spectrometry
NCE	New Chemical Entity
NDA	New Drug Application
NDAC	New Drug Advisory Committee (India)
NHP	Nonhuman primate
NIH	National Institutes of Health
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
OLAW	Office of Laboratory Animal Welfare
PDP	Preclinical Development Plan
P-gp	P-Glycoprotein
PK	Pharmacokinetics
PM	Project Manager
PMDA	Pharmaceuticals and Medical Devices Agency (Japan)
PREA	Pediatric Research Equity Act
P-SEAT	Protocol Safety and Efficacy Assessment Template
PSP	Pediatric Study Plan
QAU	Quality Assurance Unit
QCU	Quality Control Unit
R&D	Research and Development
RH	Relative Humidity

RHI	Regulation for Health Investigation (Mexico)
RR	Respiratory Rate
SADC	South African Development Community
SFDA	State Food and Drug Administration (China)
$t_{1/2}$	Elimination Half-Life
TGA	Therapeutic Goods Administration (Australia)
TK	Toxicokinetic
T_{max}	Time to Maximum Concentration
TPP	Target Product Profile
TRL	Technical Readiness Level
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
WOCP	Women of Childbearing Potential

INTRODUCTION

This document is an update to the Small Molecule Generic Preclinical Development Plan first prepared by SRI in December 2007, with subsequent updates in 2012 (Revision 1) and 2015 (Revision 2). While most of the basic principles of drug development are unchanged, a number of specific regulations and guidelines have been published that have led to changes in the way certain assays are conducted and in how data are transmitted to the U.S. Food and Drug Administration (FDA). These changes are noted in this document where applicable. In addition, because of a growing need to provide background to Investigators on early development activities conducted in parallel with drug discovery research, a new section on Drug Discovery and Lead Optimization has been added.

The 2012 update (Revision 1) included a separate Appendix outlining the general process and regulatory requirements in other countries. Compilation of this document was outsourced to a third party (INC Research) who had expertise in international regulatory guidelines. Updating of this document was not conducted as part of this current revision, but in reviewing the guidelines for major regulatory agencies (EMA, PMDA), there appear to be no significant changes in the regulatory requirements for other countries. The original 2012 review is therefore included here, unchanged, for completeness.

In addition to this generic small molecule Product Development Plan, the National Institute of Allergy and Infectious Diseases' (NIAID's) Preclinical Services also includes a service to generate compound-specific Product Development Plans. Drug candidates are typically in the early lead optimization stage of the drug discovery and development process when the National Institutes of Health (NIH) considers a request for a product-specific Product Development Plan (PDP) and a Target Product Profile (TPP), along with an outline of the key Investigational New Drug (IND)-enabling studies with go/no-go decision points.

It is useful to define "small molecule" for the purpose of this generic product development plan, however, a precise definition is impossible. Inevitably, some entities will fall on the indistinct boundaries between small and large, or small and biological. Many in vitro and in vivo studies discussed in this plan may be equally relevant to large or biological molecules, however, the specific requirements for their use in this context are not discussed here. Generally, a small molecule is a molecular entity that has a specific, defined chemical structure achievable in high purity by synthesis, semi-synthesis, or isolation from a biological producer. In addition, a small molecule has little, if any, secondary structure and no tertiary structure. This definition rules out most polymeric mixtures, tinctures, all traditional biomolecules and their conjugates, such as antibodies, proteins, lengthy peptides, and RNA, and biological systems such as vaccines and viral vectors, as well as cellular systems. While the definition excludes many biomolecules it includes purified biological metabolites of fermentation and their semi-synthetic derivatives. A small molecule drug will typically have molecular weight <1000 g/mol and engage in a specific interaction with a biological target, although neither of these criteria is strictly necessary. Ultimately, the properties of a small molecule drug must be consistent with its intended route of administration, site of action, and safe and efficacious dose in humans.

The sections that follow outline the key steps required to advance a drug candidate to Phase 1 clinical trials and provide an outline of the various data gathering steps for completion of an IND application suitable for U.S. Food and Drug Administration (FDA) submission.

I. 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 chemical entity 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 sections, 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 New Drug Application (NDA). Completion of these studies to demonstrate safety and efficacy in both animal models and humans and filing of the NDA are the final steps before market introduction. Although the details involved in each stage depend on the type of pharmaceutical product, the general development process is similar for all drugs regardless of dose administration form (oral, parenteral, topical, etc.).

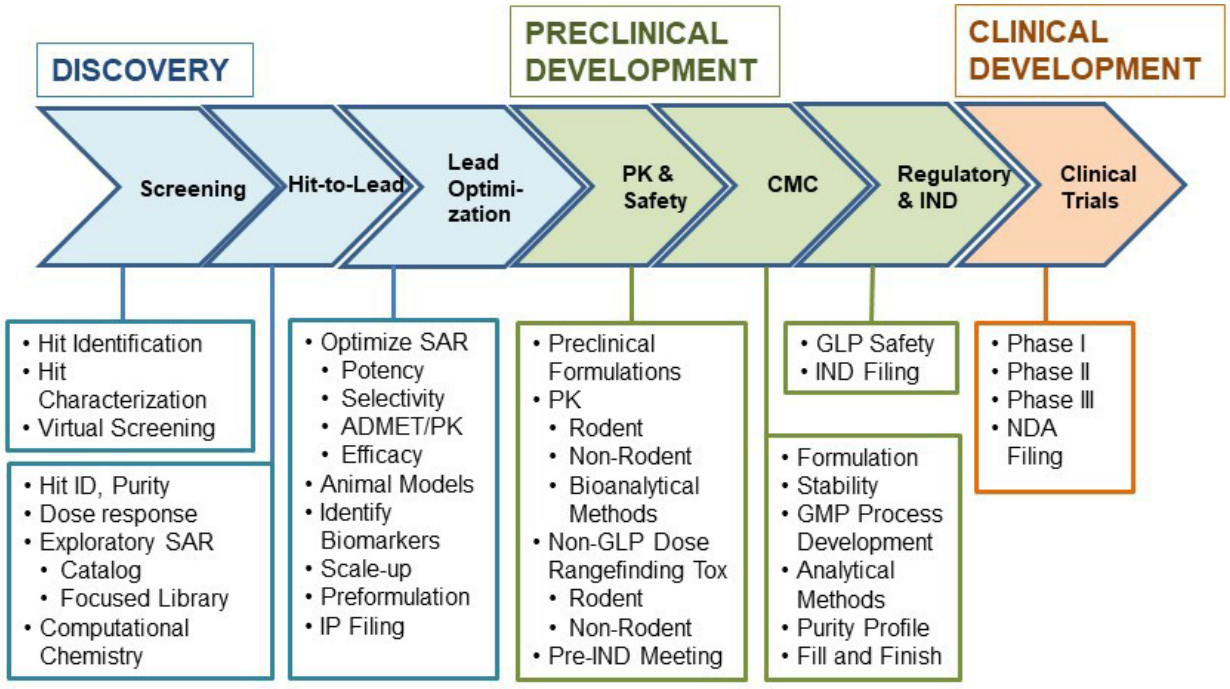

Figure 1. The drug discovery and development process.

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.
EXAMPLES OF INFECTIOUS DISEASE RELATED, PRODUCT-SPECIFIC 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
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

Note that the process for development of biological products, leading up to a Biologics License Application (BLA), are similar, but with some noteworthy differences. The process for biological agents falls outside the scope of this document. A separate document outlining the process of development of monoclonal antibodies is also available that was prepared in 2010, but has not been updated since. This is available on request from your DMID Program Officer.

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.

For new chemical entities, the lead candidate is still considered in the discovery phase. The next steps for developing the drug will be further discussed in this document. Repurposing of approved drugs may be able to skip particular steps if the dosing regimen and route are unchanged from that of the approved drug.

A. 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. A few examples are included in the links below:

https://api.army.mil/e2/c/downloads/404585.pdf
https://www.medicalcountermeasures.gov/trl/integrated-trls/

While TRLs are popular within some Department of Defense programs, 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 2 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 2.
TECHNOLOGY READINESS LEVELS[1]

TRL #	Description	Development Stage[2]
TRL 1	**Review of Scientific Knowledge Base** 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** 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)** 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. **3A** Identify target and/or candidate. **3B** Demonstrate in vitro activity of candidate(s) to counteract the effects of the threat agent. **3C** Generate preliminary in vivo proof-of-concept efficacy data (non-GLP [Good Laboratory Practice]).	**Screening** **Hit Confirmation**
TRL 4	**Candidate Optimization and Non-GLP In Vivo Demonstration of Activity and Efficacy** 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. **Animal Models:** Initiate development of appropriate and relevant animal model(s) for the desired indications. **Assays**: Initiate development of appropriate and relevant assays and associated reagents for the desired indications. **Manufacturing:** Manufacture laboratory-scale (i.e., non-GMP [Good Manufacturing Practice]) quantities of bulk product and proposed formulated product. **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 2. 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). **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** 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. **Animal Models:** Continue development of animal models for efficacy and dose-ranging studies. **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. **Manufacturing:** Initiate process development for small-scale manufacturing amenable to GMP. **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. **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. **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** **CMC**
TRL 6	**GMP Pilot Lot Production, IND Submission, and Phase 1 Clinical Trial(s)** 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. **Animal Models:** Continue animal model development via toxicology, pharmacology, and immunogenicity studies.	**CMC** **Regulatory & IND Submission**

TABLE 2. TECHNOLOGY READINESS LEVELS[1]		
TRL #	**Description**	**Development Stage[2]**
	Assays: Qualify assays for manufacturing quality control and immunogenicity, if applicable. **Manufacturing:** Manufacture, release, and conduct stability testing of GMP-compliant bulk and formulated product in support of the IND and clinical trial(s). **Target Product Profile:** Update TPP as appropriate. **6A** Conduct GLP non-clinical studies for toxicology, pharmacology, and immunogenicity as appropriate. **6B** Prepare and submit full IND package to FDA to support initial clinical trial(s). **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)** Scale-up and initiate validation of GMP manufacturing process. Conduct animal efficacy studies as appropriate. Conduct Phase 2 clinical trial(s).[3] **Animal Models:** Refine animal model development in preparation for pivotal GLP animal efficacy studies. **Assays:** Validate assays for manufacturing quality control and immunogenicity if applicable. **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. **Target Product Profile:** Update TPP as appropriate. **7A** Conduct GLP animal efficacy studies as appropriate for the product at this stage. **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** 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 NDA. **Manufacturing:** Complete validation and manufacturing of consistency lots at a scale compatible with U.S. government requirements. Complete stability studies in support of label expiry dating.	**NDA Market Approval**

TABLE 2. TECHNOLOGY READINESS LEVELS[1]		
TRL #	**Description**	**Development Stage[2]**
	Target Product Profile: Finalize TPP in preparation for FDA approval. **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. **8B** Prepare and submit NDA to the FDA. **8C** Obtain FDA approval or licensure.	
TRL 9	**Post-Licensure and Post-Approval Activities** **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. **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." Other than human safety studies, no additional clinical data may be feasible or ethical to obtain.

II. 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. The draft document can be found at:

http://www.ncai-cc.ccf.org/skills/documents/U.S.%20FDA%20Target%20Product%20Profile%20Guidance%20Document%20(2007).pdf.

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.

Despite the draft status, this document is a useful tool when planning for product development.

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 3 presents an example of an abbreviated TPP that would be appropriate for an oral therapeutic to treat or prevent an infectious disease.

TABLE 3.
EXAMPLE OF A TARGET PRODUCT PROFILE

Product Targets	Minimum Acceptable Result	Target
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	Oral	Oral
Dosage Form	Tablet or capsule	Tablet or capsule
Regimen	1–2x/day	1x/day
Efficacy	90% pathogen inhibition/survival	100% pathogen inhibition/survival
Bioavailability	10%	>20%
Safety	No observed adverse effect level (NOAEL) 10-fold human dose	NOAEL 100-fold human dose
Storage Conditions	Temperature: 25ºC Humidity: 60% relative humidity (RH)	Temperature: Extended periods of >37°C Humidity: 60% RH
Shelf-life Stability	2 yr	5 yr

A. 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 search for new chemical entities for a particular target may be quite different depending on whether it is intended for oral administration (e.g., via tablet or capsule) vs. intravenous (e.g., sterile injectable, auto-injector, IV-drip, etc.) vs. a topical formulation (e.g., gel, cream, spray-on) vs. ocular (e.g., eye drops, ocular injection). Physicochemical properties and specifications (solubility, pH, stability, crystal structure, salt vs. free base, etc.) can impact the suitability of a drug for eventual human use, but can be controlled early in the program during discovery and lead optimization. Therefore this aspect of the TPP should be central to the medicinal chemistry strategy of the program.

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. 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 (mg) supplies may not be practical if later-stage manufacturing requires kg-level manufacturing.

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 small molecule for urinary tract infections), 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.

III. DRUG DISCOVERY AND LEAD OPTIMIZATION PHASE

For the purpose of this document, drug discovery is defined as including the activities from hit identification through to lead optimization, and does not include the basic research that typically comes before identification of potential therapeutic classes begin. Drug discovery is typically treated as a pre-development phase in which basic research leads to new drug targets, and molecules against these targets are discovered and tested. Drug discovery can also focus on improvements to existing drug classes and compound scaffolds. This phase is usually considered to be separate from preclinical development because there are no specific regulatory requirements, and because it can be characterized as a highly iterative process that is unique to each molecule or scaffold. Nevertheless, there are a variety of general principles to be taken into consideration to assure that new molecules are viable potential therapeutics.

There is no one right way to conduct a discovery program, and much of the decision making will revolve around relative costs of assays, liabilities or special considerations with certain drug classes, cost of synthesis, timelines, and other factors. As an example, when a relevant animal efficacy model is very difficult or expensive (e.g., Ebola testing in rhesus macaques), significant

preparation for this study (development of a suitable formulation, PK testing in macaques, extensive in vitro screening) will likely occur before the efficacy model. In contrast, if an efficacy model is a simple 24 hour study in a mouse, it might be feasible to conduct an early efficacy study before conducting any PK studies. Nevertheless, all drug discovery programs share the ultimate goal of identifying and optimizing a lead candidate and entering into a preclinical development phase. Therefore, preclinical development programs and the TPP should serve as a guideline throughout the discovery process. A generalized flowchart of the overall process is provided in Figure 2.

In all drug discovery efforts, the process is iterative, with a combination of in silico modeling, chemical synthesis, in vitro potency and in vitro/in vivo ADMET being used in tandem to identify promising molecules to move into in vivo efficacy studies. Or perhaps more accurately, the purpose is to identify compounds with unfavorable properties, and eliminate them from the discovery pathway.

While every discovery program has its own unique characteristics, the points below are applicable to all discovery programs and should be considered for each program to determine how and when to incorporate them into each program.

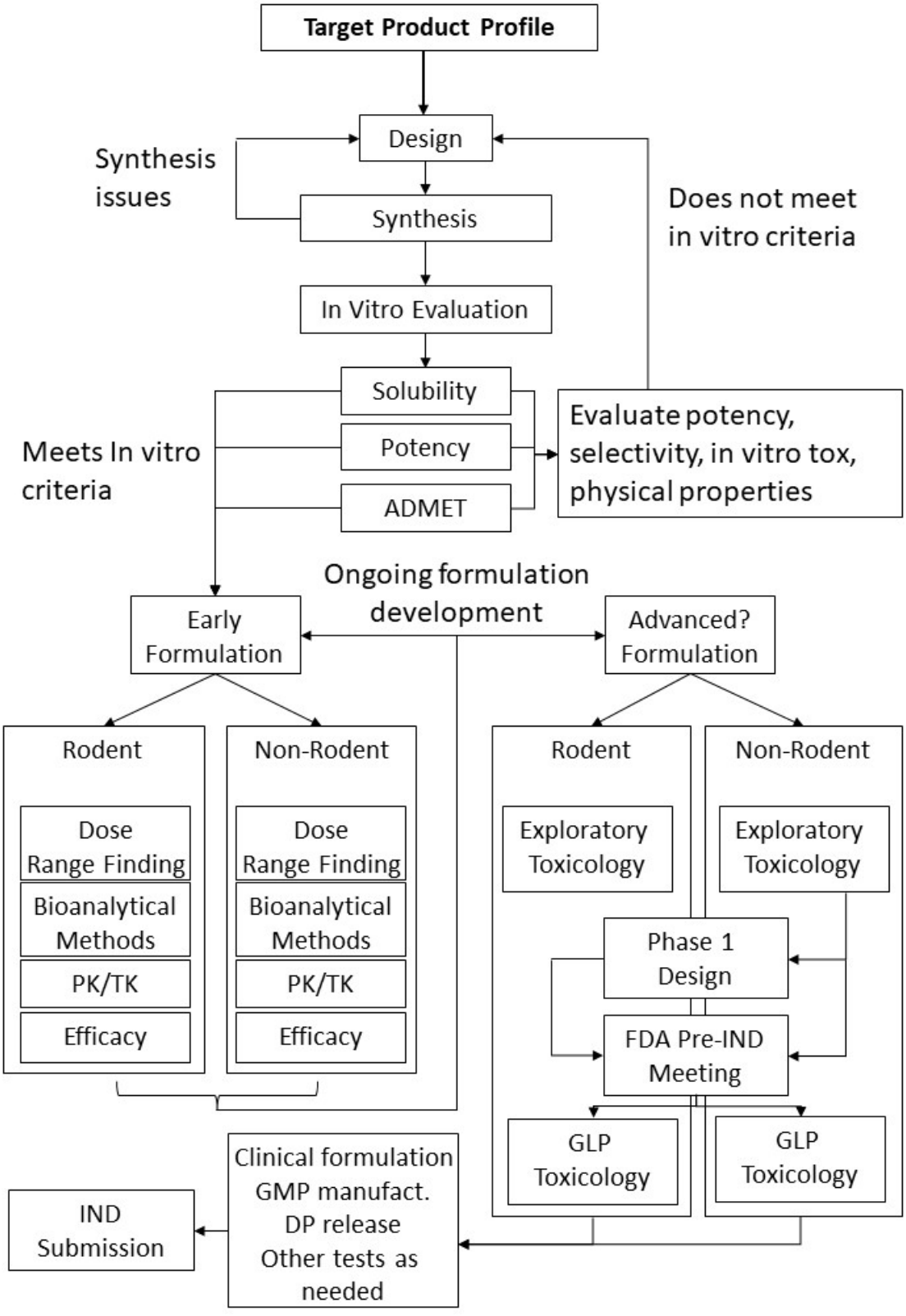

Figure 2. Flowchart of Drug Discovery and Preclinical Development

A. Selection of Appropriate Chemical Characteristics

Given the nearly infinite number of potential chemical structures, there is no one right way to pick compounds as candidate pharmaceutical compounds. Table 4 below summarizes some of the general characteristics to consider during the drug discovery phase.

TABLE 4.
CONSIDERATIONS FOR INDENTIFICATION OF SUCCESSFUL LEAD COMPOUNDS

Category	Consideration	Mitigation Technique
Chemistry and In Vitro Testing		
Administration Route	Are physicochemical properties consistent with proposed clinical route of administration?	In silico property predictions Preformulation studies
Solubility	Is the solubility consistent with what would be needed for IV or other parenteral routes?	In silico property predictions Solubility screens; need for making a corresponding prodrug or salts
Synthesis	Are reagents, catalysts and intermediates safe to work with at large scale and readily available at feasible costs?	Literature review; vendor analysis; further process development
Pharmacokinetics	Is the structure likely to have pharmacologic properties consistent with intended route and use	In vitro ADMET studies
Protein Binding	The efficacy of the drug may be impacted by increased binding to plasma proteins.	In vitro plasma protein binding studies in human and animal plasma
Hydrolysis	Prodrugs are sometimes easily hydrolyzed to their insoluble parents	Formulation studies
Toxicity	Are there off-target toxicological mechanisms that would lead to preclinical development failure?	Hemolysis assay (for IV drugs) Mammalian cell toxicity testing Target enzyme assays In silico analysis hERG inhibition testing
Off-Target Binding	Is the structure likely to be promiscuous in binding to a broad range of receptors?	In silico analysis; receptor binding assays
Metabolism	Is the structure prone to common metabolism that could either inactivate or produce reactive metabolites?	In silico analysis; metabolic stability studies
Structural Alerts	Are there any chemical structural alerts (identified in silico or other methods) that suggest potential reactivity of parent or metabolites?	In silico analysis; genetox screens
Class Liabilities	Are there any known safety or other liabilities of this chemical class?	Literature review In silico analysis
In Vivo Testing		
Rodent PK	Does drug have acceptable PK properties for the intended dose route: half-life, total exposure, bioavailability, Tmax, Cmax, etc.	Rodent PK screens Formulation to aid bioavailability

TABLE 4.
CONSIDERATIONS FOR INDENTIFICATION OF SUCCESSFUL LEAD COMPOUNDS

Category	Consideration	Mitigation Technique
In Vivo Efficacy	Does the drug demonstrate acceptable efficacy in a relevant animal model	Appropriate animal efficacy models conducted by a relevant route of exposure
Rodent Toxicity	Does the drug demonstrate any unexpected safety liabilities; is there an acceptable therapeutic index	Mouse or rat does escalation and repeat-dose screening studies Include relevant biomarkers or histopathology of target organs as appropriate

B. In Vitro Efficacy

The initial step in establishing efficacy of new pathogen treatments is based on methods to assess inhibition of the intended target. There are many quantifiable parameters that give information regarding the effectiveness of a drug. These include: inhibition of the pathogen target (inhibition constant, Ki); selectivity for pathogen versus host targets; inhibition of pathogen growth (EC50, MIC); cidality; ratio of cytotoxicity to efficacy in vitro (therapeutic index), synergy and drug interaction; kill-curve kinetics, and resistance frequency studies. Each of these different parameters gives valid information about the properties of an antimicrobial drug agent and can help to determine its potential clinical utility relative to other drugs or in combination with other drugs.

Using in vitro assays for evaluating compounds for activity against a pathogen is critical and developing standard operating procedures for generating reliable and reproducible in vitro data sets is required. The standard operating procedures will consider variable conditions such as pathogen input, kinetics of infection and the culture conditions such as cell passage number and their impact on the effective concentration for growth inhibition. Table 5 summarizes some of the key characteristics of in vitro efficacy/potency assays.

TABLE 5.
COMMON IN VITRO ASSAYS TO EVALUATE ANTIMICROBIAL ACTIVITY

Assay	Approach	Advantages	Application
Viral Assays			
Cytopathic Effect (CPE) Inhibition Assay	Determine effective drug concentration (EC_{50}) using cell culture or molecular methods	Rapid, inexpensive	First-tier screening, all programs; early identification of compound efficacy
Viral Yield Reduction (VYR) Assay	Determine drug concentration that inhibits virus production in mammalian cell culture	Rapid, inexpensive	Second-tier screening, follow-up for compounds with activity in the CPE assay.
Median Cellular Cytotoxicity Concentration (CC_{50})	Determination of drug dose that induces cell death as compared to the EC_{50} to determine the therapeutic index.	Rapid, inexpensive	First-tier screening, all programs; early identification of compound efficacy
Determination of Inhibitory Quotient (IQ)	Determination of the serum adjusted EC_{50}	Rapid, inexpensive	Determination of plasma and intracellular product concentrations; second tier screening
Bacterial and Fungal Assays			
Minimum Inhibitory Concentration (MIC) Testing	Pathogens are cultured in either broth or agar in the presence of different dilutions of the test compound	Flexible assay for many bacteria and/or fungal targets; CLSI standard methods are available for several pathogens	First-tier screening, all programs; useful to predict anti-microbial activity
Minimum Effective Concentration (MEC) Testing	Pathogens are cultured in either brother or agar in the presence of different dilutions of the test compound	Reserved for fungal targets; CLSI standard methods are available for several pathogens	First-tier screening, all programs; useful to predict anti-microbial activity

C. In Vitro ADMET

In vitro assays that measure a variety of parameters related to absorption, distribution, metabolism, elimination and toxicity (ADMET) have become an invaluable tool in assessing whether new molecules are likely to be viable pharmaceutical candidates. When used in conjunction with chemical synthesis and in vitro efficacy, they provide a rapid tool for narrowing the search to molecules that are likely to have appropriate properties.

ADMET assays are most commonly used to prioritize/deprioritize compounds for preclinical development after in vitro efficacy has been identified (i.e., in moving compounds from "hit" to "lead"). The importance of each assay is dependent on the specific target indication. For example, for drugs intended for oral administration, absorption assays (PAMPA, Caco-2) are critical for determining the probability of systemic uptake, but are not particularly useful for IV

or topical drugs. CYP inhibition is most important for drugs expected to be given in combination with other therapeutics (e.g., HIV, TB). Metabolic stability can identify compounds that will be rapidly metabolized by the liver. As an example, a drug with a short metabolic stability (minutes, not hours) and poor absorption, is likely to be very poor candidate for oral administration.
Plasma protein binding can indicate the degree to which drugs are bound to proteins, which can limit their delivery to some organs (e.g., the brain), but binding may be unimportant for some systemic therapeutics. Cytotoxicity assays are broadly useful for all drugs to identify any early safety liabilities.

The specific sequences and priorities of the various in vitro ADMET assays will vary by project and will depend on the TPP for a particular drug discovery effort. Generally, lower cost and more rapid test assays will be applied in the earlier tiers of the test funnel, whereas employing the more expensive and slower testing procedures will require that compounds meet specific metrics before being profiled in those assays. An additional consideration at this stage is the amount of compound required for each assay, with those requiring minimal compound given priority. Table 6 presents a summary of the most common ADMET assays in standard use and the rationale for their use.

TABLE 6.
ADMET ASSAYS COMMONLY USED IN DRUG DISCOVERY AND PRECLINICAL DEVELOPMENT

Assay	Approach	Advantages	Application
Absorption Assays			
Aqueous Solubility*	In silico and/or experimental use of common vehicles to determine compound solubility limits	Rapid, inexpensive	First-tier screening, all programs; early identification of solubility
Parallel Artificial Membrane Permeability Assay (PAMPA)	Incubate drug with cell-free membrane system	Rapid, inexpensive	First-tier screening, all programs; predicts GI absorption
Caco-2 or MDCK Cell Assays	Determination of bidirectional permeability to cell monolayer	Cell models with transporter/efflux proteins	Oral drug programs, especially when efflux/transporters may be active; useful for predicting ability to cross intestinal lining
Clearance/Metabolism Assays			
Metabolic Stability	Liver microsomes, S9 or hepatocytes, human and laboratory species used to determine rate of metabolism of drugs by the liver	Flexible assay for all hepatic enzymes; species differences indication	First-tier screening, all programs; useful to predict in vivo half-life of drugs

TABLE 6.
ADMET ASSAYS COMMONLY USED IN DRUG DISCOVERY AND PRECLINICAL DEVELOPMENT

Assay	Approach	Advantages	Application
CYP Inhibition	Screen inhibition of 7 major CYPs in human liver microsomes	Rapid, sensitive assay	First-tier screening, all programs; can predict potential drug-drug interactions in vivo
Distribution Assays			
Plasma Protein Binding	Ultrafiltration or dialysis of human and animal plasma incubated with drug	Detection of high protein binding that can limit penetration into CNS or other tissues	First-/second-tier assay for CNS programs; with subsequent screening for other discovery programs
Toxicity Assays			
Cytotoxicity	Various cell lines and primary cultures (e.g., VERO, HEK, primary hepatocytes) used to identify cytotoxic effects	Ranking of compounds for relative cytotoxicity	Second-tier assay; can identify potential organ-specific or systemic toxicity

* For additional details on solubility determinations, see Formulation section (Section III.D) below.

A common panel that may be used for most lead optimization programs consists of solubility, metabolic stability in microsomes, a permeability assay (PAMPA or Caco2), plasma protein binding, CYP inhibition, and a cytotoxicity assay. A brief description of each assay in this common panel follows:

Solubility. Simple aqueous solubility is a good predictor of systemic absorption of compounds, as well as a variety of practical considerations related to ease of formulation and manufacturing. Highly insoluble compounds will pose a series of challenges throughout preclinical development, and if more soluble analogs have comparable efficacy, those will nearly always be better choices for lead compounds.

Metabolic Stability in Microsomes. In vitro metabolic stability studies help predict the relative rate of biotransformation of the test compounds which in turn is predictive of half-life and clearance in vivo. Test compounds are incubated with pooled mixed-sex liver microsomes of a relevant species (e.g., human, rat, mouse) and disappearance of the drug is measured by liquid chromatography coupled with tandem mass spectrometric detection (LC-MS/MS).

Parallel Artificial Membrane Permeability Assay (PAMPA). This assay uses lipid-coated filters to mimic the intestinal epithelium and helps in predicting the passive diffusion of drugs across the intestinal membrane. Promising results in a PAMPA assay are often followed by a Caco-2 assay.

Caco-2 Assay. The assay is generally conducted to evaluate drug movement across the Caco-2 cell monolayer in both directions. A higher rate of flux from the basal to the apical side compared with apical to basal suggests that unfavorable membrane transport properties are likely to limit drug bioavailability. An efflux ratio (ratio of basal to apical transport rate to apical to

basal transport rate) greater than 2 typically suggests the involvement of transporters (e.g., P-gp, BCRP), which can be confirmed by an efflux ratio that is less than 2 or significantly reduced in the presence of specific inhibitor. An efflux ratio less than 2 in the absence of inhibitor suggests the drug is not a substrate for transporters and that bioavailability will not be limited.

Plasma Protein Binding. Binding of compounds to plasma proteins can interfere with transport into specific tissues of the body. Plasma protein binding is particularly important for anti-infectives as only unbound molecules can easily penetrate the outer membrane of most pathogens. This assay involves incubating compounds at concentrations expected in vivo with plasma from relevant species (e.g., rat, mouse and human) using equilibrium dialysis and quantitating the percent bound by LC-MS/MS. In vivo drug exposure parameters such as C_{max} and AUC can be adjusted by accounting for the percent bound to plasma proteins to reflect exposure to the *free* drug, if the plasma protein binding is extensive (e.g. over 95%).

CYP Inhibition. Drug-induced inhibition of CYP enzymes is a useful tool for identifying compounds with potentially problematic drug-drug interactions. This entails incubating a cocktail of CYP probe substrates with multiple concentrations of the test compound, with the specific metabolites determined using a single high-throughput LC-MS assay.

Cytotoxicity Assays. Measurement of cytotoxicity is a useful predictor of in vivo toxicity. Incubation in various cell lines can provide general degrees of cytotoxocity, but use of specialized cells (e.g., kidney or CNS cells) can provide additional insights into organ-specific toxicity.

In addition to the commonly conducted assays described above, there are a variety of specialized in vitro assays that may be useful for either further examination of effects (e.g., potentially triggered by a finding in the above assays), or for specialized applications. Table 7 presents additional assays that may be important for specific programs.

TABLE 7.
ADDITIONAL ADMET ASSAYS THAT MAY BE USED FOR SPECIFIC PROGRAMS

Assay	Approach	Advantages	Application
Absorption Assays			
Solubility (biologic fluids)	Determination of solubility in simulated GI fluid	Rapid, inexpensive	First-tier screening, all programs; can determine solubility in the gut for better prediction of intestinal absorption
Clearance/Metabolism Assays			
Metabolic Stability (non-liver)	Small intestine (SI) microsomes, or other sources used to identify organ-specific metabolism	Prediction of presystemic metabolism	First-tier screening, especially critical for oral drugs; can determine stability in specific target organs

TABLE 7.
ADDITIONAL ADMET ASSAYS THAT MAY BE USED FOR SPECIFIC PROGRAMS

Assay	Approach	Advantages	Application
Metabolite Identification	Liver or S9 preparations used to generate metabolites followed by LC/MS identification	Preliminary information about expected metabolites; guides additional synthesis to block metabolism	Useful for classes of compounds that are highly metabolized or to identify active or toxic metabolites
Plasma Stability	Incubation with plasma from human and laboratory species	Detection of plasma instability problems	Second-tier screen, unless structure suggests plasma instability (e.g., ester)
Transporter Assays ("FDA Nine")	Cells expressing single transporters	Rapid assays for both substrates and inhibitors, detection of rapid clearance	Second-/third-tier screen, important for CNS drugs
CYP Induction	Screen induction in hepatocytes or reporter model	Detection of species-related induction	Third-tier screen, except for compounds structurally related to known inducers
Reaction Phenotyping	Identification of the CYP, UGT, SULT or other enzyme is responsible for metabolite formation	Determination of specific isoform of CYP, UGT, SULT, or other enzymes primarily responsible for metabolism	Second-tier assay; identification of compounds metabolized by one major enzyme (associated with drug interactions and toxicity)
Distribution Assays			
Free Brain Levels	Determination of binding to brain homogenates	Correlation with the level of compound available for drug effect	For CNS programs
Red Blood Cell: Plasma Partitioning	Determine ratio of drug in RBC vs. plasma	Allows determination of delivery of drug to the site of pathogen	For bloodborne parasites and other pathogens who live within RBCs
Toxicity Assays			
Hepatotoxicity	Primary hepatocytes: mitochondrial function (ATP or MTT), or membrane permeability (LDH release)	Detection of hepatotoxic compounds; demonstration of species differences	Applicable for compounds suspected of liver toxicity
Genotoxicity	Ames mutagenesis, mouse lymphoma or micronucleus assays	Rapid, small amount of drug required, metabolic activation used	Useful when compounds have "structural alerts" for mutagenic properties
Reactive Metabolite Formation	Trapping of reactive metabolites with GSH and detection using LC-MS/MS	Early detection of reactive metabolites related to toxic effects	Useful when structures suggest reactive metabolites are likely

TABLE 7.
ADDITIONAL ADMET ASSAYS THAT MAY BE USED FOR SPECIFIC PROGRAMS

Assay	Approach	Advantages	Application
Cardiac Effects	Ion channel effects in hERG cells	Early signal of potential cardiac effects	For compounds where cardiac effects are a potential liability
Receptor Binding	Assessment of compound binding to panel of receptors	Early identification of off-target receptor binding that could lead to organ-specific toxicity	For compounds with little toxicity data available for that chemical class
Mitochondrial Toxicity	Assessment of effects on critical mitochondrial functions	Early identification of mitochondrial toxicity effects	For certain classes of compounds (e.g., fluoroquinolones) with known mitochondrial toxicity liabilities

Applications of these assays will depend on the specific compound and indication. For example, determining RBC partitioning is useful for bloodborne parasites such as malaria, because the assay will determine which compounds are most likely to reach effective concentrations within the RBCs. Metabolite identification may be warranted if a metabolic stability study demonstrates rapid elimination of a parent drug in the presence of liver microsomes.

Sometimes specialized in vitro assays targeting specific mechanisms of organ system toxicity may be used. These are more relevant at the discovery and lead optimization stage, though they can also be useful for mechanistic evaluations when toxicities are identified during preclinical development. For example, several classes of anti-infective compounds have known liabilities for causing mitochondrial toxicity. Many non-nucleoside reverse transcriptase inhibitors and fluoroquinolones have severe mitochondrial effects that have resulted in FDA "black box" warnings on marketed drug products. There is a growing body of literature on new in vitro models for assessing the potential of drugs to induce mitochondrial injury. These assays involve treatment of various cell lines in culture, followed by ELISA-based analysis of several key functions of mitochondria.

Other assays may be appropriate for classes of compounds with a history of organ-specific safety concerns (e.g., aminoglycosides and renal injury). The use of these early screening assays can identify potentially problematic compounds before investing significant time and funding into advancing them into preclinical development.

3-D, Organ-on-a-Chip, and Other Specialized In Vitro Systems. There is growing interest in a new generation of cell culture models that use 3-D cell culture systems (usually grown on a scaffold); precision cut organ slices; organ-on-a-chip systems, often with multiple systems (e.g., lung, heart, liver) interconnected through microfluidic systems; 3-D "printing" of organ systems (e.g., assembly of custom livers using an ink-jet printer technology); and other new technologies. These systems have tremendous promise for specific research applications, but as of this writing are still experimental, relatively expensive to run, sometimes limited in use by intellectual property restrictions, and not suitable for most drug discovery programs. Nevertheless, the

significant investment in this field is sure to produce improvements in speed, cost and applicability, and this is a trend to watch over the next decade.

D. Early Formulation Development

It is important early in drug discovery to begin consideration of specific formulations that will need to be developed. While the final clinical formulation may be years away, suitable formulations will be required to initiate research work, but will evolve throughout the discovery, preclinical and clinical development process until a final marketed product is available. Early in discovery, simple dissolution in DMSO may be adequate to conduct in vitro potency assays, but by the time early animal studies (range finding toxicity, efficacy) are conducted, a reasonable formulation to allow dosing by the route of clinical interest should be established. These formulations may initially be suitable for animals, but may also include excipients not yet approved for human use. By the time GLP studies in animals are conducted, the formulation used should resemble, or in many cases be identical to, the formulation proposed for administration in a Phase I clinical study. It is not uncommon to conduct GLP safety studies using the actual GMP drug product (e.g., a sterile injectable product in a syringe vial), though this not a strict FDA requirement. The types of appropriate formulations will depend on the indication, target population, desired site of delivery chemical properties of the active ingredient, and a host of other factors. Formulation for Phase I studies may be simple, and not intended for commercialization (e.g., powder in bottle with instructions to add water for oral administration). As the product progresses through Phase II and Phase III studies, the formulation should approach what the ultimate end product will be that will seek final market approval from the FDA. All of these points should be considered at the time the TPP is developed.

Dosage formulation and design is a process to develop a product that contains the correct amount of drug in the right form and maintains its chemical and biological integrity so it will be delivered over the proper time and at the proper rate for a desired clinical route of administration. The design of a dosage formulation involves a series of trade-offs to produce the desired properties for the finished product. Compromise is sometimes necessary because desired properties, such as resistance to mechanical abrasion or friability, are competing with other desired parameters, such as rapid disintegration and dissolution time. In addition, for clinical development of formulations, the processes selected must meet criteria for scale-up to commercial quantities, and the entire operation must meet validation requirements.

A full discussion of formulations for preclinical development studies is included later in this document (see Section IV.C). For lead optimization-related activities (in vitro and in vivo efficacy, in vitro ADMET, early toxicology/PK), a broader range of vehicles and formulation components may be considered than would be appropriate for use in human clinical trials, and this approach will allow compounds to progress to preclinical development more quickly. Nevertheless, it is important to understand that delaying development of clinically suitable formulations might accelerate the discovery phase, but cause delays, backtracking, or costly program failure if the preclinical candidate is found to be unsuitable for use in a human formulation when preparing for clinical development. It is therefore a balancing act to determine how and when to invest in formulation development to maximize the probability of eventual product success without unduly hindering the discovery process.

Formulation development for orally administered drugs often has more flexibility, because compounds can be administered as suspensions, and there are a wide variety of suspending and

solubilizing vehicles commercially available. In contrast, a poorly soluble compound may be easily administered orally as a suspension, but could also have very poor oral bioavailability. Parenteral administration (IV, IM, SC) requires sterility, which can add additional challenges for poorly soluble compounds. For in vitro assays, compounds may be highly soluble in DMSO or acetone, but precipitate out when added to culture media. Some vehicles that are suitable for short-term use in PK and efficacy studies can result in adverse effects in toxicology studies. Nonionic emulsifiers (e.g., Tween 80, Poloxamer) may be suitable for studies in rats, but can produce anaphylactic reactions in dogs. DMSO is relatively well tolerated by animals via oral, IV, IP and other routes, and may be appropriate in limited cases, but it is highly recommended to find alternatives to DMSO whenever possible as it can change membrane permeability in ways that produce unrealistic and irrelevant uptake by tissues.

Development of formulations for inhalation delivery (e.g., a powder administered via a metered-dose inhaler) is particularly challenging. Formulations for inhalation are complex research projects on their own, irrespective of the drug being delivered. The respiratory tract of mammals has evolved to keep particles out of the respiratory system, so development of aerosol formulations focuses on techniques for maximizing deposition of particles into the lungs. Even very effective delivery systems often achieve only ~20% delivery to the lungs. (For a good review of this field, see Newman, 2017). Those planning products that are delivered via inhalation should engage an expert in pulmonary delivery systems very early in the discovery phase to maximize the possibility of success.

Table 8 provides some general guidelines to consider when developing formulations for use at various stages of discovery.

TABLE 8.
COMMON SOLVENTS AND APPROACHES FOR DISCOVERY STAGE FORMULATIONS

Aqueous Solubility	Example Solvent/Vehicle	Challenges
In Vitro Efficacy and ADMET		
> 50 mg/ml	Water, culture media, saline	None
1-50 mg/ml	DMSO, acetone	Precipitates out at higher concentrations
< 1 mg/ml		Difficult to test under any situations
In Vivo Efficacy and PK		
> 50 mg/ml	Saline, water, drinking water	None
1-50 mg/ml	PEG-500, Tween 80, Carboxymethylcellulose, DMSO, corn oil	None
< 1 mg/ml	Poloxamer, Tween 80, Intralipid	Difficult for IV; at higher doses, thick suspensions can be difficult to gavage
In Vivo Toxicology		
> 50 mg/ml	Saline, water, drinking water	None
1-50 mg/ml	PEG-500, Carboxymethylcellulose	some vehicles (oils, DMSO) can cause changes in some clinical chemistry parameters

TABLE 8.
COMMON SOLVENTS AND APPROACHES FOR DISCOVERY STAGE FORMULATIONS

Aqueous Solubility	Example Solvent/Vehicle	Challenges
< 1 mg/ml	Poloxamer, Tween 80, Intralipid	Difficult for IV; at higher doses, thick suspensions can be difficult to gavage; vehicles may have some adverse effects such as anaphylaxis in dogs

E. 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.

F. Early Rodent Pharmacokinetics

Early rodent pharmacokinetics studies are intended for rapid assessment of in vivo PK of a compound to guide subsequent efficacy studies, or to pick an analog with the best PK parameters. Species selection is guided by the animal species that are available for relevant infection models and/or downstream pharmacology/toxicology plans. A typical experiment employs a sufficient number of animals to provide adequate blood samples. For early screening, a single sex is commonly used (two sexes are included in subsequent confirmatory and GLP studies should the candidate progress). A sample study design for rodents is shown in Table 9. The compound is administered either at two dose levels by a single route, or by a single dose level by two routes. Common routes of exposure used in early studies are po, iv, im, sc, and ip. Early PK is nearly always done in rodents which are inexpensive and require small drug quantities. There may be specialized cases where other species are desirable (e.g., hamsters, guinea pigs, rabbits, ferrets, pigs, non-human primates). The study designs for these species are similar, with appropriate numbers of animals, taking into account blood volume, drug requirements, and other factors. Typically nonhuman primate (NHP) studies will be conducted in 1 to 2 animals per sex per dose group, with smaller species using 3-5 animals/sex/group/timepoint. Because of the limited blood volume of mice, and the inability to install jugular vein catheters, multiple cohorts of mice may be required (e.g., 3 groups of 3 mice = 9 mice to obtain 6 timepoints).

TABLE 9. DESIGN FOR PK STUDIES IN RATS AND MICE			
Group	**# Animals/ Group**	**Dosage Route**	**Blood collection time**
1	3 Male Rats 9 Male Mice	iv	Predose, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24
2	3 Male Rats 9 Male Mice	po	Predose, 0.25, 0.5, 1, 2, 4, 8, 12, 24

If the intended clinical route of administration is IV, then the study is typically conducted only by the IV route. If an alternate route is intended for the clinic (e.g., oral, intramuscular) then the intended route plus an IV group is included. This allows calculation of bioavailability, which is expressed as po exposure relative to the iv exposure.

Blood samples after administration of the test article are collected at multiple time points (e.g., predose, 5, 15, 30 min and 1, 2, 4, 8, 12, 24 hr). Ideally, (1) sampling covers a sufficient period of time and equal number of timepoints in each of the uptake and elimination phases, and (2) the sampling period encompasses approximately five elimination half-lives, or 95% of the dose.

The following endpoints are also evaluated:

- Mortality/Morbidity: Checked daily.
- Clinical Signs: Immediately postdose and 2–4 hr after treatment.
- Body Weights: Study Day 0 prior to dose administration.
- Plasma Drug Levels: At timepoints shown in the blood collection schedule.
- Pharmacokinetic parameters are calculated (Table 10).
- Pharmacodynamic parameters may also be collected (see PK/PD section below)

TABLE 10.
COMMON PHARMACOKINETIC PARAMETERS

Parameter	Description	What is it used for?
C_{max}	Maximum plasma concentration	The highest plasma concentration that is achieved after dose administration. Important for both assessing efficacy of some antibiotics as well as determining safe levels relative to safety.
T_{max}	The time C_{max} occurs	When is the highest concentration observed ?
AUC	Area under the concentration vs. time curve	An indicator of total exposure. This is often (but not always), the most important measure of how much drug is reaching the systemic circulation.
F	Bioavailability	Determine how much of a non-intravenous (e.g., oral) dose reaches the systemic circulation compared with the IV route
C_0	Extrapolated maximum plasma concentration after IV	An estimate of the amount of drug in plasma immediately after an IV injection, determined by extrapolation. Useful for estimating a peak blood level due to the inability to actually measure instantaneous blood peaks after injections.
$t_{½}$	Terminal elimination half-life	How long is the drug staying in the circulation. Short half-life drugs will require frequent dosing.
Cl	Total clearance	The volume of plasma from which a drug is completely removed relative to time.
V	Apparent volume of distribution	Represents whether a drug stays in plasma or distributes to other compartments. Useful for estimating how much drug is in tissues vs. blood.
MRT	Mean residence time	The average time a molecule stays in the body. A different measure than half-life of how long drug exposure may occur and is relevant for calculating binding time to targets.

G. In Vivo Efficacy

There is an increasing need for drugs and dosage regimens with optimal antimicrobial activity and a good safety and tolerability profile to ensure a high likelihood of therapeutic success in patients. Preclinical in vitro and in vivo efficacy (pharmacodynamic) studies and pharmacokinetic studies can provide crucial data to select doses and dose regimens to achieve efficacious drug exposure while also limiting toxicity and development of resistance.

For nearly all pathogens it is critical to demonstrate efficacy in an appropriate animal model under conditions (dose level, dose route, frequency of administration, conditions of use) that are relevant to treatment of human disease. The major goal of these nonclinical studies is to determine drug exposure-response relationships which help select the appropriate clinical dose regimen to achieve the target exposure, and hence, efficacy in patients. In vivo efficacy evaluation typically follows identification of early “hits” using in vitro potency assays, early-

stage toxicity evaluations, development of an appropriate formulation and determination of favorable pharmacokinetics. A comprehensive list of all available animal models is outside the scope of this document as there are hundreds of possible infection models that range from mouse (many bacterial pathogens) to ferrets (influenza) to woodchucks (hepatitis) to macaques (anthrax, Ebola) and other species. The design and endpoints examined can include pathogen load, survival, clinical observations, histopathology, specific biomarkers, or other endpoints. Table 11 briefly summarizes some of the key endpoints in these efficacy models, and the advantages and disadvantages of each.

TABLE 11.
CATEGORIES OF ANTI-INFECTIVE IN VIVO EFFICACY ASSAYS

Assay	Approach	Advantages	Disadvantages
Quantitation of Pathogen Load Over Time	Bacterial/Fungal: Determination of Log CFU. Viral: Determination of the serum adjusted EC_{50}	Rapid, inexpensive, direct measure of scale of infection	Does not address symptoms or pathology
Survival and clinical Signs	Evaluation of clinical endpoints (body temp, hypoactivity, etc.) in treated vs. untreated animals	Detailed clinical assessment, correlation to human symptoms	More expensive; requires staff experienced in clinical grading of signs
Histologic Examination of Tissues	Determination of pathology of treated vs. untreated animals.	Detailed evaluation of a specific target organ	Expensive; requires detailed knowledge of disease pathology
Biomarker Assessment	Measurement of a specific tissue biomarker as a surrogate for disease	Rapid, inexpensive, direct measure of impact on a specific organ target	Addresses only a single symptom, not severity of infection

These endpoints may be combined, for example, assessing clinical signs, viral load, a liver biomarker, and liver histopathology in a hepatitis model.

While survival is sometimes used as an endpoint (e.g., how many mice die without treatment vs. with treatment), in practice, for ethical and other reasons, death alone as an endpoint should be avoided whenever possible. Typically, animal models relying on mortality have euthanasia criteria, and measurement of time to reaching euthanasia criteria are a better, more humane, and more quantitative indicator of efficacy than simply death/survival. Use of clinical signs (e.g., labored breathing, severe hypoactivity) or time to appearance of clinical signs provide a richer data set and are more humane than allowing animals to die on test.

Efficacy models are frequently conducted with collection of plasma or other tissues for determination of pharmacokinetics in the infected model. These are subsequently used for PK/PD assessments (see next section).

H. PK/PD Assessments

It is critical to understand the relationship between pharmacokinetics (PK) and pharmacodynamics (PD) in assessing efficacy of anti-infective drugs. A simple way to differentiate between PK and PD is that pharmacokinetics is what the body does to the drug and

pharmacodynamics is what the drug does to the body. With many anti-infectives, pharmacodynamics is the effect produced on the pathogen responsible for the infection, though some drugs may also work by impacting the host response (e.g., therapeutics that decrease a cytokine storm response in the lungs).

To develop PK/PD models one will typically require in vitro efficacy data (e.g., bacterial MIC or viral PFU reduction assays), in vivo PK and in vivo efficacy data.

MIC, or minimal inhibitory concentration, is the lowest concentration of the test agent at which visible growth of the specific organism is completely inhibited. MIC is usually determined by incubating the test compound with clinical isolates of specific strains using a broth microdilution assay.

PK studies need to be conducted in the specific animal disease model in infected and uninfected animals, as the infection may alter the PK characteristics of the compound. A range of dose levels need to be selected to account for any nonlinearity in exposure with increasing doses. Characterization of the PK in the deeper infection sites is important, e.g., determining distribution to the bronchioalveolar lavage fluid (BALF) and correlate plasma and BALF PK with PD endpoints. It is recommended to determine the plasma protein binding potential of the test compound as only the unbound or free drug is available to interact with the biological target.

The relationship between drug exposure and response can be quantified using static or dynamic in vitro and in vivo infection models. Examples of in vitro PK/PD models include static concentration (SCTK) assay, dynamic one-compartmental model using chemostats and dynamic two-compartmental models using hollow fiber in vitro infection system. The most commonly used in vivo PK/PD models are the murine thigh and lung infection models (Bulitta et al, 2019).

The MIC values for different clinical isolates vary widely; hence, the PK/PD parameters determined are "indexed" to the MIC. The quantitative relationship between a PK parameter and a PD parameter in antimicrobial PK/PD analyses and MIC is called a PK/PD index or driver. The three main indices used to predict antimicrobial activity in PK/PD studies are illustrated in Figure 3 and Table 12 below (Asin-Preito et al, 2015).

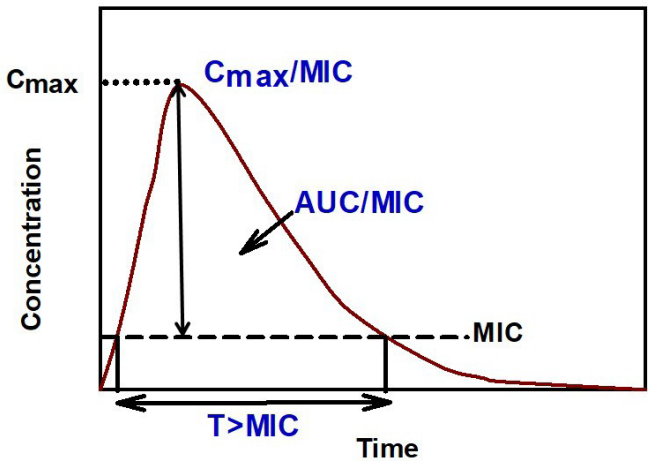

Figure 3. PK/PD indices associated with the efficacy of antimicrobial agents

TABLE 12.
PK/PD INDICES USED IN ANTIMICROBIAL DRUG DEVELOPMENT

PK/PD Index	Definition
*f*AUC/MIC	Ratio of the area under the unbound drug concentration-time profile to MIC
%*f*T>MIC	Percentage of time that the unbound drug concentration exceeds the MIC over a 24-hr period
fC_{max}/MIC	Ratio of the maximum unbound drug concentration to MIC

Three major patterns of activity have usually been observed with antimicrobial compounds (Asin-Prieto et al, 2015), which are listed and described in Table 13.

TABLE 13.
PATTERNS OF ANTIMICROBIAL ACTIVITY WITH CORRESPONDING PK/PD INDICES

Pattern of Antimicrobial Activity	PK/PD Index	Examples of Antimicrobial Drugs (Target Magnitude of PK/PD Index)
Concentration-dependent killing and persistent effect	fC_{max}/MIC and *f*AUC/MIC	Aminoglycosides (C_{max}/MIC=10) Quinolones (AUC/MIC=125)
Time-dependent killing and no or very short persistent effect	%*f*T>MIC	ß-lactum antibiotics Penicillin (*f*%T>MIC=50–60) Cephalosporins (*f*%T>MIC=60–70) Carbapenems (*f*%T>MIC=40–50)
Concentration-independent killing and persistent effect	fC_{max}/MIC and *f*AUC/MIC	Tetracyclines (AUC/MIC = 25) Vancomycin (AUC/MIC=400) Azithromycin, Clarithromycin (*f*AUC/MIC=25)

Dose Fractionation efficacy studies, performed in vitro or in vivo, are used to determine which parameter is most relevant for a specific anti-infective. Dose fractionation changes C_{max}/MIC and %T>MIC but not AUC/MIC. In some cases, different dosing schedules will be used to achieve the same cumulative exposure (i.e. same AUC/MIC). Here, a more frequent dosing schedule splits the doses and C_{max}/MIC will decrease proportionate to dosing frequency and the %T>MIC will increase. Combining several doses and administering less frequently will increase C_{max}/MIC and decrease %T>MIC (Gumbo et al 2015). For some drugs, efficacy is optimized if the drug is administered more frequently, maximizing %T>MIC (time-dependent). For others, efficacy is optimized by administering the drug less frequently and maximizing the C_{max}/AUC (concentration-dependent). In other cases, the dosing schedule is not important as long as the same cumulative dose is given during the dosing interval (AUC/MIC linked).

The correlation between efficacy (log change in CFU) and each of the three PK/PD indices is usually determined by nonlinear least-squares multivariate regression analysis, derived from the Hill equation. The coefficient of determination (R^2) is used to estimate the variance due to regression with each PK/PD index (Lepak et al, 2020). The values of the PK/PD indices required for bacteriostasis and for a 1 and 2 $\log_{10}$ reduction in bacterial burden is determined. This value is assumed to be host-independent and is used to project a human dose for the specific pathogen.

I. Early Rodent Safety

Drug toxicity accounts for about one-third of all drug failures; it is therefore important to determine potential safety liabilities early in the discovery process. While formal dose-range finding (DRF) and IND-directed GLP safety studies will eventually be conducted on candidate lead compounds, conducting early toxicity screens with promising analogs can quickly identify which molecules are most likely to survive subsequent safety screening. Such studies can be conducted in small numbers of animals, in a single sex, with limited endpoints, and can be designed to discriminate between multiple analogs from the same structural class. Specific endpoints can be included depending on the specifics of the class. For example, aminoglycosides are known to have significant potential for renal toxicity. Inclusion of kidney histopathology or urinary biomarkers of renal functions (e.g., KIM-1, Clusterin, Cystatin C, beta-2-microglobulin) in early rodent safety studies might also provide useful early warning signals of potential renal toxicity.

The types of safety studies conducted will depend on the purpose of the study and the stage of preclinical development. Early studies are nearly always conducted in rodents (rats, sometimes mice), but in special cases may be done in other species such as guinea pigs or hamsters, depending on what the efficacy model species is, or compound class-specific sensitivities. Table 14 below, summarizes the basic categories of safety studies, when they are typically conducted, and the purpose and design of the studies.

TABLE 14.
CATEGORIES OF SAFETY STUDIES

Type of Study	Purpose	Design	When to Conduct
Dose Escalation	Identify early tolerable doses to support efficacy and PK studies	Very small (N=1-3 animals/group); clinical observations and mortality; match species to efficacy and PK study	Before efficacy and PK
Limited Repeat DRF	Confirm that tolerable doses identified in dose escalation are tolerated when delivered over multiple days	Very small (N=1-3 animals/group); clinical observations and mortality; match species to efficacy and PK study	Before efficacy to identify dose levels for multi-day animal model studies
Dose Escalation (preparing for IND-directed study)	Identify tolerable doses to support repeat dose-range finding study	Very small (N=1-3 animals/group); clinical observations and mortality; match species to IND-directed study	After efficacy and PK, before IND-directed studies.
Repeat DRF (preparing for IND-directed study)	Identify preliminary MTD and NOAEL; used to set doses for IND-directed study.	Small (N=3-5 animals/group); often includes a TK satellite; clinical observations, mortality and clinical pathology included; sometimes minimal histopathology added; match species to IND-directed study	After efficacy and PK, before IND-directed studies.

**TABLE 14.
CATEGORIES OF SAFETY STUDIES**

Type of Study	Purpose	Design	When to Conduct
Biomarker/Target Organ Screen (preparing for IND-directed study)	To answer specific mechanistic questions about drug safety	Designs vary depending on specific purpose; typically 3-5 animals/group; often includes specialized clinical pathology and histopathology of target organ of interest or other endpoints (e.g., EKG, FOB)	If range finding study demonstrates unexpected toxicity; for classes of compounds with known liabilities
IND-Directed*	For submission as part of IND application	Large studies with main, recovery and satellite TK; all endpoints evaluated; conducted under GLP compliance	Prior to submission of IND.

* See discussion of IND-directed studies later in this document.

Safety assessment, while traditionally thought of as part of preclinical development, often overlaps with the lead optimization phase. These "discovery toxicology" studies are conducted exactly like the early safety assessment studies on the path to IND-enabling studies, with the understanding that if adverse effects, or poor PK/efficacy are seen, the studies will need to be repeated with new compounds.

The designs of safety studies are described in detail in Section F below. Typically, one begins with a simple rodent escalation study to determine the maximum tolerated dose following a single administration by a relevant route. The data from these studies are typically limited to mortality and clinical signs, and are used to set dose levels for efficacy and PK studies.

If safety is considered to be a liability that is greater than poor PK or efficacy, it may make sense to conduct head-to-head safety studies using multiple analogs. For example, a particular set of aminoglycoside analogs might all be suspected (or known) to possess comparable PK and efficacy properties, but concern about kidney toxicology would lead one to screen several analogs early to identify potential safety liabilities. The design of a simple 4-day toxicity screening study of 4 analogs is presented in Table 15 below.

**TABLE 15.
SCREENING TOXICITY STUDY OF FOUR STRUCTURALLY SIMILAR ANALOGS**

Treatment Group	Compound	Dose (mg/kg)*	# of Animals
1	Analog #1	50	3M
2	Analog #1	250	3M
3	Analog #2	50	3M
4	Analog #2	250	3M
5	Analog #3	50	3M
6	Analog #3	250	3M
7	Analog #4	50	3M
8	Analog #4	250	3M
		TOTAL	**24M**

*Because of differences in molecular weights, dose levels are often adjusted to be equimolar, thereby removing differences related to salt form.

- Each test article will be administered via an appropriate route (oral, iv, sc, im, intranasal, etc.) once daily for 4 days to male Sprague Dawley rats
- Clinical observations will be performed daily, ~ 2 hr post dose. Animals will be examined for gross motor activity and observable changes in appearance (i.e., ataxia, convulsion, moribundity, labored breathing, ruffled fur, hunched posture)
- Body weights will be recorded on Day 1 and on Day 5 prior to euthanasia
- Blood will be collected prior to necropsy for clinical chemistry and hematology evaluations. **Optional:** additional blood-based biomarkers of specific organs of toxicity can also be evaluated if appropriate
- **Optional:** Urine can be collected by placing rats in metabolism cages the night before necropsy and evaluated for specific renal biomarkers
- **Optional:** If a specific target organ is anticipated, that organ can be collected at sacrifice and evaluated for histopathologic lesions

Dose selection for this type of multi-analog screening study will depend on the chemical class and the intended use of the data, and may require a preliminary escalation study to narrow the dose levels, but the example above (i.e., doses of 50 and 250 mg/kg) is fairly typical of the range of toxicity for most anti-infective drugs. The design above requires about 1.4 g of each analog. If that quantity is challenging to produce early in the discovery stage, the same design can be conducted with mice with about 120 mg of each analog.

Other early safety screens may also be appropriate, depending on the intended use of the drug, the structural class, data obtained from efficacy or other studies, etc. For example, for a drug intended to treat meningitis, a simple preliminary CNS screen such as a limited functional observational battery, might provide useful insights to the discovery process.

J. Summary of Discovery Requirements

Drug discovery is the phase between basic research and preclinical development when compounds advance from being a “hit” for a molecular target, to the point where a compound is identified as a “lead” compound that has been shown to have both the desired biologic activity and the properties that will allow it to be a commercially viable pharmaceutical. As part of this analysis, basic chemical synthesis, formulation, safety and pharmacokinetic properties will need to be evaluated. Many compounds fail in preclinical development not for lack of efficacy, but for adverse safety signals, poor pharmacokinetics, chemical scale-up issues, or other problems. It is therefore critical to identify these potential liabilities early, and to either find solutions to the problems or to discover new molecules with fewer liabilities.

The degree of information and data available is expected to evolve throughout the discovery process and into the preclinical and clinical development phases. Table 16 below provides a checklist of questions to be considered throughout the discovery stage. While each research problem should be tailored to the specific compound, indication and use, these general guidelines should be considered throughout the process.

TABLE 16.
CHECKLIST OF CONSIDERATIONS THROUGHOUT THE DRUG DISCOVERY PROCESS

Issue	Questions to Ask	Remediation
Chemical Synthesis		
Reagent Availability	Are unusual reagents with supply limitations or unreasonable costs required?	If cost or supply are prohibitive, consider alternative synthesis routes.
Stability	Are synthesized compound stable for at least 90 days refrigerated?	If compound is unstable, what part of molecule is degrading, and can modifications be added to stabilize it?
Reactive or Electrophilic Groups	Are there structural alerts suggesting adverse covalent binding to macromolecules?	Use in silico tools to predict and eliminate problematic structural groups early
Explosive or Toxic Intermediates	Are reagents or intermediates used that pose any hazards?	Consider alternative reagents or synthetic pathways
Formulation		
Simple Solubility for In Vitro Studies	Is the drug soluble in water, DMSO or acetone?	Consider alternative salt forms if compound has poor solubility
Development of a Suitable In Vivo formulation	Can a homogeneous suspension be prepared for oral dosing or a particle-free solution for parenteral routes?	Consider alternative formulation components
In Vitro Potency		
Active at Pharmacologically Relevant Doses	Is MIC in a range that is likely to be reached at the target site?	Ideally screen for molecules with lower MIC
Activity Against Alternative Strains	Is activity seen only against common wild type strain vs. drug-resistant or alternatives strains?	Conduct second tier battery against diverse strains of greater clinical interest
In Vitro ADMET		
Stability	Does the compound have adequate stability in plasma and microsomes?	If stability is poor in both, consider chemical modifications to structure
Absorption	Does compound have properties suggesting adequate absorption (especially critical for oral drugs)?	Increasing solubility with alternate salt form or formulation may help
Drug-Drug Interactions	Does drug induce or inhibit critical metabolizing enzymes?	Consider to what extent the target population will be impacted by this
In Vivo Efficacy		
Dosing Regimen	Is efficacy observed with a dosing regimen that is feasible for the target patient population?	If frequent dosing required, this might be due to PK; novel drug delivery systems may help
Therapeutic Index	Is efficacy observed in the absence of significant adverse effects?	Consider risk: benefit if adverse effects noted at efficacious doses; alternative is to change dosing regimen

TABLE 16.
CHECKLIST OF CONSIDERATIONS THROUGHOUT THE DRUG DISCOVERY PROCESS

Issue	Questions to Ask	Remediation
Pharmacokinetics		
Bioavailability	Does drug reach the intended target in an animal model to achieve an efficacious dose?	Alternative salt forms (to improve solubility) or novel formulations may increase delivery to target site
Pharmacokinetics	Are the pharmacokinetics conducive to a practical dosing regimen in patients?	Short half-life may be extended with time-release formulations
Safety		
Cytotoxicity	Are cytotoxic effects seen at therapeutic-relevant concentrations?	Confirm relevance of cytotoxic effects in animal safety models
Toxicokinetics	Are adverse effects seen in animals at plasma levels comparable to efficacious levels?	Can fractionated or continuous dosing maintain efficacious doses with less toxicity
Target Organs of Toxicity	Are adverse effects seen in critical target organs at clinically relevant dose levels?	CNS and cardiac effects very hard to resolve; other effects may be tolerable for short-term dosing if recovery occurs.
Reversibility	Do adverse effects resolve following a recovery period?	Irreversible toxicity is very hard to work around with FDA; consider a different molecule

IV. PRECLINICAL DEVELOPMENT PHASE

A. Overview of the Preclinical Development Process

The immediate goal of any preclinical drug development project is to obtain evidence of the potential safety and efficacy, in humans, of a new chemical entity (NCE). Successful completion of the project ultimately leads to an IND application to the FDA for approval to initiate human clinical trials.

It is common practice and highly recommended to request a Pre-IND meeting with the FDA to discuss the proposed preclinical and clinical development plans. This meeting is typically held after discovery activities are complete, a lead compound has been selected, and preliminary animal efficacy, safety and PK studies have been conducted.

A typical PDP will consist of six major efforts:

1. manufacture of the DS containing the API, and manufacture of the final DP, under pilot conditions and eventually under FDA current Good Manufacturing Practices (cGMP) conditions;
2. preformulation and formulation development (dosage design);
3. analytical and bioanalytical methods development and validation;
4. metabolism and PK profiling;
5. toxicology assessment (both safety and genetic toxicology and possibly safety pharmacology); and

6. other considerations.

Additionally, post-IND stability studies are expected to cover the duration of the DP usage during clinical trials. The IND application is essentially a description of the results of all these interrelated activities. The effective scheduling of the various tasks involved in the broader effort and incorporation of clear go/no go decision points is critical to completing a timely and cost-effective development program. Figure 6 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 1 clinical trial. A Gantt chart presented later in this document (Figure 8) illustrates the estimated schedule of activities to prepare the pre-IND and IND submissions and to initiate the Phase 1 clinical trial.

No acceptable PDP can be drafted without reasonable knowledge of the anticipated clinical plan. It is standard practice to plan backward from the product label indications intended for FDA approval to design an appropriate Phase 3 trial; the nature of that trial will determine the appropriate Phase 2 clinical design, which will define the appropriate Phase 1 design. To draft an appropriate preclinical plan, the design of the First in Human (FIH) Phase 1 trial is critical because the FDA GLP toxicology studies must mimic, at a minimum, the initial intended human use.

Without exception, Investigators are advised to plan for a pre-IND meeting with the FDA to obtain concurrence from the agency that it considers the intended pharmacology/toxicology studies, manufacturing plan and proposed clinical program are acceptable in relation to the intended initial clinical use. This meeting should generally take place after the first toxicology dose range-finding studies and before the initiation of the definitive GLP studies or cGMP manufacturing. The details of the pre-IND meeting will be discussed in greater detail below.

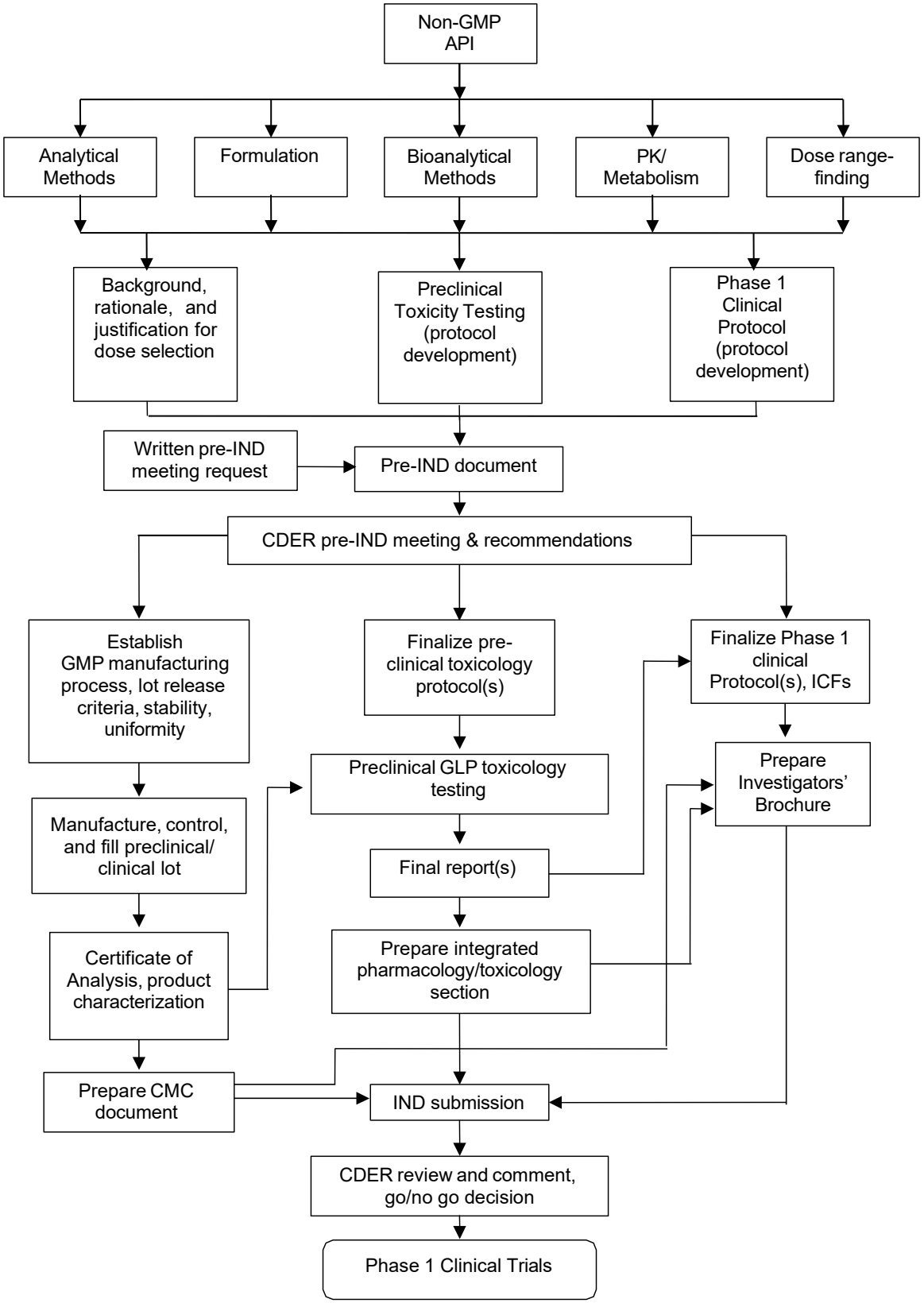

Figure 6. Flow chart of preclinical development steps to Phase 1 trial for a small molecule.

B. Manufacturing

B.1 Overview

Manufacture of the drug substance and subsequent formulation is required in virtually all preclinical and clinical development programs. Indeed, no drug development program of any design can proceed without an adequate supply of DS of suitable quality for the intended purpose. Drug may be required for non-GLP efficacy studies, for early formulation development, for toxicology studies such as dose range-finding studies or for the more rigorous IND-enabling GLP toxicology and safety studies. Depending on the intended use, material must be supplied in various quantities and at various purity levels. At the start of a typical preclinical development program, a sponsor may face the challenge that the API has probably been synthesized only at the laboratory scale, in quantities ranging from milligrams to several grams. Synthetic chemists often synthesize a compound in a quantity large enough to enable in vitro or possibly in vivo testing, without optimization of chemical processes, such as selection of reagents, solvents, reaction and workup conditions. As a consequence, most, if not all, laboratory-scale syntheses are not amenable to direct larger scale or cGMP production. Process development to reduce or eliminate process impurities, improve reaction yields, reduce cost, and improve process safety is usually needed before larger batches can be synthesized. This effort can be very much a research project.

Once the NCE is declared as a preclinical candidate for drug development, a series of activities begin in parallel. One of these is the development of a stability-indicating assay of the DS. The assay must be robust enough to separate the DS from its impurities and degradation products in a variety of sample matrices. This assay forms the cornerstone for all evaluations of the NCE during the preformulation and formulation development stages of the preclinical and subsequent clinical development processes.

While formulation work is ongoing, scale-up synthesis of adequate supplies of cGMP DS may begin. The cGMP synthesis work is performed in two phases. The synthesis first consists of a bench-scale run or demonstration batch, which is often done on $1/10^{th}$ of the scale of the cGMP planned batch size. The demonstration batch synthesis is designed to confirm the soundness of the synthetic method and establish a process suitable for the scale-up. The actual cGMP synthesis is only undertaken when the method has been demonstrated to work and all the intermediates and final product can be characterized. All of the important intermediates are routinely purified and characterized, not only increasing the yield on the next step, but also ensuring that the final synthetic product is of the current identity. The compounds are prepared by unequivocal methods in gram quantities, and they are fully characterized by a meaningful combination of techniques, including infrared and ultraviolet-visible spectroscopies, melting point or boiling point, elemental analysis, nuclear magnetic resonance (NMR), high performance liquid chromatography (HPLC), thin layer chromatography, and mass spectrometry (MS). During the demonstration batch synthesis, the Quality Control Unit (QCU) collects samples to characterize as reference standards for the cGMP synthesis and to set the specifications for the intermediates and DS. The final products are thoroughly purified and characterized, and then a Certificate of Analysis (CoA) is generated that includes descriptions and specification limits of the tests, methods, and results that establish the identity, purity, quality, and strength of the

product. The compounds are generally purified by titration, recrystallization, and column chromatography (normal and reverse phase, including preparative HPLC).

Some clinical trials may require the use of a placebo. A placebo is identical to the drug product except that it does not contain the drug substance. The appearance should be indistinguishable from the drug product, and there should be no components in the placebo that are not also present in the drug product, though proportions of the excipients might change. For example, if the drug product is a capsule that is a white, size 0 capsule containing 250 mg of azithromycin, with dextrose and mannose as a filler, the placebo should be a white, size 0 capsule containing no azithromycin, but including a sufficient quantity of dextrose/mannose to achieve the same weight and appearance as the drug product.

All aspects of a cGMP synthesis are reviewed and audited by the QCU and Quality Assurance Unit (QAU). The QCU is responsible for assuring that all raw materials and released products meet the mandated specifications. The QCU typically reports to the same management as the manufacturing unit. The QAU is responsible for assuring that all FDA and other regulatory guidelines are met. The QAU reports to either a different or higher management organization to avoid a conflict of interest. The QCU issues Certificates of Analysis and other analytical materials whereas the QAU releases statements assuring compliance of the manufacturing and of the QCU.

The QAU review encompasses all aspects of the manufacturing cycle and includes inspection of the manufacturing facilities, completed batch records, and the manufacturing program, as well as verification of the CoA. At the conclusion of the cGMP synthesis campaign, QCU prepares a final CoA summarizing product specifications and results of analysis, stability testing, and other evaluations of the DS. When everything is determined to be within acceptable specifications, QAU releases the DS and the DS is shipped.

The FDA is no longer accepting paper copies of IND submissions, and electronic submissions in the CTD format are required. The specifications for drug manufacture are included in a section entitled “Module 3: Quality.” The CMC has two major subsections: one for the DS and the other for the drug product (DP). The information in the CMC must assure the reviewers of the proper identification, quality, and purity of the DS and the strength of the DP. Impurities and quantity of impurities should be identified and limits established. Also, sufficient chemical and physical stability data or a commitment to collect such stability data for the DP should be provided to cover the duration of the planned clinical investigation. For Phase 1 clinical studies, the CMC section should focus on providing sufficient information to allow the safety of subjects in the proposed clinical study to be evaluated. Insufficient safety data on either the DS or the DP can be the basis for a clinical hold.

B.2 Drug Substance Manufacturing

The API is the active drug that is desired for therapeutic use, but in practice, is synthesized as a “drug substance” (DS) that contains water, salts and other impurities. Therefore, while API is the theoretical material being synthesized, the actual material produced is a DS that contains some quantity of the API. The amount of DS required is dependent on toxicity and the species selected for animal studies. For example, a relatively toxic or highly potent compound intended for testing in mice and rabbits might require only 10–20 g for the entire preclinical safety

package. In contrast, a non-toxic material intended for testing in rats and dogs could require up to 5 kg of DS. Toxicology studies may use "GMP-like" material, which is produced according to processing procedures that yield similar purity and product quality intended for the ultimate cGMP clinical DS but is not made under formal cGMP compliance. This initial material will be used for the preliminary toxicology studies. The synthesis will be used to find approaches to reduce the synthetic costs enough to make the drug economically feasible for commercial use, including use in developing nations, if appropriate. In addition, this synthesis effort (Phase 1) will be used to further refine and streamline the methods so that a technology transfer to a cGMP manufacturer, who will synthesize kilogram quantities of the DS for the clinical studies, can be easily accomplished. It will also be used to provide reference standards for analytical methods development.

B.3 FDA Guidance for Pharmaceutical cGMP

The FDA's final report *Pharmaceutical cGMPs for the 21st Century – A Risk-Based Approach* published September 2004, presents the FDA's initiative intended to modernize regulation of pharmaceutical quality for veterinary and human drugs and select human biological products such as vaccines. This report is available for download at:

https://www.fda.gov/media/77391/download.

A progress report was published May 2007. This report is available at:

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

Working in collaboration with the International Conference on Harmonisation (ICH), several guidelines were published following the 2004 final report. The Guidance for Industry*: Quality Systems Approach to Pharmaceutical cGMP Regulations* was published October 2006 and is available at:

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070337.pdf.

The FDA indicates that its intent is to integrate *quality systems* and *risk management* approaches into its existing programs with the goal of encouraging industry to adopt modern and innovative manufacturing technologies.

In May 2006 the ICH Guidance for Industry: *Q8 Pharmaceutical Development* was published with an annex added to the parent Q8 document and published November 2009. This can be found at:

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073507.pdf.

The guidance describes suggested contents to be considered during the development and life cycle of the drug candidate and presents recommendations adopted by regulatory bodies under the ICH process. However, the scope of the guidance indicates that it does not apply to contents of drug products during the clinical research stages but cautions that the principles in the guidance are important to consider during these stages.

On June 2006, FDA published Guidance for Industry: Q9 Quality Risk Management, intended to present a systematic approach to quality risk management. This can be found at:

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073511.pdf.

The intention is to provide specific guidance on the principles and some of the tools of quality risk management that can enable more effective and consistent risk-based decisions, by both regulators and industry, regarding the quality of drug substances and drug products across the product lifecycle.

C. Preformulation Studies and Formulation Development

C.1 Overview and Preformulation

After DS manufacture, the next major hurdle in a preclinical and clinical development program is often the formulation development. As noted in Section III.C. above, formulation is a continuous process that begins in discovery phases and evolves through the discovery and preclinical development phases, all the way to clinical development and ultimately to market approval. This section focuses primarily on the formulation steps required for IND-enabling animal studies and Phase I clinical studies.

The specific physicochemical properties of the DS will dictate which formulation options are available. Typical data gathered in the preformulation process include physicochemical characterization and solubility. A compound with suitable pharmaceutical qualities needs to have appropriate solubility and permeability, either as an inherent property or achieved via formulation manipulations. The choice of formulation excipients depends on the solubility or insolubility of the DS as well as degradation pathways, stability, and excipient compatibility.

Physical characterization of DS pertains to measurement of fundamental solid state properties such as melting point, crystallinity, surface properties, and particle size. DS chemical characterization pertains to experimental determination of the ionization constant, partition coefficient, intrinsic solubility, pH solubility, pH stability, and stability as defined in ICH guidelines.

In vitro drug permeability testing may also be performed to understand the inherent permeability characteristics of a drug, which might be used during the formulation stage to determine the need to incorporate permeation enhancers. Such studies are important when the oral bioavailability of the drug is low.

The forced degradation study is considered a vital analytical aspect of the preformulation program for small molecules. Some of the applications of the studies include to optimize formulations, to develop and validate stability-indicating methods, and to set up specification of degradants or impurities. The objective of the stability study is to determine the API stability by exposing active DS to a variety of experimental conditions, including the formulation per se, heat, light, oxygen, and other parameters. The stability of an API (and the associated DS) is determined using stressed (accelerated) storage conditions, in which the drug substance is subjected to room and elevated temperature/humidity storage for a specified time. The drug substance is sampled periodically for analysis of its physical and chemical integrity.

Preformulation stability studies are designed to address both solution (in pharmaceutically acceptable solvents/co-solvents and pH based buffer systems) and solid-state stability.

Preformulation studies encompass the determination of the fundamental physical and chemical properties of the drug substance that will dictate the possible approaches in formulation development. Table 17 illustrates a typical preformulation study design highlighting methodologies and potential information that can be gained. Once the formulator understands the key factors affecting the stability, appropriate excipients, packaging, and storage conditions can be developed to enhance the stability of the drug substance and formulated products.

TABLE 17.
PREFORMULATION STUDY DESIGN

Drug Characteristic	Method	Potential Information
1.HPLC Assay	HPLC- typically a short assay method and a second stability indicating method is developed.	API quantitation in DS and formulation
2.Thermal Characterization	DSC, melting point apparatus	melting, polymorphism, hydrates, solvates
3.Dissociation Constant	Experimental/calculated	Solubility and stability as a function of pH
4.Partition Coefficient	Octanol extraction/calculated	Permeability, excipient selection
5.Solubility in Aqueous Buffers	Experimental/calculated	Bioavailability selection
6.Solubility in Solvents	Experimental	Vehicles and DS extraction from formulation matrix
7.Hygroscopicity	DVS	Bound water content
8.Polymorphism	DSC, TGA, XRD	Crystal information, hydrates, solvates
9.Particle size and Morphology	Microscopy	Surface area, packing, homogeneity, dissolution
10.Flow Properties (for Tablet/capsule)	Bulk density, flow, and compression properties	Choice of excipients and granulation procedures
11.Excipient Compatibility	Preliminary screen by DSC Confirmation by HPLC data	Informed choice of suitable excipients

C.2 Solid Dosage Formulation Development

Designing solid dosage formulations requires two major activities: identifying the excipients most suited for a prototype formulation of the drug, and evaluating mechanical properties such as flow and cohesive capabilities that affect disintegration and dissolution.

Blending is important for uniform mixing of DS with inactive ingredients. When powders containing more than one component are mixed, in-process blend uniformity must be established to demonstrate that a unit dose sampling will yield a suitable average or mean concentration of that component and standard deviation around the mean. If difficulties are encountered in uniform mixing with simple blending using V-blenders, alternative processing such as wet granulation can be considered; the additional influence of milling and sieve analysis on the particle size and drying stability should also be studied.

Flow Properties. Flow properties are discussed in detail above in the preformulation section. Other processes may be used to attain uniform blending or increased drug packing in a capsule/tablet. Granulation is the process in which primary powder particles are made to adhere together to form larger, multiparticle entities called granules. Granulation normally commences after initial dry mixing (blending) of the necessary powdered ingredients (excipients and drug) so that a uniform distribution of each ingredient through the mix is achieved. Granulation is employed to (a) prevent segregation of the constituents of the powder mix, (b) improve the flow properties of the mix, and (c) improve the compaction characteristics of the mix.

Extrusion/spheronization is a multistep process used to make uniformly sized spherical particles that pack tightly. The process of extrusion/spheronization can also be used to increase the bulk density, improve flow properties, and reduce the problems of dust usually encountered with low-density, finely divided active and excipient powders. Extrusion/spheronization is a more labor-intensive process than other forms of granulation and should therefore only be considered when other methods are either not satisfactory for that particular formulation or are inappropriate (i.e., when spheres are required). This process involves the separate steps of wet massing, followed by extrusion of the wet mass into rod-shaped granules, and subsequent spheronization of the granules.

C.3 Production of Typical Solid Oral Dosage Forms

Capsules. The most common oral DP form and one of the easiest and fastest ways to evaluate safety and efficacy in humans is the capsule dosage form. Capsules may be hard or soft, with hard capsules more common, and these are primarily made from gelatin. An appropriate bulking agent is selected based on an excipient compatibility study, blended with the DS, and filled into the capsule. Capsules of gelatin easily disintegrate in the gastrointestinal tract, permitting the gastric juices to permeate and reach their contents. Preformulation studies are performed to determine if all of the formulation's bulk powders may be effectively blended together as such, or if they require reduction in particle size. Excipients, which include diluent, filler, and disintegrants, are then incorporated. Milling or micronization of the drug might be required to achieve good bioavailability. FDA is increasingly suggesting the use of gelatin from non-bovine sources for capsules.

Hard gelatin capsules can also be filled with nonaqueous solutions. Typically the major components of nonaqueous formulations are medium-chain triglycerides, purified oils, and so forth. To prevent leakage, hard gelatin capsules are subsequently banded.

Tablets. Developing tablets requires extensive excipient screening and selection. Processing equipment and aids also must be investigated. Tablets are primarily made by compression, though a limited number are prepared by molding. Compressed tablets are manufactured with tablet machines capable of exerting great pressure and compacting the powdered or granulated tableting material. Types of tablets include compressed, multiple compressed, sugar-coated, gelatin-coated, enteric-coated, buccal, chewable, effervescent, immediate-release, extended-release, and instantly disintegrating. Various coatings are used for both aesthetic and delivery purposes. Enteric coated solid dosage forms are useful when the drug is known to degrade in the stomach or is known to be preferentially absorbed in specific regions of the small intestine.

Powder-in-Bottle. Powder-in-bottle drug products consist of a known mass of active ingredient packaged in a unit container. The contents are typically reconstituted with a liquid vehicle at the clinical site resulting in a solution or suspension. This product type offers the convenience that the dose amount (volume) can easily be adjusted as needed. However, a DS with bitter or unacceptable taste or odor characteristics may not be suitable for this dosage form, although sometimes this may be overcome by addition of sweeteners to the reconstitution solution. Additional short-term stability, solubility, and suspendibility studies are also needed.

C.4 Liquid Dosage Formulation Development

Solutions intended for oral administration usually contain flavoring and coloring agents (frequently referred to as "flavorants" and "colorants") to make the medication more attractive and palatable for the patient. Sometimes solubility and permeability limitations of orally administered drugs necessitate the use of liquid oral formulations, since larger volumes of solubility and permeability enhancing additives, which are mostly in solution form, can be included. Emulsions and miscellar formulations are especially attractive for drugs that are poorly soluble and /or poorly bioavailable. As with solid dosage formulation, preformulation and excipient compatibility data are required.

C.5 Parenteral Dosage Form Development

Drugs that are destroyed or inactivated in the gastrointestinal tract or too poorly absorbed to show efficacy will need to be administered parenterally. Parenteral administration via intravenous, intramuscular or subcutaneous injection is preferable when faster drug response is required and if low oral bioavailability is an issue. Preformulation and excipient compatibility are important as with the other formulations described above. Additionally, this product class is subject to additional demonstration of sterility.

C.6 Alternate Dosage Form Development

A variety of other routes of administration exist and can be utilized if found to be more efficacious. Examples include sublingual (under the tongue), transdermal (via skin surface), intranasal (through nose), aural (ear), aerosol (through the lung), vaginal, rectal, etc.

C.7 Development of Stability Data

Accelerated Stability of Pilot Batches. Once the process of making a dosage form is established, pilot batches of the material are made and placed on an accelerated stability testing schedule to determine the recommended storage conditions and expected storage stability of the DP.

Because drugs for infectious disease may be used in very warm and humid climates, stability under these accelerated/stressed conditions is critical. Samples intended for long-term ambient storage will be stored for varying periods of time under two experimental conditions: 25°C at 60% relative humidity (RH) and 40°C at 75% RH. Table 18 describes the conditions that will be used to test the effects of different stability conditions on the formulations.

TABLE 18.
TYPICAL DESIGN FOR ACCELERATED STABILITY TESTS OF PRELIMINARY FORMULATIONS

Storage Condition	Evaluation Period (weeks)					
	Initial	1 week	2 weeks	4 weeks	8 weeks	12 weeks
25°C/60% RH	X	X	X	X	X	X
40°C/75% RH	—	X	X	X	X	X

Products intended for long-term storage at refrigerated (5°C) or frozen (20°C) conditions are studied at those conditions and the accelerated conditions are one step up, i.e., 25°C for refrigerated and 5°C for frozen products.

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) dissolution for solid dosage forms.

D. Analytical and Bioanalytical Method Development and Validation

Starting from the initial drug discovery phase and through to market approval, analytical chemistry methods are established and modified throughout the entire lifecycle of the development process. These applications can be categorized into two major subdivisions: pharmaceutical analysis (DS and DP) and bioanalysis. Pharmaceutical analysis involves the measurement of an analyte in a neat bulk sample or formulation, and bioanalysis is the quantification of an analyte in a biological matrix.

D.1 Analytical Method Development

Reliable analytical methods are required to test and qualify incoming components, in-process materials, bench formulations, DS, DP, cleaning samples, and stability samples. Methods must be accurate, precise, and specific to be suitable for quality control under GLP and cGMP conditions. In addition, FDA and ICH guidelines require stability testing on each lot of DS and DP in early-stage clinical development. Later, once the manufacturing processes are fully validated and storage conditions and expiration dates are set, the stability testing program can be reduced as long as there are no changes to the materials, processes, packaging, or manufacturer. 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 DP.

Analytical support consists of two phases: research and development (R&D) and regulated studies (GLP/cGMP). The R&D phase includes analytical method development and analytical support for preformulation and formulation process development. The remainder of the analytical work is conducted according to GLP and cGMP guidelines and is performed with well-documented methodology and set performance characteristics and acceptance criteria. The specifications of the tested materials will evolve as the preclinical and clinical drug development processes progress, and the specifications may become more stringent (e.g., greater purity, less batch-to-batch variability); therefore the performance characteristics and reproducibility of the analytical methods must improve as well.

It is essential to use methodology validated to test the particular DS to be used in clinical manufacturing. The method must be specific, meaning it must be able to determine the active ingredient unequivocally without the interference of other co-existing substances such as impurities, and it must have sufficient sensitivity to determine impurities at a level below the specification requirement. At the stage of clinical manufacturing, a reference standard must be designated for assay of the active drug. The assay method must be validated for specificity, linearity, accuracy, precision and sensitivity. Assay methods are verified under the ICH guideline *Q2(R1) Validation of Analytical Procedures: Text and Methodology.* Microbiological test methods, such as the United States Pharmacopeia (USP) *USP <61> Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests* and *USP <62> Microbiological Examination of Non-sterile Products: Tests for Specified Microorganisms*, are well established. They can be followed and used in the testing of DS for clinical manufacturing. The release specifications for a cGMP DS typically include the following tests, at a minimum:

- Appearance
- Identity (by two or more methods, e.g., NMR, MS, IR)
- Chromatographic purity (generally HPLC)
- Water content
- Elemental impurities (Heavy metals)
- Residue on ignition
- Residual solvents
- Microbial limit testing.

Methods similar to the DS method can be employed to evaluate formulations during the preformulation and formulation development stage. They can also be adopted for analyzing dosing formulations for GLP studies. In this phase, the DS method should be evaluated and modified, if necessary, to ensure the vehicle excipients or diluent will not affect the analysis in the particular formulation. Sample preparation procedures should be developed for the trial formulations to ensure that the active drug are sufficiently soluble and stable in the test solutions, and the active drug can be completely released and recoved from the vehicle excipients.

In GLP studies, all test articles are required to be tested and their identity, purity and stability must be confirmed. Before the dose formulations are applied to the GLP studies, the concentration, homogeneity, and stability of the dosing formulations must be tested and confirmed. These attributes must be verified at least once at the beginning of a preclinical development program. These studies provide evidence that the drug 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 suspension remains homogeneous 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 analysis on representative preparations are

necessary. This might typically include analysis of the Week 1 and Week 4 preparations of a 28-day study.

The suitability of a compound for pharmaceutical use requires establishment of its identity, purity and stability, as well as knowledge of its chemical and physical properties. The purpose of analyzing a formulation or a drug product is to verify the content and potency of the active component, determine its impurities and degradation products, verifying its dosage uniformity and other quality attributes such as dissolution for solid dosage form or sterility for parenteral dosage forms. Finally, the attributes are to be tested in stability studies to establish a shelf life for the formulation or drug product. It is important to ensure that materials with defined and well controlled quality are used in all studies and that they conform to applicable FDA regulatory requirements.

D.2 Bioanalytical Method Development

Physiological fluids such as blood, plasma, or urine are analyzed to determine the disposition of a DS administered to a test animal or patient by measuring the API in the relevant biologic matrix over time. All bioanalytical assays begin with an extraction step to remove the compound of interest from the matrix. There are a variety of liquid and solid-phase methods for isolating the compound of interest for analysis. This extraction step is typically straightforward in plasma, but can be complicated when measuring drug levels in tissues, because different cell types, connective tissues, and other compounds present may interfere with measurement of the analyte of interest. Both the extraction method and analysis method need to be optimized as part of the method development and validation.

Aliquots of blood may be sampled over time to determine therapeutic drug concentration ranges. Often the goal is to assess the overall absorption, distribution, metabolism, and excretion (ADME) of the API. 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 linearity 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 with the analysis, enzymes in the biological fluid that are capable of metabolizing the analyte, plasma proteins to which the analyte can bind, concomitant drugs that might interfere in the analysis, etc. All these factors must be considered when planning a bioanalytical method.

The most widely used technique for quantitation of the API is liquid chromatography followed by mass spectrometry (LC-MS/MS), because it incorporates a separation step before quantitation. This technique is particularly useful for analysis of bulk drugs in the presence of excipients, dosing vehicles, and biological matrices, and for the determination of impurity and degradation profiles. A well-developed method is highly specific for the component of interest and will be a function of the column, detector, and mobile phase composition. Selection of an appropriate detection system will provide the sensitivity and linear range needed for analysis of the wide variety of sample types encountered during both preclinical and clinical drug development, including bioanalytical, cleaning, placebo and control, dissolution, assay, and purity samples. A literature search for related compounds and analogues can often aid the initial selection process and minimize extensive method development; this should be undertaken as part

of the method development phase. As with analytical chemistry method development of DS and DP, the analytical method conditions selected will undergo further optimization and validation, depending on the conditions of use and regulatory phase of development.

D.3 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 the FDA Bioanalytical Method Validation Guidance for Industry, May 2018, available at:

https://www.fda.gov/media/70858/download.

The validation process should address the following key parameters:

a. All parameters are determined using calibration standards prepared in biological matrix fresh on each day of validation. Calibration standards are spiked with analyte over at least 7 concentrations and the resulting curve / weighting used to determine unknown concentrations in Quality Control (QC) samples.

b. Accuracy and Precision: Tested using QC samples at ≥ 4 concentrations, including at the lower limit of quantitation (LLOQ). Accuracy is how far removed the actual concentration is from the nominal concentration. Accuracy is measured individually, and the mean accuracy assessed within a batch and across ≥ 3 batches for a full validation. Precision (%CV) is determined based on how variable the nominal concentration values are, both within a batch and across ≥ 3 batches".

c. Specificity: The analyte of interest will be present in samples from a wide variety of sources and with excipients, degradants, impurities, and matrices with the potential for interfering backgrounds. Additionally, analytes are frequently measured in biological fluids (e.g., serum, saliva, urine, homogenized tissue) that can vary among patients or in an individual patient 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 if co-administered compounds are known to be given.

d. 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.

e. Linearity and Range: The range in which the assay result is proportional to the concentration defines the functional limits of the assay.

f. Stability of analyte in biological matrix, including freeze-thaw stability in matrix, room temperature stability, long term storage stability, autoinjector (reinjection) stability, post-preparative extract stability, and whole blood stability (for plasma assays).

g. Stock and spiking solution stability.

h. Matrix effects: Assess the effect of the matrix on signal enhancement or suppression in LC-MS/MS based assays, using ≥ 6 unique lots of matrix, including the effect of hemolysis or lipemia (human) in plasma assays.

i. Recovery: The actual amount of analyte and internal standard found in an extracted matrix sample is compared with a matrix sample spiked post-extraction with analyte and internal standard at concentrations intended to represent the theoretical amount of these compounds in the extracted sample.

j. Incurred sample reanalysis (ISR) is performed during study sample analysis to determine the reproducibility of results in samples that have already been analyzed. In preclinical studies, ISR will be conducted with each animal species used. In clinical studies, ISR will be performed in FIH studies, bioequivalence and bioavailability studies, proof-of-concept studies, drug-drug interaction studies, or any study where PK assessment is the primary endpoint. ISR results will be reported as part of the bioanalytical report for the appropriate toxicology or pharmacokinetic study.

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

E. Metabolism and Pharmacokinetic Profiling

E.1 Pharmacokinetics Overview

Pharmacokinetics (PK), 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. Poor pharmacokinetics used to be the major cause of drug failures, halting up to 40% of all compounds prior to the 2000s. This failure rate has decreased dramatically in the past two decades thanks to better early predictive models (Kola & Landis, 2004). As a result, most drug discovery programs now incorporate early screens for desirable "drug-like" properties to optimize the selection of successful candidates. These approaches are discussed in Section III.C. above. After evaluation of these screening data in concert with efficacy results, compounds that are predicted to have favorable PK properties are studied further using in vivo animal models. PK results obtained from animals provide one of the important data sets used for dose selection in human clinical trials.

PK parameters are derived from the measurement of drug concentrations in the plasma or blood and provide 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. Oral and IV administrations are compared to determine oral bioavailability of drug if the oral route is the anticipated route of administration in the clinic. Plasma protein binding is a significant factor in the PK of therapeutic agents in vivo. A basic assumption in PK is that only unbound drug is available for uptake by tissues, including the red blood cells and plasma proteins that transport the blood through systemic circulation. The unbound fraction, therefore, directly affects the onset, duration, and intensity of a drug's pharmacological and/or toxicological effects. Tightly bound drugs may be essentially unavailable or may serve as a storage site that slowly releases the agent. In vitro estimates of plasma protein binding obtained under physiologic conditions may provide an excellent estimate of in vivo binding. It is not necessary to delineate

all PK and metabolism characteristics of a molecule at the time of IND submission, since the first clinical study will focus on the PK and metabolism of the agent in humans; however, in vitro techniques for studying human metabolism are recommended by the FDA, and given the importance of these results for understanding the disposition of the drug in humans, most preclinical development plans incorporate these studies as early as possible, generally at about the same time as the first animal PK studies.

E.2 Recommended Pharmacokinetic and Bioavailability Studies

Initial animal studies focus on establishing a reasonable dose range for testing in two species, and in establishing the basic PK parameters, including bioavailability, $t_{1/2}$, C_{max}, and T_{max}. These studies are typically not conducted in compliance with FDA GLP regulations, unless the drug is being developed under the Animal Rule (see Section IV.G3). 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. As previously described, the most common rodent species for small molecules is the rat, and the most common non-rodent species is the dog, but alternatives may be proposed. For purposes of this document, we assume rat and dog for all studies.

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 in order to support preclinical PK and toxicokinetic (TK) studies. Also see Sections D.2 (Bioanalytical Method Development) and D.3 (Assay Validation). The bioanalytical method needs to be sensitive enough to detect drug at the lowest dose levels used. Currently, virtually all small molecules are evaluated using LC-MS/MS systems. A suitable internal standard such as stable isotope-labeled material or a structurally related compound is often ideal for use and needs to be prepared. To validate the method, linearity, accuracy, precision, specificity, range, limit of detection, and system suitability are established. The following steps are taken for this effort:

- Develop method in rat plasma
- Validate method in rat plasma
- Cross-validate method in dog plasma (or other relevant nonrodent species)
- Prepare Method Development and Validation Report.

A major challenge for the LC-MS/MS approach is finding a suitable compound to use as an internal standard for the assay (which is critical for reproducibility in LC-MS/MS assays). It is desirable to synthesize a deuterated (Mass+1 per deuterium substitution) version of the lead candidate for this purpose.

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

It should be noted that FDA guidances and opinions on bioanalytical validation have been rapidly changing over the past decade. Approaches that were acceptable a few years ago no longer meet current standards. It is therefore critical to work with a bioanalytical laboratory that stays abreast of these rapid changes in order to assure current regulatory compliance.

Single Dose Pharmacokinetics/Bioavailability Study in Rats with Plasma Analysis. The purpose of this study is to determine the pharmacokinetics of a drug in the systemic circulation following administration by the relevant treatment route. The design for a single dose PK/bioavailability study in rats is as follows:

- Sprague Dawley rats, 3M/3F
- One IV group and two oral groups
- Blood collected and processed to plasma at 12 time points
- Analyze plasma drug concentration using bioanalytical method
- Calculate PK parameters, including area under the plasma drug concentration versus time curve (AUC), maximum plasma drug concentration (C_{max}), time to maximum concentration (T_{max}), elimination half-life ($t_{1/2}$), and oral bioavailability (F)
- A recent trend is to use a minimal number of jugular vein catheterized (JVC) rats, allowing a large number of blood collections from a minimal number of animals, therefore, decreasing costs and animal use.

Single Dose Pharmacokinetics/Bioavailability Study in Dogs with Plasma Analysis. PK/bioavailability studies should be performed in dogs as well as rats, as follows:

- Beagle dogs, 2M/2F
- One IV group and two oral groups; dose administration may be done in an escalation fashion in each group using the same animals after 1–2 week washout period
- Blood collected and processed to plasma at 12 time points
- Analyze plasma drug concentration using bioanalytical method
- Daily clinical observations and twice weekly body weights
- Clinical pathology (hematology, clinical chemistry, coagulation tests) prestudy and on Day 3
- Using noncompartmental approaches, calculate PK parameters, including area under the plasma concentration versus time curve (AUC), observed peak plasma concentration (C_{max}), time of C_{max}, T_{max}, terminal half-life ($t_{1/2}$), and oral bioavailability (F). Using the IV group data, the systemic clearance (Cl) and apparent volume of distribution (Vd) will also be calculated.

F. Safety Assessment

F.1 Introduction

Despite numerous technical advances in the science of toxicology and attempts to develop in silico screening, the primary methods used to assess safety of candidate pharmaceutical products remain single- and repeat-dose toxicology studies conducted in rodent and nonrodent laboratory

animal species. Although pilot and dose range-finding studies need not be conducted under formal GLP compliance, definitive pivotal studies must be conducted under GLP regulations and performed to meet the testing requirements of the FDA and the ICH M3 (Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals**)** and other related ICH guidance. The ICH M3 guidance is available at the following site:

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m3r2-nonclinical-safety-studies-conduct-human-clinical-trials-and-marketing-authorization.

Although the basic protocols and procedures for toxicology studies have not changed significantly in the past 40 years, new molecular and biochemical endpoints are now more frequently included to predict potential adverse effects in humans. These endpoints include measurements of cell proliferation, oncogene activation, micronuclei, immunotoxicity, gene expression, mutation markers and a variety of new, target-organ specific biomarkers.

The FDA typically requires submission of data from three genetic toxicology assays for IND applications: (1) gene mutation in bacteria, including four strains of *Salmonella typhimurium* and one strain of *Escherichia coli* (Ames test); (2) a mammalian cell assay such as cytogenic evaluation of in vitro micronuclei or chromosomal damage in mammalian cells or mutations in a cell line such as mouse lymphoma cells; (3) in vivo chromosomal damage in rat or mouse hematopoietic cells (micronucleus assay). The genetic toxicology requirements for an IND have standardized around simple Ames/in vitro micronucleus tests combined with a micronucleus assessment in blood or bone marrow collected from repeat-dose rodent toxicology studies, thereby saving significant cost and use of animals. While other combinations of tests may be proposed in a pre-IND meeting with the FDA, justification for using a non-standard set of tests will be required.

Toxicology studies are typically conducted in two species, one rodent and one nonrodent. The most commonly used species for small molecule anti-infective therapeutics are rats and dogs, though other large animal species may be appropriate for specific products or therapeutic applications. For example, topical products are frequently evaluated in minipigs or rabbits. Ocular products are frequently evaluated in rabbits. While NHPs have sometimes been used for small molecules when dogs are deemed inappropriate (e.g., when the drug causes severe emesis in dogs), 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" (see FDA, 2022 in Refences).

Preliminary toxicity studies are often performed as part of the lead compound selection process (see discussion of discovery program toxicology assessments in Section III). For IND-directed safety studies, only two complete GLP-compliant safety studies are generally required. The route of administration of these studies must be the same as the proposed clinical route. For example, if the proposed clinical route is oral, the drug may be administered by gavage to rats and by capsule or gavage to dogs. In most cases it is not acceptable to substitute an alternative route of exposure solely for convenience reasons (e.g., intraperitoneal injection in place of intramuscular). In some cases, a novel route of exposure (e.g., intravaginal or intraocular) might be supplemented with an additional intravenous study to provide information on the "worst case scenario" if there was a large systemic exposure to a drug due to overdose, misdose, or other reason.

The duration of administration and dose regimen must at a minimum conform to the proposed clinical protocol. FDA typically prefers preclinical safety studies done at increased frequency and dose level than what will be used in the clinic. For example, to support a Phase 1 clinical trial of an antibiotic intended to be given for 7–10 days, the FDA typically requires a toxicity study of 14 days' duration followed by a 1–2 week post-treatment recovery phase. It is common to conduct a toxicity study of 14–28 days to provide support for a longer dosing period. This provides increased flexibility in designing clinical trials, and can provide the support for subsequent Phase 2 trials that could extend past the initial 7–10 day dosing period.

The frequency of dosing (e.g., three times per 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. For example, a drug intended to be given once a month for 3 months might be given once a week for 4 weeks. While more common for cancer therapeutics than infectious disease drugs, preclinical programs can often match complex dosing regimens; e.g., 1-hr infusion, 3 times a week, for 3 weeks.

Safety tests should be performed with cGMP or "GMP-like" drug whenever possible. (See discussion in Section IV.B.2. concerning purity of drug substance to be used in GLP safety studies.) While this is not an 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 cGMP and non-GMP batches, the safety studies could be considered invalid.

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. Indeed, toxicology studies should be designed such that frank toxicity is observed in the highest dose group. Pilot studies typically identify a maximum tolerated dose for a particular species, and then this dose is used in the definitive, IND-directed safety study. Ideally, a dose is selected that produces clear adverse effects (body weight loss, clinical or histopathology changes), but not mortality or severe clinical signs that require veterinary intervention. A recent trend, especially in large animal studies, is to include a recovery group only for the controls and the high dose. This approach decreases the number of animals used, as well as the overall cost, but comes with risks. If a histopathology finding is seen at all dose levels, and recovery is not seen in the high dose group, there is no way to determine if recovery would occur at lower doses, likely resulting in a repeat of the entire study. Recent Pre-IND submissions to the FDA with this approach has resulted in the FDA suggesting a compromise of including recovery groups for the two highest dose levels, but not the lowest level, with the rationale that the lowest level is expected to have no adverse effects, and seeing effects at this dose could halt the preclinical development program, whether recovery is seen or not.

If clinical trials will include large populations of males and/or females of reproductive age, reproductive toxicity testing is required for later phase clinical trials. Reproductive toxicity studies are generally not required for Phase 1 trials unless the therapeutics are specifically intended for the treatment of pregnant women; however, for some diseases (e.g., malaria) where early clinical trials are expected to include women and potentially children, FDA is now requesting reproductive studies before Phase I is initiated.

Another consideration when testing anti-infective therapeutics is that while Phase 1 clinical trials are typically performed on a single drug, many anti-infectives are used in combinations with other drugs in the clinic. Before Phase 2 clinical trials in infected populations are initiated, separate safety testing of drug combinations may be required, using designs similar to those described below. This is very common in treatments directed at TB and HIV. This effort, which essentially doubles the overall cost of the toxicology section described here, is a factor to be evaluated in the overall preclinical development cost of therapeutics expected to be given in combination.

Juvenile animal models may be requested when drugs are likely to be used exclusively in children, with no adult clinical data being generated. When there are ample adult clinical data available, the requirement for juvenile animal studies are typically waived. Studies in juvenile animals (ranging from weanling through to early sexual maturity) were initially required in both rodent and non-rodent species, but the most recent FDA recommendations are to conduct juvenile studies in rats only. The designs are similar to those used for adult animals described below, but the use of very young animals introduces a variety of technical challenges including scheduling births so that animals of a closely synchronized age are used, limitations in blood volume that can be collected from very small animals, etc. Because this area of regulatory guidelines is rapidly evolving, it is recommended to discuss plans for safety testing to support pediatric clinical trials as part of the pre-IND meeting with the FDA (see Section V.F, Pediatric Research Equity Act and Compliance).

In the sections that follow, we describe typical protocol designs that are used for evaluation of small molecules. While these designs provide a general framework for study conduct, the reality is that each testing program will have its own unique parameters and should be designed by an experienced toxicologist and pharmacologist to optimize it for the particular compound and therapeutic indication of interest.

F.2 Recommended Range-Finding Studies

Single Dose Range-Finding Study in Rats. The purpose of this study is to identify dose levels for subsequent toxicology studies. Dose range-finding will require the following:

- Sprague Dawley rats, 3M/3F per dose group; control and three dose levels
- Single dose administration
- Daily clinical observations and twice weekly body weights
- Clinical pathology (hematology, clinical chemistry) on Day 3
- Gross necropsy on Day 8; no tissues retained for histopathologic evaluation.
- When compound is in short supply, a common modification of this study is to do it with N=1 or 2 rats, and possibly single sex. Dosing is done in an escalation format, starting with low doses and increasing by 50-100% per level (e.g., 10, 20, 50, 100, 150 mg/kg), depending on observed clinical signs. This takes more time and is less efficient (and therefore generally more expensive), but preserves test compound.

7-Day Repeat Dose Range-Finding Study in Rats. The objective of the 7-day repeat dose studies in rats and dogs is to identify dose levels to use in the definitive IND-directed studies. The design for these studies is as follows:

- Sprague Dawley rats, 3M/3F per dose group; control group and three dose levels
- Administration once daily for 7 days
- Daily clinical observations and weekly body weights
- Clinical pathology (hematology, clinical chemistry) on Day 8
- Full gross necropsy on Day 8
- Tissues retained for potential future evaluation.
- It is common to also include PK collection times as part of this study, via a satellite TK group, to evaluate changes in PK profiles with repeated dosing.

7-Day Repeat Dose Range-Finding Study in Dogs. The studies in dogs are similar to those in rats:

- Beagle dogs, 1M/1F per dose group; control and three dose levels
- Daily dose administrations for 7 days (Days 1–7)
- Daily clinical observations and weekly body weights
- Clinical pathology (hematology, clinical chemistry, coagulation) prestudy and on Day 7
- Organ weights and weight ratios determined at necropsy (Day 8)
- Histopathologic evaluation of all animals.
- Sometimes preceded by a single-dose escalation study, and often the preliminary pharmacokinetics study (see description in previous section) serves as both a PK and a dose-finding study. Alternatively, the repeat-dose study can be run in a staggered, escalating format. This takes longer and generally costs more, but preserves compound and minimizes the risk of severe adverse effects in dogs seen if immediately starting with multiple doses.
- It is common to also include PK collection times as part of this study, to evaluate changes in PK profiles with repeated dosing.

F.3 GLP Safety Studies

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 and are 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 stability, homogeneity, and concentration of dose formulations.

G. Repeat-Dose Toxicity Studies

The two 28-day studies described below may be conducted as 14-day studies if the proposed clinical treatment period is less than 14 days. For example, an antibiotic intended for 5–10 daily doses would not require a 28-day study; a 14-day treatment phase would be considered adequate.

Since 2017, FDA has required all IND-directed animal safety studies to be submitted in the Standard for Exchange of Nonclinical Data (SEND) format. Certain mechanistic and safety pharmacology studies, as well as reproductive toxicology, carcinogenicity and other animal studies, are exempted from this requirement at this time, but this additional step is now mandatory for routine repeat-dose toxicology studies and would be included as part of the reporting deliverables for the studies below. Additional tests may be added to the SEND requirement list at a future date, but FDA has not, at this time, given any indication of when this will occur and what assays will be included.

28-Day Repeat-Dose Toxicity Study in Rats, with Functional Observation Battery (FOB) Endpoints and Micronucleus Evaluation (GLP). The toxicity study in rats is performed as follows:

- Sprague Dawley rats, 15M/15F per dose group for main study; control and three dose levels
- A satellite TK group; 9M/9F per dose group; three dose levels of test article
- Administered drug via dosage regimen determined in preliminary studies, i.e., once daily for 4 weeks
- Clinical observations daily; body weights and food consumption weekly
- Clinical pathology (clinical chemistry, hematology) on the day of scheduled sacrifice (Days 29 and 42)
- Neurobehavioral assessments such as the Functional Observation Battery (FOB) evaluation on Day 1. (This assessment can also be conducted and submitted as a separate study.)
- Plasma drug levels on 3M/3F per group (six time points) on Days 1 and 28
- Ophthalmology prestudy and before necropsy
- 10M/10F per group necropsied on Day 29 and 5M/5F per group necropsied 14 days after the last dose (Day 42)
- Organ weights and weight ratios determined at necropsy
- Histopathologic evaluation of high-dose and control tissues; target organs evaluated in mid- and low-dose groups
- Urinalysis in the week of necropsies

- Micronucleus evaluation included in 28-day repeat-dose toxicity study in rats; bone marrow harvested at scheduled sacrifice at the end of the treatment period. This is an important addition to this protocol, since ICH guidelines now permit bone marrow micronucleus assays in conjunction with repeat-dose toxicity studies. Acridine orange stain is used to unequivocally differentiate DNA from RNA and to eliminate basophil-derived artifacts. 2,000 polychromatic erythrocytes per animal scored for micronuclei.
- It is becoming more common to add the *pigA* mutation marker via flow cytometric evaluation. This optional genetic toxicology endpoint may be requested by the FDA, depending on structural alerts of the compound or other data suggesting a potential mutagenic risk.

28-Day Repeat-Dose Toxicity Study in Dogs, with Cardiovascular Endpoints (GLP). The design for toxicity studies in dogs includes cardiovascular evaluations:

- Beagle dogs, 5M/5F per dose group; control and three dose levels
- Administered drug via dosage regimen determined in preliminary studies, i.e., once daily for 4 weeks
- 3M/3F per group necropsied on Day 29; 2M/2F necropsied on Day 42
- Clinical pathology (clinical chemistry, hematology, coagulation) prestudy and Days 29 and 42
- Ophthalmology prestudy and before necropsy
- Electrocardiographic evaluation prestudy, after dosing on Day 1 or Day 28, and prior to recovery necropsy (Day 42) if any findings are seen on Day 28. This endpoint is optional, and may be replaced with a separate cardiovascular telemetry study.
- Plasma drug levels (six time points) on Days 1 and 28
- Organ weights and weight ratios determined at necropsy
- Histopathologic evaluation of high-dose and control tissues; target organs evaluated in mid- and low-dose groups
- Urinalysis at necropsy.

H. Genetic Toxicology Studies

The genetic toxicology component of the IND requires, in most cases, three studies:

- Bacterial mutagenesis (Ames test)
- A mammalian cell assay (in vitro micronucleus, in vitro chromosome aberrations or mouse lymphoma mutagenesis
- Rodent bone marrow micronucleus

As mentioned in previous sections, the bone marrow micronucleus endpoint is now routinely included as part of the repeat-dose rodent toxicology study. This leaves just two in vitro tests to

be submitted as part of the IND. The in vitro micronucleus assay is described as this has become the most commonly conducted of the three mammalian cell tests.

H.1 Bacterial Mutagenesis (Ames) Assay (GLP)

The Salmonella/E. coli Reverse Mutation Assay (Ames Test) using four Salmonella strains and one E. coli strain is performed as described below.

- Preliminary dose-range finding experiment with and without metabolic activation (MA) over a wide range of doses with one *Salmonella* strain and one *E. coli* strain
- Mutagenicity experiment conducted with four *Salmonella* strains and one *E. coli* strain (one experiment)
- Six dose levels, with and without metabolic activation
- Three plates per dose; positive, negative, and sterility controls
- Use of plate incorporation

Note that conducting bacterial mutagenicity is often problematic when evaluating antibiotics, because potent anti-bacterial agents will demonstrate significant cytotoxicity against the tester strains, resulting in an inability to complete the mutagenicity testing. Likewise certain antibiotics (e.g., fluoroquinolones) have a mechanism of topoisomerase or gyrase inhibition that results in bacterial DNA strand breaks. These compounds work, by definition, as bacterial mutagens, and will give positive results in these assays. In either case, it may be necessary to conduct supplemental studies in mammalian cells such as the mouse lymphoma mutagenesis assay.

H.2 In vitro Micronucleus in CHO cells (GLP)

The in vitro micronucleus assay in CHO cells is performed as described below.

- Dose range-finding experiment with single cultures using 5 -8 concentrations and solvent control using a 3 hr exposure with and without S9 and 21 hr exposure without S9. 500 cells for proliferation index will be scored for cytotoxicity.
- Two experiments are performed in duplicate cultures with at least 4 concentrations and solvent and positive controls.
- The initial experiment is performed with 3 hr exposure with and without S9 and a harvest time of 1.5 cell cycle.
- If negative results are obtained, a confirmatory experiment is performed with 21 hr treatment without S9 and 3 hr treatment with S9. If positive results are obtained, a confirmatory experiment is performed with 3 hr treatment with and without S9.
- 500 cells for proliferation index and 2000 binucleated cells for micronuclei will be scored for solvent and positive controls and for each dose level.

I. Safety Pharmacology

Safety pharmacology studies have traditionally been considered on a case-by-case basis, but whereas these tests were previously considered optional, the FDA and other regulatory agencies are increasingly requiring these studies for all IND submissions. The list of required safety pharmacology studies typically includes assessment of the central nervous system (CNS), cardiac and respiratory systems. The CNS assessment is often covered by the FOB assay included as part of the repeat-dose rat toxicity study, but may be conducted as a separate study. The cardiac assessment may be covered by the electrocardiogram (ECG) assessment in dogs as part of the 28-day dog toxicity study, but increasingly, regulatory agencies are requesting continuous ECG monitoring by telemetric monitored dogs or non-human primates for a period of 24 hr or longer. In addition, the hERG assay (an in vitro test for ion channel effects of drugs, which is useful as an early screen, described below) is being requested as part of some regulatory submissions, especially in Europe, and it is frequently requested by the FDA as well. Likewise, rat respiratory effects of drugs are frequently requested. Study outlines for the dog cardiovascular telemetry, hERG, and rat respiratory assays are described below. The specific requirements for these tests should be determined as part of the pre-IND process with the FDA.

As with the repeat-dose toxicology studies above, all studies will be conducted with analytical chemistry evaluations of the dose formulations.

Cardiovascular Telemetry Study in Dogs. The objective of this study is to conduct a non-invasive cardiovascular telemetry study to determine any potential cardiovascular effects of a single oral administration of a test article to determine a no observed adverse effect level (NOAEL) in male and female beagle dogs. The study can also be performed with a virtually identical design in NHPs if desired, but NHPs are rarely used in this assay for small molecules. A single dose will be administered to each dog and the effect of the drug on the heart will be determined by ECG, measuring heart rate (HR), respiratory rate (RR), body temperature (using implanted microchips) and blood pressure.

- 3M/3F per dose group; control and three dose levels
- Vehicle group will be treated first; on the next day the same 3M/3F will be treated with low dose after a one week (depending on the $t_{1/2}$ of the compound) washout period; the same animals will be assigned to mid-dose group; and so forth
- Body Weight: Prior to each dose administration for the purpose of dose volume calculation
- Mortality/Morbidity: Daily
- Clinical Observations: 2–4 hr post dose on each dosing day
- Body Temperature: At four time points (e.g., 30, 60, 120 and 240 min)
- ECG, HR, RR: These parameters will be measured for conscious dogs continuously for up to 24 hr and will be evaluated at approximately seven time points surrounding the T_{max} of the drug (e.g., pretest, 2, 30, 60, 120, and 240 min and 24 hr post dose)
- Blood Pressure: Will be measured at pretest and at four time points around the T_{max} of the compound

- Clinical Pathology: Blood will be collected for hematology, clinical chemistry, and biomarker evaluations prestudy and ~48 hr after each dose
- Necropsy: This is a survival study; no necropsy is planned. In the event of mortality, necropsy and histopathology may be performed

hERG Assay. The objective of this study is to assess in a mammalian cell line effects of a drug on the (**h**uman **E**ther-à-go-go **R**elated **G**ene) that codes for the potassium ion channel. Effects in this assay are considered to be a predictor of possible QT prolongation effects. This assay, which has previously been used as part of drug discovery efforts, is now required for virtually all IND submissions of small molecule drugs.

- Four concentrations per test article; three replicates per concentration
- Positive and vehicle controls are included

Respiratory Safety Pharmacology Study in Rats. The objective of this study is to determine the effects of the drug on respiratory parameters in the rat.

- Eight male rats per group; control and three dose levels plus a reference (positive) control are used
- Ventilatory parameters will be evaluated following single administration to conscious, unrestrained rats using barometric plethysmography
- Following dosing, animals will be continuously monitored for respiratory parameters from 30 min up to 240 min after dose administration (values averaged over 5 min intervals)
- Respiratory parameters include respiratory flow, respiration rate, tidal volume, minute volume, inspiratory and expiratory time, peak inspiratory and expiratory flow, and relaxation time

J. Other Considerations

J.1 Biomarker Assessment and Feasibility

A biomarker is an indicator of a particular disease, or a pharmacologic/toxicologic response that can be used to measure the progress of disease or the effects of treatment. An NIH working group committed to the following definition: "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention" (Biomarkers Definitions Working Group, 2001). A biomarker's potential and feasibility should be considered and evaluated because biomarkers may be an indication of the effectiveness of protection prior to exposure. Biomarkers are particularly important for agents for which clinical trials on affected populations will not necessarily be possible. For example, a measure of inhibition of a particular marker protease, if feasible for evaluation in tissues from treated animals, would be of particular value in assessing the effects of the lead candidate. In addition, such a biomarker, if it can be collected by a noninvasive method (e.g., blood, urine, saliva) may be suitable for use in Phase 1 clinical trials

as an early indicator of drug action in humans. A simple and obvious example of this is that a drug designed to lower cholesterol levels should, indeed, result in a reduction in circulating cholesterol in nonclinical studies.

Development of biomarkers is typically conducted as part of the drug discovery R&D program, and it should be considered as a potential research area to pursue in parallel with preclinical and clinical development. There may be cases where inclusion of a specific biomarker in GLP safety studies will provide additional value. This is relatively uncommon in infectious disease therapy, as the "target" of drug action is typically a pathogen. There may be exceptions for host response markers; e.g., measurement of specific cytokines following treatment with drugs intended to diminish the impact of cytokine storms following infection.

There is increasing use of safety biomarkers to provide better assessment of drug-induced adverse effects. ELISA-based kits for assessment of new renal and cardiac markers are available, and other biomarkers for assessment of other drug-induced toxicities (vasculitis, testicular injury, etc.) are in development. A number of clinical trials are also ongoing to evaluate whether some preclinical markers can be translated into human clinical use. It is therefore important to work with testing laboratories that remain current in this area. The Predictive Safety Testing Consortium (PSTC), a working group managed by the Critical Path Institute, consists of currently 12 pharmaceutical companies, the FDA, and several partner organizations including the Foundation for NIH and SRI International, and is developing, validating, and seeking regulatory approval of a broad range of safety biomarkers.

J.2 Animal Rule Development Projects

Of particular 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 small pox; and obiltoxaximab, raxibacumab, ANTHRASIL and BioThrax® for anthrax.

V. REGULATORY CONSIDERATIONS

A. 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 19 summarizes the key components included in a pre-IND meeting package.

TABLE 19.
INFORMATION FOR PRE-IND

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

TABLE 19. INFORMATION FOR PRE-IND	
Information needed	**Description**
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)

B. 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:

http://www.fda.gov/regulatoryinformation/guidances/ucm125335.htm.

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.

C. 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 7). 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.

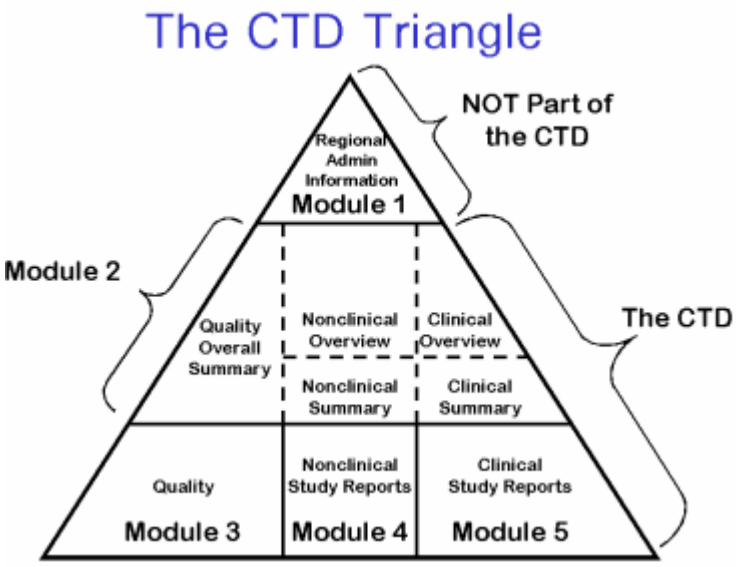

Figure 7. The CTD triangle.

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 NDAs 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 NDA" 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.

D. 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:

https://www.ich.org/page/ich-guidelines,

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 (link provided above) 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 20. 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 21, 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 20.
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.
In vitro genotoxicity	Yes	Studies that investigate the potential of a drug to induce mutations or chromosomal aberrations; studies are conducted in bacteria (Ames assay) and mammalian cells.
In vitro metabolism	No	Studies that compare the changes in drug structure in different metabolizing systems such as hepatic microsome and hepatocytes. One purpose is to identify cytochrome P450 (CYP), which may metabolize the drug in the clinic, allowing for assessment of potential drug interactions.
In vitro protein binding	No	Studies that measure the binding of the drug to serum proteins of humans and species used for repeat dose toxicity studies.
Pharmacokinetics	Typically No Yes for animal rule	Studies that investigate the process of drug absorption, distribution, metabolism and excretion. 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. 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.
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.
Safety pharmacology	Yes	Studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in and above the therapeutic range.
Secondary pharmacology	No	Studies that investigate the effects of a drug on the function of nontarget organs or parameters.
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 21. SUMMARY OF FIH REQUIREMENTS BY COUNTRY

Country	ICH	Manufacture			Nonclinical								Clinical			
	ICH Compliance	preGMP/Pilot Batch	cGMP Batch	Import Drug for FIH Study?	Primary Pharmacology	Safety Pharmacology	PK & In Vitro Metabolism	In Vitro Protein Binding	Toxicokinetic Evaluations	In Vitro Genotoxicity Studies	Acute Toxicity Studies	Repeat Dose Toxicity Studies	Protocol	Investigator's Brochure	Informed Consent	Women of Childbearing Potential*
Argentina	Yes	ND	ND	Yes	GLP	GLP	ND	ND	GLP	GLP	GLP	GLP	Yes	Yes	Yes	Allowed
Australia	Yes	NC	NC/C	Yes	Non GLP	GLP	No	No	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
Brazil	Yes	ND	ND	Yes	GLP	GLP	ND	ND	GLP	GLP	GLP	GLP	Yes	Yes	Yes	Allowed
Canada	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
Chile	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	ND
China	Yes	NC	C	No	GLP@	GLP	Non GLP	Non GLP	GLP	GLP	GLP	GLP	Yes	Yes	Yes	Allowed
Colombia	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	ND
EMA	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
India	Mostly Yes	NC	NC/C	No	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
Japan	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
Mexico	Yes	NC	NC/C	ND	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
Peru	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	ND
Russia	Yes	NC	NC/C	No	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
South Africa	Yes	NC	NC/C	No	Non GLP	GLP	Non GLP	Non GLP	GLP	GLP	Non GLP	GLP	Yes	Yes	Yes	Allowed
United States	Yes	NC	NC/C	Yes	Non GLP	GLP	Non GLP	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

E. 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:

www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM078932.pdf.

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 Ctrough 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.

F. 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.

VI. ESTIMATED SCHEDULE

Figure 8 presents a Gantt chart illustrates the estimated schedule of activities to prepare the pre-IND and IND submissions and to initiate the Phase 1 clinical trial for a typical small molecule anti-infective drug. This schedule outlines the "best-case scenario" for a drug that is well characterized and encounters no significant problems in safety, efficacy, manufacturing or supply chain. The reality is that few drugs ever achieve this best-case status, and invariably, some issue will result in either delays or diversions to conduct additional research project to address problems that occur.

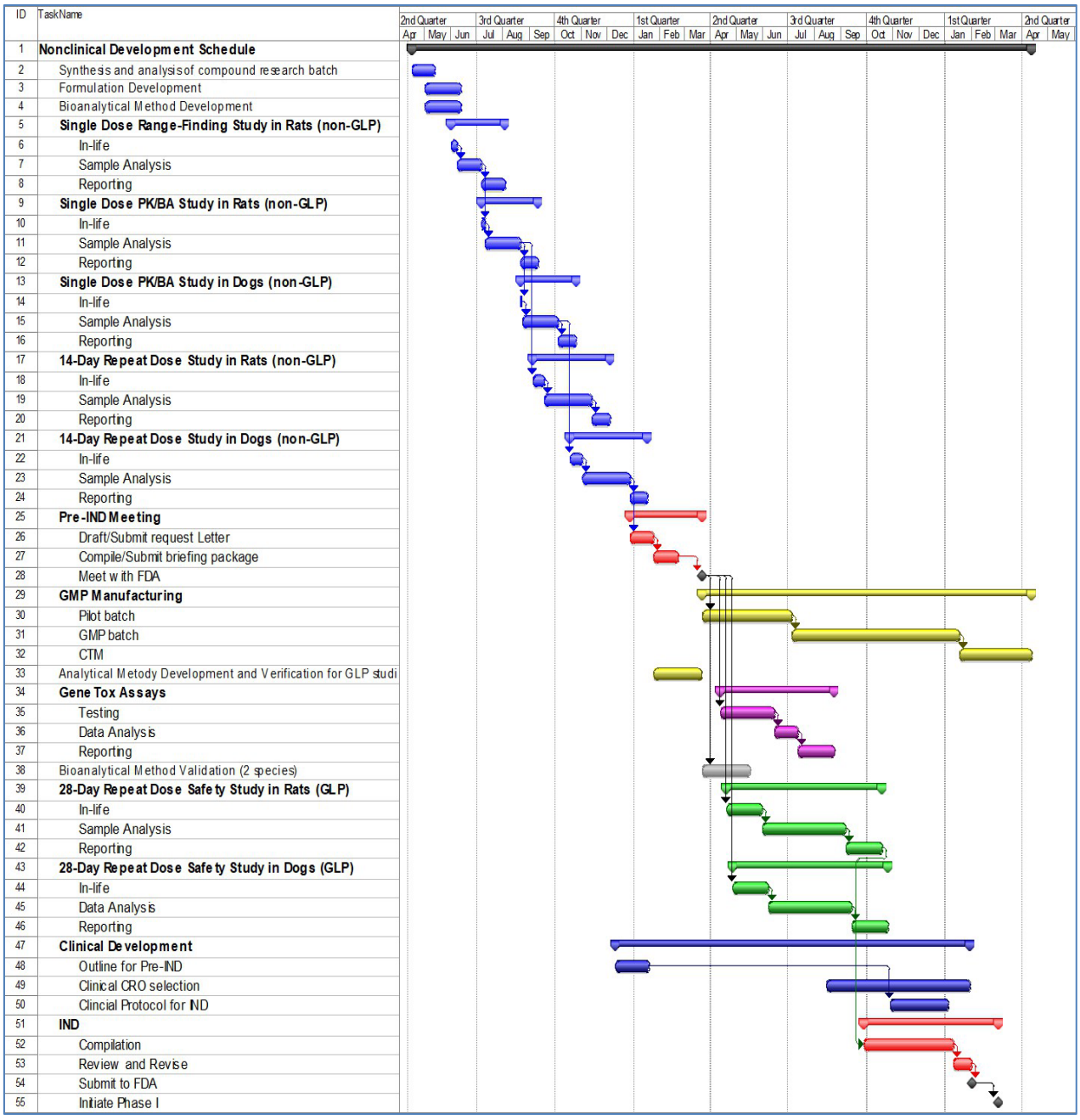

Figure 8. Estimated schedule.

VII. PROJECT MANAGEMENT AND RISK MITIGATION

A. 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, NDA, 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.

B. Risk Mitigation

Discovery and preclinical development of anti-infective therapeutics is a complicated process involving multiple scientific fields, regulatory constraints, GLP/cGMP 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.

VIII. SUMMARY OF RECOMMENDED TASKS AND APPROXIMATE COSTS

Table 22 summarizes the anticipated costs at a typical contract research organization (CRO) for the preclinical development tasks recommended for a typical small molecule development program. Not included in this table is the cost of efficacy studies, which are disease-specific, with a huge range of potential costs depending on the animal model used. Indeed, efficacy studies are considered a necessary prerequisite for initiation of a development program as outlined in this document. The preclinical development program outlined below assumes that efficacy studies have been completed and that the lead candidate has been shown to have efficacy in an appropriate animal model.

The costs for clinical trials are not included in this table as the design for trials can vary widely based on therapeutic indication, conduct in healthy volunteers vs. patients with disease, and location of trials (U.S. vs. overseas). Phase 1 clinical trials can cost as little as $250,000 and as much as several million dollars, depending on design parameters.

TABLE 22.
STUDIES AND COST ESTIMATES

Task/Study	Cost Estimate
Stage I: Synthesis and Manufacturing	
Chemical Synthesis (3-6 step) of non-GMP Batch (100 g)	$50,000–$100,000
Stage II: Pharmacokinetics and Range-Finding	
Bioanalytical Method Development (two species)	$30,000–$60,000
Comparative In Vitro Metabolism	$15,000–$30,000
CYP Inhibition and Induction	$ 60,000–$100,000
Drug Transporter Assay	$ 20,000–$40,000
Stage III: Pharmacokinetics and Range-Finding (concluded)	
Single Dose Range-Finding Study in Rats	$25,000–$35,000
Single Dose PK/Bioavailability Study in Rats with Plasma Analysis	$55,000–$90,000
Single Dose PK/Bioavailability Study in Dogs with Plasma Analysis	$80,000–$125,000
Preformulation/Formulation Development	$100,000–$350,000
7-Day Repeat Dose Range-Finding Toxicity & TK in Rats	$75,000–$100,000
7-Day Repeat Dose Range-Finding Toxicity & TK in Dogs	$120,000–$175,000
Stage IV: Pre-IND Meeting and GLP Studies*	
Preparation of pre-IND Package/pre-IND Meeting	$50,000–$75,000
Synthesis of “GMP-like” Batch of ~250 g; Synthesis Optimization	$75,000–$250,000
Synthesis of 1.5 kg of cGMP DS (includes Tech Transfer)	$150,000–$500,000

TABLE 22. STUDIES AND COST ESTIMATES	
Task/Study	**Cost Estimate**
Bioanalytical Method Validation (two species)	$125,000-$175,000
28-Day Repeat Dose in Rats with TK, FOB, MN & Recovery (GLP)	$400,000–$550,000
28-Day Repeat Dose in Dogs with TK, CV & Recovery (GLP)	$850,000–$1,200,000
Mouse Lymphoma Assay (GLP)	$60,000-$75,000
Ames Assay (GLP)	$30,000-$40,000
Dog Cardiovascular Telemetry Study (GLP)	$150,000–$200,000
hERG Assay (GLP)	$35,000–$50,000
Rat Respiratory Safety Pharmacology (GLP)	$50,000–$70,000
Stage V: Clinical Manufacturing and IND Submission	
Clinical Manufacturing of cGMP Drug Product (includes methods development, validation, and stability testing)	$600,000–$1,000,000
Drug Product Stability (to 3 years)	$200,000-300,000
IND Preparation (including electronic submission)	$150,000–$250,000
Total:	**~$3.5M–$6.0M**

* All GLP study costs include FDA mandated analytical chemistry support

The preclinical cost of development represents only a very small fraction of the full cost of advancing a drug from discovery to market approval. DiMasi et al. (2016) estimated that the fully capitalized cost of taking a new drug from discovery to market (including amortizing all of the failures for the program) to be $2.558 billion (in 2013 dollars). For emerging pathogens (e.g., SARS-CoV-2), this number can be greatly reduced with Emergency Use Authorization (EUA) or other tools that the FDA may apply to streamline approvals at times of a global pandemic. Anyone planning to develop a drug and advance it to market should consider a business plan as early as possible. A common strategy is to use internal institute, private or government funding through Phase I clinical trials, then attempt to partner the compound with a pharmaceutical company at about the time of Phase II clinical trials.

IX. CONCLUSION

Drug development is a high-risk/high-reward activity, where even the most successful companies fail far more often than they succeed. The key to success in the early (preclinical) stages of development is to create a clear plan, based on the TPP and the ultimate intended clinical use, for advancing the drug. Literally every decision about how the drug is used in efficacy studies, how it is tested for safety, how pharmacokinetics and pharmacodynamics are evaluated, how it is manufactured and packaged, should revolve around the question of "How will this drug be used in a clinical patient population." A clearly focused intended clinical use, combined with a competent team of experts in chemistry, manufacturing, formulation, safety, pharmacology and regulatory submissions, will greatly decrease the risk of failures, delays, and excessive expenditure of funds to advance products from early discovery through to clinical use.

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- Carol Green, Ph.D., D.A.B.T., Sr. Director, Nonclinical Development

APPENDIX A.

SUMMARY OF INTERNATIONAL REGULATORY REQUIREMENTS FOR FIRST IN HUMAN (FIH) BY COUNTRY

INTRODUCTION

The sections that follow describe, country-by-country, the specific requirements for initiation of First in Human (FIH) studies. While requirements are similar across the world, individual countries have minor differences, and different mechanisms for handling applications. This assessment was prepared by INC Research for the 2012 plan update (Revision 1). Updating of this country-by-country guidance falls outside the score for this revised plan, but there have been no significant changes that we are aware of since the original assessment, and these guidelines are therefore still considered to be fairly current.

ARGENTINA

Argentina participates in the Global Cooperation Group of the ICH. A specific agency within the Ministry of Health regulates clinical trials—the Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT). Argentina follows ICH recommendations on Good Clinical Practice (GCP) and requirements. The clinical trial regulations may be accessed at the ANMAT website at:

http://www.anmat.gov.ar/principal.asp.

This agency has the power to authorize and monitor all clinical trials in Argentina. Its regulations contain detailed provisions outlining the obligations of sponsors and investigators and the participation of independent ethics committees or review boards. ANMAT also performs audits of clinical trials. All sites must be certified by ANMAT.

There is a 60-day review period for ANMAT to evaluate the FIH study application.

AUSTRALIA

Australia is a member of the Global Cooperation Group and its regulations follow ICH guidelines. Clinical trials of medicines and medical devices conducted in Australia are subject to Commonwealth Government regulation administered by the Therapeutic Goods Administration (TGA). A list of relevant guidance documents can be found at the Therapeutics Goods Administration website and include guidance documents for nonclinical studies:

https://www.tga.gov.au/guidance-23-nonclinical-studies

and clinical studies:

https://www.tga.gov.au/clinical-trials.

There are two schemes under which clinical trials involving therapeutic goods may be conducted: the Clinical Trial Notification (CTN) Scheme and the Clinical Trial Exemption (CTX) Scheme. The choice of which scheme to follow (CTN or CTX) lies firstly with the sponsor and then with the Human Research Ethics Committee (HREC) that reviews the protocol and provides advice to the "Approving Authority," which decides whether the trial is allowed to proceed. The determining factor for an HREC is whether the Committee has access to appropriate scientific and technical expertise in order to assess the safety of the product.

As a general rule, Phase 3, 4, and bioavailability/bioequivalence studies of medicines are usually suited to the CTN Scheme. The CTN Scheme can also be an option for earlier phase (1 and 2) studies if there is adequate preclinical review available (especially of safety).

BRAZIL

Brazil participates in the Global Cooperation Group of the ICH. As per Brazilian Regulations, Phase 1 studies have the same regulations and requirements as Phase 2 and 3 studies, i.e., they should be submitted for approval to a Local Ethics Committee, the National Ethics Committee (Comissão Nacional de Ética em Pesquisa; CONEP) and the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária; ANVISA). A listing of the documents and procedures are available in a document that can be downloaded at:

http://conselho.saude.gov.br/resolucoes/2004/Reso340.doc.

The submission to the Local Ethics Committee is performed electronically through a National System called "Plataforma Brasil." This submission is performed through the sites selected, so during the site selection process it is important to check if the sites are registered in the system. After local approval of site coordinator, submission to CONEP is performed by local Ethics Committee. This can take 60 to 120 days.

The initial submission to ANVISA can be done in parallel with local submissions. It takes 3 months to obtain ANVISA approval; however, CONEP approval is required to start initiation visits. The ANVISA approval includes import license, so the importation process can begin even if CONEP approval is not in place yet. It is necessary to request an import license for each importation.

New sites can be added during the review process. If new sites are added after study approval, ANVISA and CONEP are merely notified of the extra sites. They both provide an acknowledgement of receipt. In the case of ANVISA, they provide a "special communication" or "comunicado especial." New sites have to be detailed in this letter (this takes 2 months). CONEP takes 1–2 months to add a new site.

The approval timeframe is the same for Phase 2 and 3 studies (around 7 months). Special attention must be paid to this timeframe when considering that Phase 1 trials are shorter than additional trials.

Brazil follows ICH recommendations on data requirements and GCP. Depending on the importance of the project, in view of urgency and in the absence of other therapeutic methods, the Local Ethics Committee may approve projects that have not fulfilled all clinical pharmacology phases.

In Brazil, clinical research subjects cannot receive any payment to participate in a clinical trial; however, it is allowable to reimburse patients for any transportation or meal expenses.

COLOMBIA

Columbia participates in the Global Cooperation Group of the ICH through the Pan-American Network for Drug Regulatory Harmonization. Its regulations follow ICH guidelines and GCP. Clinical trials are overseen by the National Institute for Drug and Food Surveillance (Instituto Nacional de Vigilancia de Medicamentos y Alimentoa; INVIMA). In Columbian FIH trials,

regulatory and ethics board approvals are done in sequence. Research Ethics Board approval is required prior to submitting the clinical trial application to INVIMA. Ethics Committee approval generally requires 1 to 2 months, depending on the institution.

INVIMA approval requires from 60 to 75 days (they have monthly scheduled meetings published on the official web page). Following approval, the import license may be requested. The import license will cover all importations included in the INVIMA approval (blanket import permit). After INVIMA approval is granted, notifications of extra sites can be provided to INVIMA without the need for additional review. On average, the process takes 6 to 6.5 months. Sites must be certified in GCP by INVIMA.

CANADA

The basic requirements for Health Canada submissions are outlined on the Health Canada website at:

http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/clini/index-eng.php.

Sponsors are encouraged to hold a pre-CTA (Clinical Trial Application) meeting with Health Canada prior to submitting a CTA. Investigational products should be manufactured under cGMP in compliance with ICH guidelines. Health Canada does not have any additional regulations regarding the quality of the investigational product. Health Canada follows ICH M3 guidance with respect to nonclinical requirements for FIH studies. It is noteworthy that Health Canada permits the women of childbearing potential (WOCP) to participate in Phase 1 trials without requiring the Sponsor to conduct embryo-fetal developmental toxicity studies, provided adequate birth control is used:

http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/clini/womct_femec-eng.php.

Health Canada now requires documents to be submitted through the eCTD process, similar to the U.S. Electronic submission instructions can be found at:

https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/applications-submissions/guidance-documents/filing-submissions-electronically.html.

A Health Canada submission contains Module 1 and Module 2 (Health Canada specific Quality Overall Summary only) of the CTD. It may be necessary to submit Module 3 (Quality) to supplement the Quality Overall Summary if Module 3 includes additional information that is not included in Module 2 that will support the CTA review. Health Canada does not require the submission of Module 4 (Safety) or Module 5 (Efficacy) data. Health Canada is in the process of revising their submission format. The drug applicant should consult the Health Canada website prior to submission to ensure compliance with current guidelines.

Following receipt of the CTA, Health Canada will review the adequacy of the submission. If the submission is incomplete, then they will inform the Sponsor of the deficiencies and request that the Sponsor submit additional information. If the CTA is adequate, then they will issue an "Acknowledgement Clinical Trial Application" fax, generally within two business days of receipt of the complete CTA. This fax will include the date of receipt of the CTA, a File Number and a Control Number. Any future correspondence with regards to the CTA should refer the File Number and the Control Number. During the review process, Health Canada may

request additional data or revisions to the study documents. Health Canada has 30 days from the date of receipt of the full application to review and act on the CTA. However, for Phase 1 studies in healthy volunteers, Health Canada may approve the application in 7 days.

CHILE

Chile participates in the Global Cooperation Group of the ICH through the Pan-American Network for Drug Regulatory Harmonization. Its regulations follow ICH guidelines and GCP.

For FIH in Chile, the initial submission is to Regional Ethics Committees (with jurisdiction on a specific geographical area) and, in some cases, Institutional Review Boards (IRB). Submission to both committees (if applicable) can be done in parallel; however, in all cases IRB approval is needed before the approval of the Regional Ethics Committee is granted. There are no Central Ethics Committees in Chile. The approval takes approximately 45 days. Following Ethics Committee approval, a submission is made to the Ministry of Health. There is a new system in Chile called GICONA, which considerably shortens the approval times.

The initial approval from Ministry of Health (MoH; Ministerio de Salud) is considered an import license; it covers the total amount of study medication to be imported (blanket import permit). After getting the first MoH approval from the Public Health Institute, the drug may be imported into the country. Once the study medication is in the country, the MoH must give permission to use the medication included in this particular importation. The document requested to use the medication, called "Use & Disposal," takes around 10–15 working days.

After this document is obtained, the investigational product can be distributed to the sites. The approval process requires 3 to 4 months in total.

Once the approval of the first site is obtained, subsequent sites are approved as soon as they are submitted. The local regulation does not detail any requirements regarding payments to patients, although it is necessary to report patient reimbursements. Patient insurance is required.

CHINA

China participates in the Global Cooperation Group of the ICH. Its regulations generally follow ICH recommendations. The Chinese Health Authorities have published several guidance documents on nonclinical studies. Clinical trials are regulated under the Provisions for Drug Registration (SFDA Order No. 28) which is available at:

http://english.nmpa.gov.cn/2019-07/25/c_390595.htm.

Under Article 44 of SFDA Order No. 28, an overseas applicant can conduct a multi-center clinical trial in China only if the drug is already approved or in Phase 2 or 3 clinical trials overseas. The Chinese State Food and Drug Administration (SFDA) will only accept the FIH application for drug products (DP) produced in China. It is not permitted to perform an FIH using imported DP. A Clinical Trial Approval Note from the SFDA is required prior to conducting a clinical trial in China.

Sponsors are encouraged to hold a pre-CTA meeting with Health China prior to submitting a CTA, particularly if the DP is a new chemical entity. The required documents will be submitted in advance. Investigational products should be manufactured under GMP condition. The clinical trial must be conducted at institutions certified for conducting clinical trials.

Although it is permitted for WOCP to participate in Phase 1 trials, Health China will review the embryo-fetal developmental toxicity studies. Adequate birth control will be required.

At present, Health China does not require eCTD submissions; they use a hybrid paper/electronic submission. The e-application form is generated from specific software provided by SFDA, and only in Chinese. Although it is not required to submit the entire submission in electronic format, Sponsors are required to submit all summary reports in electronic format.

A diagram of the Chinese CTA procedure is presented in Figure A-1. Health China will review the adequacy of the submission. If the submission is incomplete, they will inform the Sponsor of the deficiencies and request that the Sponsor submit additional information. If the CTA is adequate, then they will issue an "Acknowledgement Clinical Trial Application" with Application Acceptance Number. It is possible for them to issue an "Acknowledgement Clinical Trial Application" without an Application Acceptance Number, then issue the note with Application Acceptance Number, within five business days of receipt of the complete CTA via courier.

The submission package will then be forwarded to the Center for Drug Evaluation (CDE). The published timeline to complete the evaluation is 80/90 days (fast track/non-fast track project). The evaluation conclusion will be sent to SFDA for final approval. Within 30 working days, the CTA note will be issued to the applicant. Please note that the timelines in this discussion do not always agree with the numbers in Figure A-1, even though the numbers are based on the SFDA website.

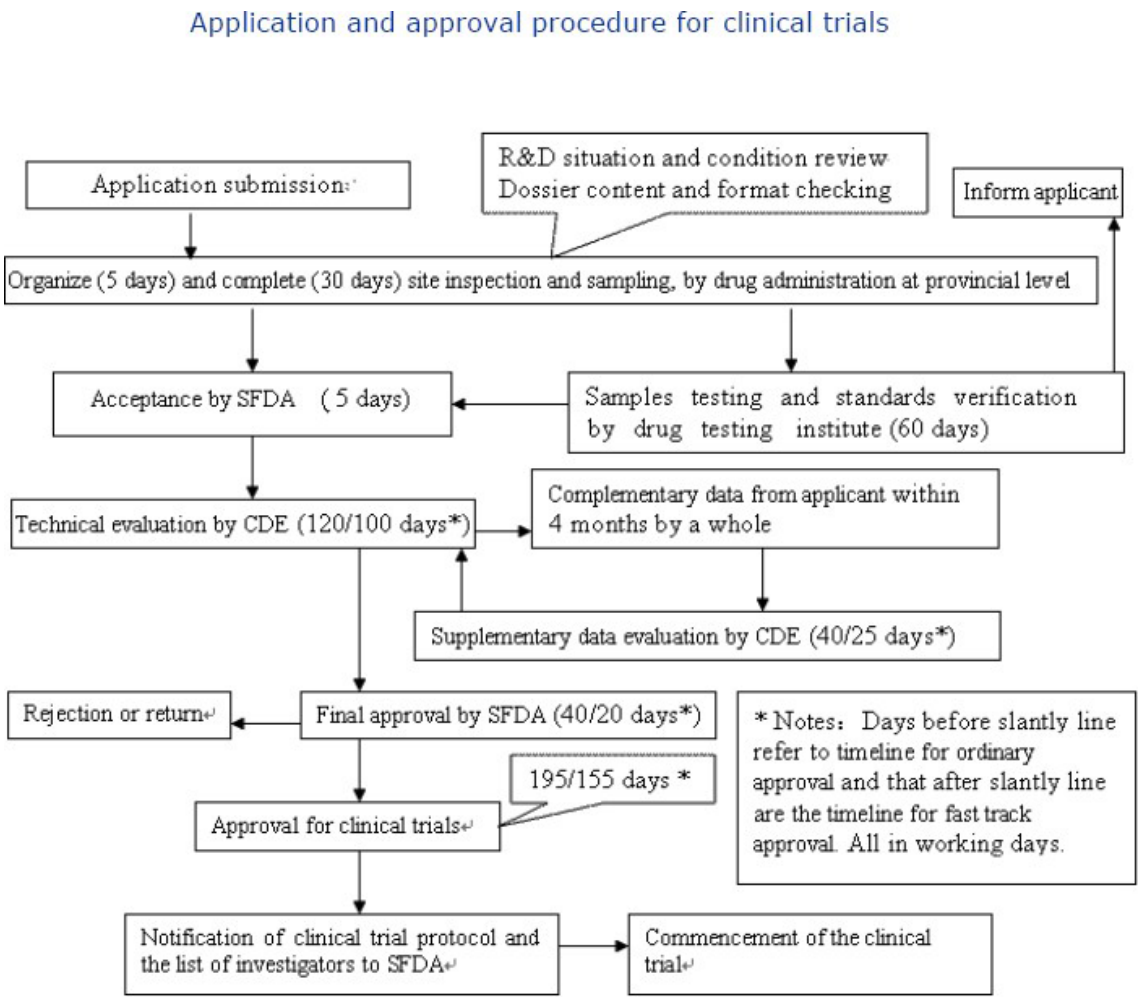

Figure A-1. Chinese CTA approval pathway.

EUROPEAN MEDICINES AGENCY (EMA)

Clinical trials in the EMA are controlled by the individual member states (competent authorities). All member states must comply with the Clinical Trials Directive

https://www.ema.europa.eu/en/human-regulatory/research-development/clinical-trials/clinical-trials-regulation,

which was originally introduced in 2001 and has been subject to a number of revisions, most recently by a requirement designed to assure that imported test substances are manufactured to GMP. All clinical trials in the EU must be registered in the EudraCT database at:

https://eudract.ema.europa.eu/.

Sponsors must have a legal entity registered within the EU.

Multinational clinical trials can be coordinated to assure a uniform assessment by the differing competent authorities through the Voluntary Harmonised Procedure controlled by the Clinical Trials Facilitation Group:

https://laegemiddelstyrelsen.dk/en/news/reassessment-of-reimbursement-of-medicines-news-archives/voluntary-harmonisation-procedure-vhp-for-the-assessment-of-multinational-clinical-trial-applications/.

National procedures differ depending on the specific location(s) of the clinical trial, particularly with respect to which documents are assessed by the competent authority and which by the local ethics committee.

The EMA follows ICH M3 guidance with respect to nonclinical requirements for FIH studies at:

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002720.pdf.

For a single dose FIH study, in vitro metabolism, protein binding, extended single dose toxicity studies in two species (including toxicokinetics) and an assay for gene mutation (Ames test) may be all that is needed. WOCP can be included, provided adequate contraception and pregnancy testing are included.

Applications should include an Investigational Medicinal Product Dossier (IMPD), which must comply with CHMP/QWP/185401/2004; this can be found at:

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003484.pdf.

with respect to the quality data for small molecules. A separate guidance for biologicals is available at:

http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/05/WC500127370.pdf.

Nonclinical data (and clinical data) can be submitted by cross-reference to the (GCP compliant) Investigators' Brochure. Although there is an option to submit a full review of the nonclinical data in the IMPD, this is not commonly used, as it would require that the Brochure be updated and re-submitted every time there is new data. The format is similar to that of a CTD nonclinical overview, apart from paragraph numbering. There is no requirement to submit the study reports, but any references to nonclinical study reports must be complete and the reports should be available if requested.

INDIA

Although India is a member of the ICH Global Cooperation Group, it does not specifically follow ICH GCP principles. The Indian GCP guidelines are available at:

https://rgcb.res.in/documents/Good-Clinical-Practice-Guideline.pdf.

India deviates from the ICH M3 guidance, so the reader should consult this site for nonclinical requirements as well.

The approval for clinical trials, import licenses, and export licenses is provided by the central health authority, Drug Controller General of India, (DCGI), whose official website address is:

https://cdsco.gov.in/opencms/opencms/en/Home/.

The Indian regulatory system is an evolving system and has continuously responded by bringing about positive changes in its way of functioning and approval timelines through actively working with biopharmaceuticals and clinical research industry players. The reader is advised to consult with the regulatory authorities to ensure compliance with regulatory requirements. The current guidance documents for Approval of Clinical Trials and New Drugs can be found at:

https://cdsco.gov.in/opencms/opencms/en/Drugs/New-Drugs/.

Following CTA submission, the DCGI staff will evaluate the completeness of the dossier. If any data is missing, then the DCGI will notify the Sponsor of the need to submit additional data within 45 days of submission of application. If the dossier is complete, it will be forwarded to the appropriate New Drug Advisory Committee (NDAC) of Key Opinion Leaders, which consists of therapeutic experts and pharmacologists for various therapeutics indications who will be reviewing all CTA applications along with the Scientific Challenging Protocols. The NDAC is expected to provide its expert opinion on CTA dossiers within 6 weeks of referral of applications.

Once the NDAC opinion is in place, then DCGI staff will also evaluate the CTA dossier and will process the application for approval. This last stage of processing the application, including the final round of signatures, may take an additional 4–5 weeks. Hence the entire approval process from the submission to approval will take approximately 22–24 weeks. The DCGI average approval time is 6 months, but may be longer, depending on the reviews of individuals or committees and the questions asked. At times, requests for special provisions have been made:

- Limits on enrolling elderly subjects
- Justification for placebo controls
- Potential enrollment caps of 20–30% of the total required target enrollment

A special license may be required to export biological samples. This could require an additional 6 to 9 months post approval.

The study protocol should include budget and source of funding. Study subjects should be satisfactorily insured against any injury caused by the study. FIH studies using a drug manufactured outside of India are not permitted unless "the drug is of special relevance to the health problem of India." Reproductive toxicity studies are required prior to enrolment of WOCP.

JAPAN

Japan is a full participant in ICH. Studies must follow GCP and ICH recommendations. The home page for the Pharmaceuticals and Medical Devices Agency (PMDA) is:

http://www.pmda.go.jp/english/index.html.

An outline to the Japanese guidance (English translation) on drug development steps can be found at:

https://www.pmda.go.jp/english/review-services/outline/0001.html.

The guidance specifically cites United States FDA and EMA guidance on FIH studies. Performing an FIH study using an imported DP is not permitted. This should not be confused with frequent

requests by PMDA that foreign companies conduct Phase 1–2 studies in Japan; they are not FIH studies.

Before a clinical trial can be conducted, a CTN should be submitted to PMDA. When the sponsor is a foreign company, it must appoint a Clinical Trial In-Country Caretaker who resides in Japan. The Japanese clinical trials are in line with ICH. The CTN is subject to a "30-day-review" by PMDA. The CTN should include a sample of the case report form. The sample is not required if information to be contained in the case report form is explicitly stated in the protocol.

Sponsors must have insurance to compensate patients for any eventual health problems arising during the participation in any clinical trial. The sponsor is also responsible for the activities of their selected CRO.

The clinical trial must be approved by an IRB. Each medical institution has its own internal IRB, which typically meets monthly.

MEXICO

Mexico participates in the Global Cooperation Group of the ICH through the Pan-American Network for Drug Regulatory Harmonization. Its regulations follow ICH guidelines and GCP. The Federal Commission for the Protection against Sanitary Risk (Comisión Federal Para La Protección Contra Riesgos Sanitarios; COFEPRIS) regulates clinical trials. A summary of the drug registration requirements in Mexico can be found at:

https://latampharmara.com/mexico/drug-registration-in-mexico/.

Regulatory and ethics board approvals are done in sequence. Research Ethics Board approval is required prior to submitting the clinical trial application to COFEPRIS. At present, COFEPRIS only accepts paper submissions. COFEPRIS is in the process of revising its submission format. The drug applicant should consult the COFEPRIS website prior to submission to ensure compliance with current guidelines. Following receipt of the CTA, COFEPRIS will review the adequacy of the submission. If the submission is incomplete, then it will request that the Sponsor submit additional information. During the review process, COFEPRIS may request additional data or revisions to the study documents. COFEPRIS has 3 months from the date of receipt of the full application to review and act on the CTA.

COFEPRIS may audit the sites at any time. An insurance certificate endorsed by a local insurance company is required before a site can be activated. COFEPRIS permits WOCP to participate in FIH trials without requiring the Sponsor to conduct embryo-fetal developmental toxicity studies provided adequate birth control is used.

The main regulatory framework for medical products is defined in Mexican Federal Laws:

- General Health Law (Ley General de Salud)
- Health Law Regulations (Reglamento de Insumos para la Salud)
- Official Mexican Standards (Norma Oficial Mexicana).

The authority responsible for enforcing this regulatory framework is COFEPRIS, which is part of Mexico's Ministry of Health. COFEPRIS grants medical licenses to manufacturers in Mexico and oversees good manufacturing practices. Clinical trials are regulated by the Regulation for Health Investigation (RHI), which is enforced by the Ministry of Health and COFEPRIS. The RHI provides the guidelines and standards for the clinical trial protocol.

Drug registration in Mexico requires submission of a formal application dossier to COFEPRIS using the CTD format structure for submission.

PERU

Peru participates in the Global Cooperation Group of the ICH through the Pan-American Network for Drug Regulatory Harmonization. Its regulations follow ICH guidelines and GCP. Clinical trials are overseen by the Research and Technological Transfer General Office (Oficina General de Investigación y Transferencia Tecnológica) which is in the National Health Institute, (Instituto Nacional de Salud; INS). A description of the process for drug registration can be found at:

https://web.ins.gob.pe/es/investigacion-en-salud/acerca-de-la-ogitt/presentacion.

Additional details on procedures for quality control of pharmaceutical products can be found at:

https://web.ins.gob.pe/es/control-de-calidad-de-medicamentos/acerca-del-cncc/presentacion.

For FIH in Peru, regulatory and ethics committee (EC) approvals are done in sequence. Research EC approval is required prior to submitting the clinical trial application to INS. There is no Central EC in Peru; there are only local ethics committees. There are two types of ECs: Institutional ECs (associated with a hospital or site institution) and Independent ECs (not related to any site institutions). All ECs must be registered at INS: if an institution does not have an INS-registered EC, an Independent EC may be used. The timeline for EC approval is about 4–6 weeks.

Subjects may be compensated for participating in a clinical trial.

RUSSIA

Russia participates in the Global Cooperation Group of the ICH. Clinical trials must be conducted in compliance with the Russian GCP, which is completely in line with the ICH GCP. The Russian Ministry of Health authorizes and controls the conduct of clinical studies through the Scientific Centre for the Evaluation of Products for Medical Use.

The sponsor should submit an application both to the Competent Authority (Scientific Centre for the Evaluation of Products for Medical Use) and the Council on Ethics of the Ministry of Health and Social Development. In addition, the local research ethics committee must approve the

study. These submissions may be done in parallel; however, approval by all bodies is required before a clinical trial may begin.

FIH studies using a drug manufactured outside of Russia are not permitted; these are reserved for locally manufactured drugs. It is mandatory that the sponsor be fully insured for the conduct of clinical trials. The usual time required to start a study in Russia is 3–4 months.

SOUTH AFRICA

South Africa is not a formal ICH member, but participates in the Global Cooperation Group through the Southern African Development Community (SADC). Clinical trials conducted in the country should be in line with the South African Guidelines for good clinical practice, which are in line with the ICH guidelines, World Health Organization Guidelines and the Declaration of Helsinki. The South African GCP guidelines are available at:

https://www.sahpra.org.za/wp-content/uploads/2021/06/SA-GCP-2020_Final.pdf.

The Medicines Control Council (MCC; website at:

https://www.sahpra.org.za/)

regulates and oversees the performance of clinical trials and registration of medicines and medical devices. The MCC is responsible for ensuring that all clinical trials of both non-registered medicines and new indications of registered medicines comply with the necessary requirements for safety, quality and efficacy. The application form (Form 6.05) for a CTA is available at:

https://www.sahpra.org.za/6-05_clinical-trial-application-form_feb-2020_v6/.

The submission may be made in either electronic or paper format.

An MCC expert committee reviews applications for clinical trials and for registration of medicines and medical devices. Studies are also required to undergo review by an EC. Reports on the progress of the study are sent to the MCC on a regular basis. The timelines for review are not available on the MCC website.

The South African National Clinical Trials Register provides the public with updated information on clinical trials on human participants being conducted in South Africa.

According to South African GCP guidelines, WOCP may be used in clinical trials if ethically justifiable.