Product Development Plan for a Generic Prophylactic Vaccine for Infectious Diseases

Deliver To:

National Institute of Allergy and Infectious Diseases
NIAID/DMID/OBRRTR/Vaccine Section
5601 Fisher's Lane
Rockville, MD 20852

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OVERVIEW

The general process of vaccine non-clinical development is similar for vaccines designed as prophylactic or therapeutic treatment covering a range of indications. This document is focused on prophylactic vaccine development for infectious disease indications. The information provided in this generic Product Development Plan (PDP) guide is intended to provide an overview for the development of a vaccine candidate to help facilitate a suitable and successful regulatory submission of an IND (Investigational New Drug) application to FDA for the vaccine that will allow for initiation of human clinical trials. This guide is intended for development through Phase I human clinical trial testing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

Acronym/ Abbreviation	Definition
A/G ratio	Albumin-to-globulin ratio
API	Active pharmaceutical ingredient
ATM	Analytical Test Method
BLA	Biological License Application
CDC	U.S. Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CMC	Chemistry, Manufacturing, and Control
CMI	Cell-mediated immunogenicity
CMO	Contract manufacturing organization
CQA	Critical Quality Attribute
CRD	Carbohydrate recognition domain
DMID	Division of Microbiology and Infectious Diseases
DP	Drug Product
DS	Drug Substance
ELISPOT	Enzyme-linked immunospot assay
F	Female
FACS	Fluorescent activated cell sorter
FDA	U.S. Food and Drug Administration
GAL	Galactose
GalNAc	Galactose and N-acetyl-D-galactosamine
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
his-tag	Histidine-tagged
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonization
ID	Identity
IFN-γ	Interferon-γ
IgA	Immunoglobulin-A
IL-10	Interleukin-10
IM	Intramuscular
IN	Intranasal
IND	Investigational New Drug
IPTG	Isopropyl-β-D-thiogalactoside
ISO	International Organization for Standardization

Acronym/ Abbreviation	Definition
IV	Intravenous
L	Liter
LC-MS	Liquid chromatography-mass spectroscopy
LLOQ	Lower limit of quantitation
LOD	Limit of Detection
M	Male
MCB	Master cell bank
mL	Milliliter
NOAEL	No observable adverse effect level
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases
OBRA	Office of Biodefense Research Affairs
PCR	Polymerase chain reaction
PDP	Product Development Plan
PHS	Public Health Service
SC	Subcutaneous
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TPP	Target Product Profile
ULOQ	Upper limit of quantitation
USP	United States Pharmacopeia
WCB	Working cell bank
WHO	World Health Organization

1.0 INTRODUCTION

At the request of the Office of Biodefense, Research Resources, and Translational Research (OBRRTR), Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health, a generic vaccine Product Development Plan (PDP) guide has been written. This plan provides an overall description of activities to be considered during the development of a new vaccine candidate and outlines Investigational New Drug (IND) enabling activities.

To obtain regulatory agency approval for human clinical administration, an investigational new vaccine follows a developmental pathway in which an ongoing risk management plan should be incorporated. The risk management plan should be appropriate for the stage of development, with key personnel identifying and assessing issues, and providing input to help resolve those issues or unexpected findings. In this regard, some risks will be controllable, but others will not be.

A second useful tool is the Target Product Profile (TPP), which defines both acceptable and preferred outcomes that are used for assessing key development stage results. Careful evaluations of early- stage optimization, modification, and vaccine design activities are useful for prioritizing development activities and the use of available resources, as are cost/benefit evaluations for each investigational pursuit.

1.1 GOALS AND CONSTRAINTS OF THIS PDP

This PDP guide is focused on providing information to product innovators on the processes required to take a vaccine candidate with promising proof of concept (POC) data (including animal immunogenicity/protection data) into a Phase I clinical trial. The focus on moving into a Phase I clinical trial results in many suggestions and processes that are industry standard for early phase clinical trial material. It should be noted that these suggestions may or may not be appropriate for a specific vaccine candidate. In addition, this document clarifies nonclinical studies that should be performed as proof-of-concept studies to support entry into product development for a vaccine candidate.

Note that development of vaccines through later clinical phases and market approval are outside of the scope of this document. However, some sections may provide brief descriptions of later stage points to consider.

1.2 TARGET PRODUCT PROFILE

The purpose of a TPP is to serve as a planning tool describing parameters that must be considered to achieve success in vaccine product development. It also provides a basis for discussions between a sponsor and the FDA that may be used throughout the vaccine development process, from pre-investigational new drug application (pre-IND) or investigational new drug application (IND) phases of product development through post marketing programs. For the purposes of the TPP described herein, the focus shall be from proof-of-concept data through Phase I clinical testing, the primary objective of which is to demonstrate safety and tolerability of the vaccine candidate.

A TPP serves multiple functions. Specifically, a TPP is:

- ***A Strategic framework*** and an adaptable document used to confirm required product attributes and to highlight product development priorities.
- **A Reference for Critical Quality Attributes (CQAs),** the technical characteristics of the product that should be within appropriate limits of product identity, strength, purity and potency to ensure desired product quality."
- ***A Common tool* to convey vaccine developmental goals** or objectives within the vaccine development team and can be used later in development to communicate with investors, potential commercialization partners and other stakeholders to evaluate assets and negotiate valuations.

Benefits of using a TPP:

If strategically developed and incorporated into a pragmatic system of strategic decision-making, a TPP will decrease development risk, tighten timelines, and could facilitate regulatory approval.

The TPP, in the context of this document, is not meant to depict a format for a summary of a development

program in terms of labeling concepts, as defined in the draft guidance provided by the Center for Drug Evaluation and Research (CDER) dated March 2007;(https://downloads.regulations.gov/FDA-2007-D-0256-0002/attachment_1.doc). Instead, it provides a prospective and dynamic summary for assessing the characteristics of a candidate vaccine during stages of development. As an organized summary of key components for the potential product, the TPP serves to place the vaccine in the time frame of the development stage and to provide an assessment of its likelihood of success. As more data and information are gathered during the course of development, the TPP is updated accordingly. A TPP for a Phase I trial should take into consideration the following:

- Patient population (typically healthy subjects for Phase I vaccine trials)
- Safety/Reactogenicity
- Dose Regimen
- Onset and Durability of Protection
- Coverage (the estimated percentage of people who have received specific vaccines)
- Dosage form ± adjuvant (if applicable) and route of administration
- Dosage strength, frequency, and duration
- Measures of vaccine effectiveness, including antibody and T cell responses (if applicable)
- Delivery Device, if applicable
- Manufacturing approach, storage conditions, and stability.
- Licensure

Other Points to Consider if relevant:

- Eventual Indication if not healthy adults
- Product quality criteria appropriate for intended marketed product or use

Table 1 presents an example of a TPP oriented towards product advancement into a Phase I clinical trial in healthy subjects.

Table 1. Sample Generic Vaccine TPP oriented towards advancement into a Phase I clinical trial.

Vaccine Characteristic	**TPP for Prophylactic Use**	
	Preferred (Nice to have)	**Critical (Need to have)**
Indication for use	For immunization to prevent infectious disease	For immunization to prevent infectious disease
Target population	Identify all age-groups and population [1]Identify age groups for Phase I trial.	All healthy adults excluding pregnant and lactating women. Identify age groups for Phase I trial. Identify excluded populations.
Safety/ Reactogenicity	Safety and reactogenicity sufficient to provide a highly favorable benefit/risk profile in the context of observed vaccine efficacy; ideally with only mild to moderate, transient adverse events related to vaccination and rare serious AEs related to vaccination	Safety and reactogenicity where vaccine benefits outweigh safety risks Demonstrated safety profile is transient reactions and rare serious adverse events related to vaccination
Evidence of efficacy or biological activity (if clinical	Greater than 50% efficacy in preventing disease in healthy children, adolescents, and adults	Greater than 50% efficacy in preventing disease in healthy adults

disease endpoint study is possible)[3]	If regulatory authorization is provided without clinical efficacy data, effectiveness data are to be generated during use in a future outbreak	If regulatory authorization is provided without clinical efficacy data, effectiveness data are to be generated during use in a future outbreak to the extent possible
Measures of efficacy (If regulatory strategy is based on non-clinical efficacy data like described in the US FDA Animal Rule.)	If demonstration of clinical efficacy is not feasible, non-clinical immunogenicity and efficacy in a standardized and relevant animal model together with clinical immunogenicity and safety may be considered	If demonstration of clinical efficacy is not feasible, non-clinical immunogenicity and efficacy in a standardized and relevant animal model together with clinical immunogenicity and safety may be considered.
Dose regimen	Single-dose regimen preferred	Primary series: No more than 2 doses
Onset and durability of protection	Confers protection of 5 years or more after primary series and can be maintained by booster doses Duration of protection may be inferred from immune markers as well as documentation of breakthrough cases Rapid onset of immunity (e.g., less than 1 month)	Confers protection of at least 1 year after primary series and can be maintained by booster doses. It may be necessary to infer protection from immune kinetics.
Route of administration	Injectable using standard volumes for intramuscular (IM), subcutaneous (SC), intranasal and/or oral routes[2]	Injectable using standard volumes for intramuscular (IM), subcutaneous (SC), intranasal and/or oral routes
Coverage	Effective against 1 or more heterologous pathogen strains (If applicable)	Effective against pathogen
Product stability storage	Liquid frozen: Shelf life of at least 24 months at <-65 °C Lyophilized: Shelf life of at least 12 months stability at 2-8° C and not damaged by freezing temperatures. The need for a preservative is determined and any issues are addressed	Shelf life of at least 12 months at <-65 °C. Demonstrated stability for at least 8 hours at 2-8°C. The need for a preservative is determined and any issues are addressed
Presentation	Vaccine is provided as a liquid product in mono-dose or multi- dose (10-20) presentations with a maximal dosage volume of 0.5mL Multi-dose presentations should be formulated, managed, and discarded in compliance with regional multi-dose vial policy	Vaccine is provided as a liquid or lyophilized product in mono-dose or multi-dose (10-20) presentations with a maximal dosage volume of 1mL Multi-dose presentations should be formulated, managed, and discarded in compliance with regional multi-dose vial policy. If the vaccine is lyophilized, it is critical for it to be accompanied by paired, separate vials of the appropriate diluent
Production scale	Capacity available to manufacture vaccine expeditiously as possible following scale-up. Dosage, regimen, and cost of goods amenable to high volume and	Capacity available to manufacture vaccine as expeditiously as possible following scale-up

	affordable supply. The vaccine should be cost effective, and the price should not be a barrier to access, including in LMICs	
Registration and licensure	Licensed for use by US FDA, countries at high-risk for outbreak and WHO Pre-qualified[3]	Licensed for use by the country of use

[1] In most Phase I vaccine trials, subjects are typically adult healthy subjects. As the primary objective is to demonstrate safety, often times vaccine effectiveness is not demonstrated. However, as part of secondary or exploratory parameters, common endpoints may include serum antibody titers, serum IgG, neutralizing antibody titers, and some cellular immune responses.
[2] Must decide if will conduct Phase I clinical trial in US or elsewhere. If Phase I clinical trial is conducted in US, then must be following FDA regulations. If elsewhere, as in Europe, must follow regulations for Europe. This may impact how vaccine is manufactured and released. Regulatory requirements for US and other countries may not be identical with respect to nonclinical data package, clinical trial execution, GLP toxicology requirements. Make sure to check with appropriate regulatory experts or consultants.
[3] If proposing multiple routes of delivery, must have nonclinical data demonstrating utility of proposed route of delivery. The TPP is not intended to be all possibilities, but a profile that is evidence-supported.

1.3 RISK MANAGEMENT

With the TPP serving to define both required and desired characteristics of the vaccine product, the attention of the development team shifts to consideration of possible events or outcomes which could jeopardize achievement of the goals outlined in the TPP. Understanding these risks and implementing mitigation strategies is a critically important part of any vaccine development program. A risk management plan should be in place, with contingencies and defined plans for unexpected results or inadequate quality of service provided by vendors or subcontractors in 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. A schedule and budget should be established that determine key milestones with go/no-go decisions, as well as tolerance for allowable 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.

Discovery and development of vaccines for human use is a complicated, expensive process involving multiple scientific fields, regulatory constraints, quality compliance, and testing and reporting requirements. Unanticipated events at any stage of the process may lead to changes in strategy, timeline delays, cost overruns or worse. Identifying, assessing, and managing risks (real or potential) are integral to the developmental process. A risk management plan should thus 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 it may be possible to resolve before expending more resources on the development process. Alternatively, it may have the potential to stop the program or halt advancement of the lead candidate. The evaluation of such instances is known as a go/no-go decision point and is sometimes also referred to as an “early exit strategy”. Although not welcomed, 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 discussed above. A TPP can establish acceptable as well as preferred results 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 vaccine design should be stopped.

Contingency plans should be developed to address controllable risks. Development involves several technical groups whose responsibilities vary, with each group identifying critical tasks that, if not properly executed, will adversely affect the cost and timeline of development. One approach for doing so is to determine the tasks that are key to successfully meeting program objectives and to 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 critical production materials) or assembling a list of available consultants for troubleshooting or for providing alternative solutions, if required. Contingency planning and the depth of managing these activities 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, risk management overview should always be included at some level for any outsourcing component of the development program. Additional details on risk management with emphasis on GMP manufacturing is presented in Appendix A.

Interaction with a regulatory agency (i.e., FDA, European authorities) and submission of required documents are required when advancing a vaccine candidate from the non-clinical 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 interact with the FDA are all ways to minimize regulatory risk and to avoid a clinical hold. The route of dose administration, delivery device, dosing regimen, target population, characterization specifications, potency, and stability of the potential vaccine product 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 European 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 not to be overlooked that are equally important to the successful execution of a development program are the budget and schedule. Although these two items are somewhat controllable, they often constitute important metrics for measuring program progress and success. A schedule with a list of tasks and key milestones is recommended with a go/no-go decision to be made at each milestone. For a conservative and risk-adverse approach, tasks or studies could be initiated in sequence rather than in parallel. Doing so would lengthen the overall duration of the nonclinical development program but allow adequate time for data review and interpretation before initiating the next set of task(s). This could also result in a slower cash-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 development tasks in parallel can be riskier, this approach is frequently taken for nonclinical programs to accelerate the development process. To minimize risk, reviews with go/no-go decisions and an exit strategy should be in place for this approach. It is strongly recommended that the development team include an experienced Project Manager to focus on the timeline, financial monitoring and project planning.

1.4 TRANSITION FROM DISCOVERY TO DEVELOPMENT

Identification of a vaccine candidate is a multi-step process that involves immunogen design, screening of candidates, dose range-finding studies, vaccine platform selection, determining the utility of adjuvant (or not), route of administration, evaluation *in vitro* and *in vivo*, and vaccination schedule. Production of the immunogen is usually performed at small-scale in the research laboratory for proof-of-concept *in vitro* and *in vivo* studies. Once the preliminary data are evaluated, a lead candidate is selected, dose and vaccination schedule determined, and IND-enabling toxicology studies are planned. These studies require a larger quantity of test material, which in turn necessitates development of acceptable and reliable production methods. Manufacturing processes require substantial oversight and evaluation, and the processes are likely to be modified during the development cycle. Extensive nonclinical and toxicology studies are performed to evaluate safety, reactivity, tolerability, and efficacy before consideration of the product for human clinical testing.

Figure 1 depicts the development steps taken in developing an investigational vaccine for human Phase I clinical trials.

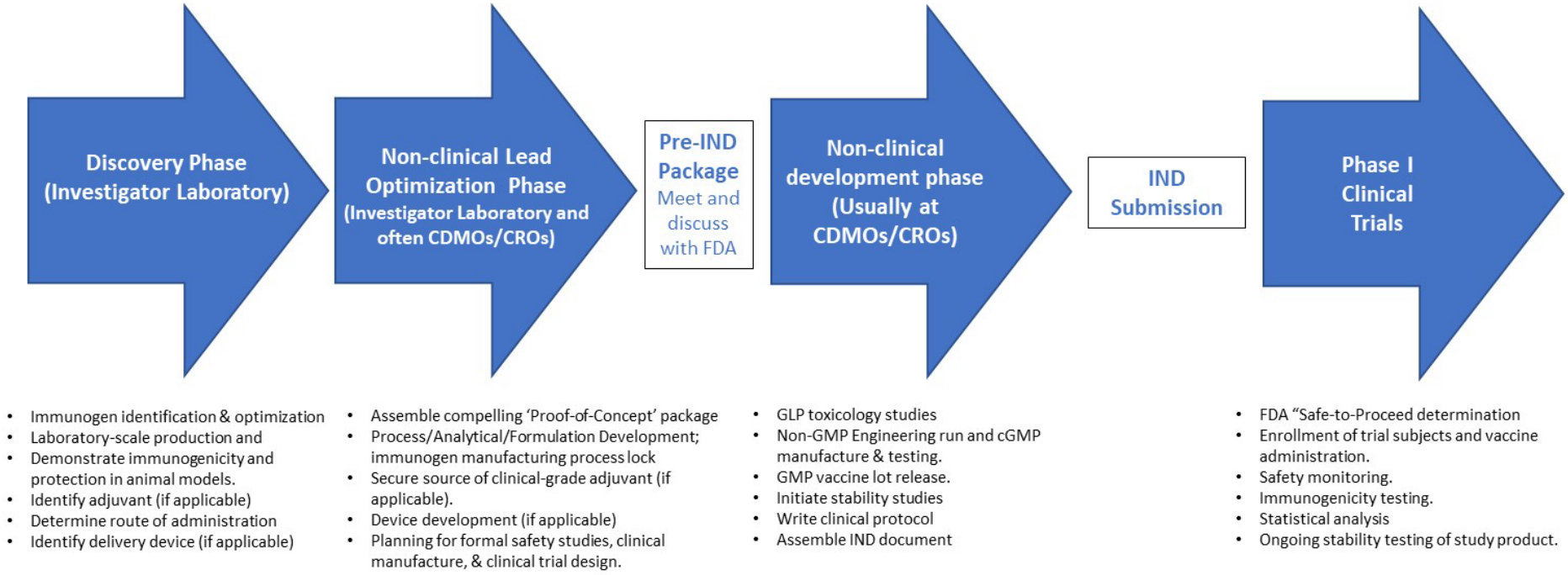

Figure 1. Non-clinical development pathway for a prophylactic vaccine. This figure summarizes key steps taken in development of a prophylactic vaccine from the immunogen discovery in the Investigator's laboratory thru Phase I clinical trials. The activities highlighted in the figure are not all-inclusive but are meant to illustrate the range of activities and disciplines involved. The Research Investigator should be prepared to support the project as the scientific subject-matter expert throughout the development process.

1.5 LAWS, REGULATIONS AND GUIDANCE GOVERNING PROPHYLACTIC VACCINE DEVELOPMENT

In the US, FDA laws, policies, and procedures are designed to ensure that drugs and vaccines are safe for their intended use. Vaccine products intended to induce immunity via prophylactic immunization are regulated by the FDA's Center for Biologics Evaluation and Research (CBER). CBER has authority for the regulation of prophylactic vaccines and other biological products. Its legal authority derives primarily from Section 351 of the Public Health Services Act (PHS Act) and certain sections of the Federal Food Drug and Cosmetic Act (FD & C Act). The statutes of the PHS Act are implemented through regulations codified in the Code of Federal Regulations (CFR) which is published annually. Regulations that are specifically applicable to vaccines and other biological products are contained in Title 21 of the CFR, parts 600 through 680 (https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-600). To protect human subjects enrolled in clinical trials, FDA has issued regulations that govern Investigational New Drug (IND) products, and these are contained in 21 CFR §312. (https://www.ecfr.gov/current/title-21/chapter-I/subchapter-D/part-312) 21 CFR 312.21(a) describes Phase I investigational trials.

Guidance documents issued by the FDA regarding nonclinical IND-enabling toxicology studies, manufacture, assay and testing, and clinical evaluation of vaccines and other biologicals do not have the force of law but are intended to provide recommendations that are current with areas of rapidly progressing science, and for specifying a degree of detail beyond what is included in the regulations. These documents are available at CBER's website. https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/vaccine-and-related-biological-product-guidances. Familiarity with these regulatory practices is essential for successful vaccine development. Also, consult with regulatory experts to ensure your development plan is in compliance with the most recent regulations. Further specific information related to pre-IND and IND processes and submissions are provided in Section 6.0.

1.6 INTELLECTUAL PROPERTY CONSIDERATIONS

Obtaining intellectual property (IP) early in vaccine development is critical. Therefore, an IP attorney should be engaged in the project in the early vaccine development stages to help define and protect ownership rights, and the team should have access to legal support at all stages of the process.

Approaches are varied but typically the focus is on composition of matter and/or method of use patents. IP is a fundamental component for the eventual commercialization of a vaccine product. Without some proprietary position, the vaccine candidate is at high risk of never being marketed. For example, intellectual property related to lipid nanoparticles (LNPs) for mRNA delivery is currently a highly litigated area, with many cases in their very early stages. The outcome of these cases will affect the evolving mRNA vaccine landscape. Another example includes limited access to specific adjuvants (see below).

Intellectual property issues also can arise related to additional patented components included in the vaccine product, platforms, and devices for delivery, such as adjuvants and LNPs for mRNA delivery. Ideally, discussions with the providers or owners of these technologies should occur as early as possible in the process to negotiate mutually acceptable license provisions. A commonly used path is to obtain permission to used licensed material and processes for R&D work and then another set of permissions for further development through non-commercial Phase I and II clinical trials. If the candidate is successful, a final agreement would need to be obtained for Phase III and commercialization of the vaccine candidate. There may be multiple collaborators involved in taking a vaccine to commercialization, and the more parties there are with an ownership interest in the product the more challenging it will be to commercialize.

Ownership of IP also may be controlled by law, by employment agreements, or by agreement of parties. The Bayh-Dole Statute (35 U.S.C 200) permits universities, non-profit organizations, or businesses receiving federal funds to pursue ownership of an invention rather than requiring inventors to assign the patent to the federal government; however, there are reporting requirements for inventions and patent activities arising from federally funded research projects. Under Bayh-Dole, the federal government does retain the right to license their inventions to additional companies if the original licensee is not making good-faith efforts to develop the technology into a usable, real-world product. For university spinout companies, the university will typically license the intellectual property to the spinout company to develop

and commercialize a vaccine. The allocation of rights in intellectual property arising from research sponsored by government, industry, or other external organizations will typically be governed by the terms of a written agreement between the institution and the sponsor. Identifying risks in engagements with third parties and including IP language in agreements at the outset is critical to limiting future challenges over ownership.

2.0 DEVELOPMENT PLAN

The immediate goal of any vaccine R&D (research and development) project is to develop a compelling data package that the vaccine candidate of interest is safe and has demonstrated evidence in stimulating/inducing immunological responses that result in protection against the target disease. This may be demonstrated through analysis of correlates of protection, protection against disease challenge, and/or in conjunction with increased immunological responses while not causing substantial and/or significant safety concerns in animal studies. When sufficient evidence of vaccine effectiveness has been established, a development plan is prepared with the objective of submitting a future IND application with regulatory authorities to secure acceptance by the FDA in initiating a Phase I human clinical trial with the vaccine lead candidate.

A typical development plan to produce Phase I clinical trial material consists of six major efforts:

- Non-cGMP (current Good Manufacturing Practices) manufacture of the vaccine, first in small or limited quantities for early *in vitro* and *in vivo* evaluation studies in which the manufacturing details of each material source and procedure used are well-described and documented
- Pre-formulation and formulation development (dosage design)
- Analytical methods development and validation
- If desired, evaluation and selection of a delivery device other than the standard of care needle and syringe method
- GLP animal toxicology/safety testing
- cGMP manufacture of the vaccine product.

Advancing the candidate from research to product development usually requires implementing a more rigorous manufacturing approach to obtain larger quantities of vaccine test material. Consideration should thus be given to a manufacturing process that is amenable to scale-up to allow the desired quantity of vaccine to be made at a reasonable cost, given the intended application and target population. The final product used for human clinical testing must be manufactured under strict quality guidelines that meet cGMP manufacturing standards. This topic is discussed further in PDP sections 2.3 and Appendix A.

The IND application is essentially a compilation of the results of all the above activities. These are not isolated, individual activities but are intertwined with each other. The interplay of the various tasks involved in the broader effort is critical to completing a timely and cost-effective development program with effective go/no-go decision points.

No acceptable development plan can be drafted without reasonable knowledge of the anticipated clinical protocol. To draft an appropriate non-clinical plan, the design of the first-in-human Phase I trial is critical because toxicology studies undertaken must satisfy the FDA's Good Laboratory Practices (GLP) standard and must mimic, at a minimum, the initial intended human use to demonstrate safety in animals. Initial non-clinical pilot dose-range-finding studies can be performed to establish preliminary results that will be useful for the definitive GLP studies. Information on optimal vaccine dosage, route of administration, delivery device, dosing frequency and immunogenicity from efficacy or effectiveness studies are important to establish the basis for the toxicology safety studies. Without exception, developers should plan for a pre-IND meeting with the FDA to obtain some assurance from the agency that it considers the intended GLP toxicology studies acceptable in relation to the intended initial clinical use. This meeting should take place after the first immunogenicity and dose range-finding studies and before the initiation of the definitive GLP toxicology studies. It is also advised that the planned cGMP manufacturing methods, testing methods, and release criteria be shared with the FDA to gain their acceptance of the overall

approach. They may require additional information and/or additional testing. The intent of the pre-IND discussion with FDA is to increase the likelihood of success in the acceptance of the IND submission by the FDA and that initiation of the Phase I human clinical trial or first-in-human (FIH) trial will advance forward smoothly.

Figure 2 outlines the interconnected steps in the non-clinical development process, including the manufacturing, safety, and regulatory activities required for IND approval and initiation of a Phase I clinical trial. For products that will use a device other than the standard of care needle and syringe method, device-vaccine compatibility and vaccine stability studies will need to be integrated in this process. More on the topic of delivery devices is described in Section 3.2.3.2.

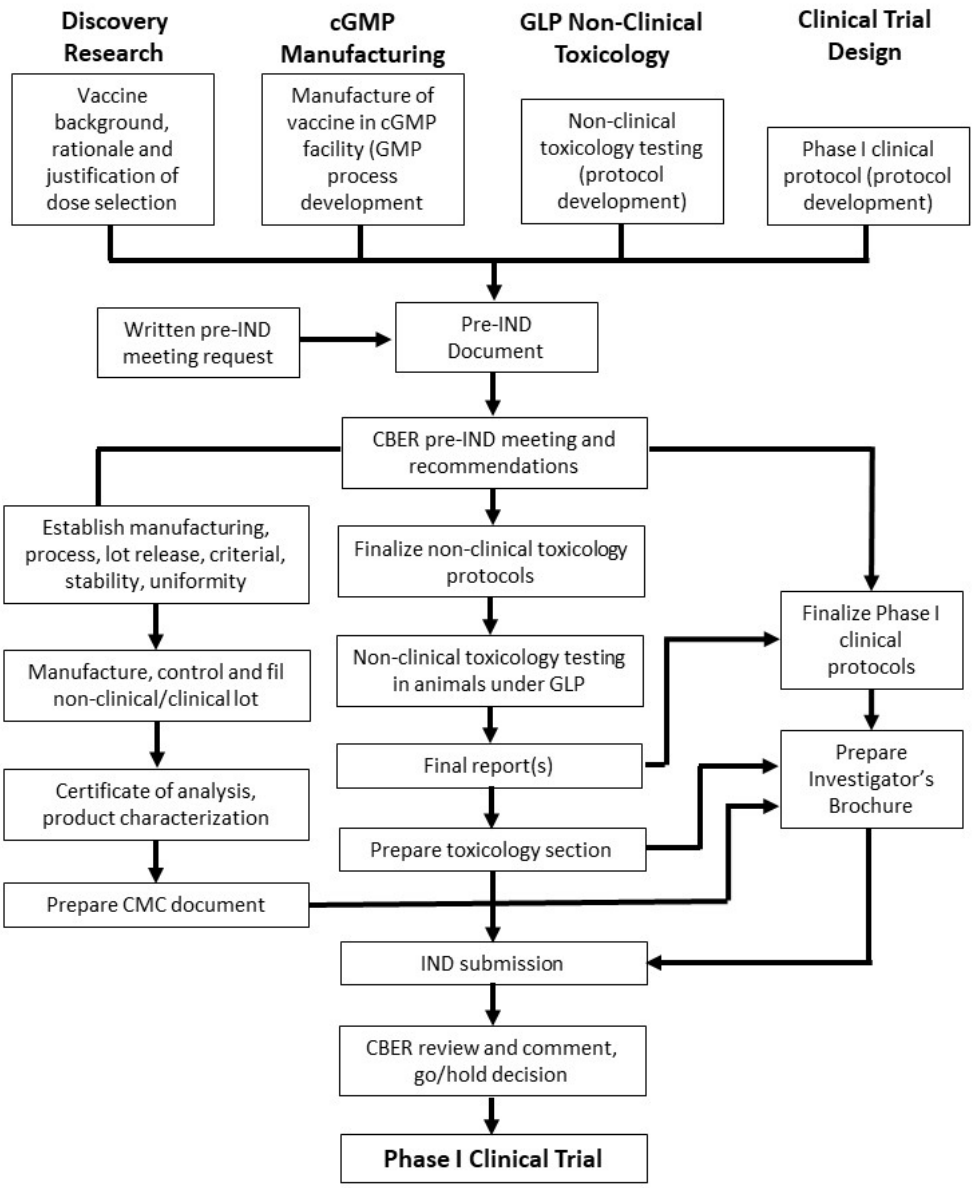

Figure 2. Integrated vaccine development pathway. This figure shows a schematic depiction of integration of Discovery/Research including proof-of-concept studies, cGMP manufacture, GLP non-clinical safety or toxicology testing and clinical trial design toward development of initial regulatory package (pre-IND package) and gain endorsement from FDA on the future development pathway to FIH clinical trial. The various steps and activities required for vaccine development leading to Phase I clinical testing are highly interconnected and complex. The Research Investigator should be intimately familiar

with the individual steps as well as the overall process, and be prepared to advise and support a large, multi-disciplinary project team.
(Adapted from Chang et al., Toxicity Testing of Vaccines, Chapter 14, in *Nonclinical Drug Safety Assessment: Practical Considerations for Successful Registrations*, Edited by William Sietsema and Rick Schwen. Publisher: FDA news, Washington, DC: Fall Church. (2007).

2.1 INDICATION AND PATIENT POPULATION

The target disease indication of the vaccine is usually identified during discovery-phase testing. Thought should be given to the patient population during development so that appropriate and relevant animal models are used for safety evaluation. The findings during non-clinical development may affect dose regimen and duration of clinical trials so careful consideration should be given to experimental designs during early phases of vaccine development.

Using the non-clinical findings, clinical studies are designed first to evaluate safety (Phase I clinical trial testing), usually determining the highest dose that can be safely given and looking for immunological responses. The Phase I safety studies are normally performed using a relatively small number of healthy adult volunteers. If the Phase I trial findings are satisfactory, testing of the candidate vaccine can proceed to larger Phase II trials, which includes controlled clinical studies in a limited number of patients that are expected to benefit from the vaccine and to determine the common short-term side effects and risks. If the Phase I and Phase II trial results appear promising, a Phase III trial is then conducted involving a larger number of subjects who could benefit from the vaccine. The Phase III studies are intended to gather additional information to evaluate the overall benefit-risk relationship of the vaccine and to provide an adequate basis for physician labeling. Successful Phase III trials lead to final FDA approval. For some new vaccines the FDA may require a Phase IV (post-approval) trial to monitor for long-term effects or for use in an expanded population sample.

2.2 GOOD DOCUMENTATION OF TECHNICAL DEVELOPMENT ACTIVITIES

Although there is not specific regulatory guidance describing the degree of documentation for work and studies conducted during the discovery and research phases of vaccine development, it is always wise to keep and maintain clear records on how studies were conducted, accurate sequence information (annotated), animal numbers per experimental group, and other critical findings so should future information be needed, details may easily be found by multiple parties. A common method of record keeping is to generate brief research reports (1-3 pages) that summarize each study (including purpose, experimental design, key findings/results, and preliminary conclusions), and references scientific investigators and location of primary data.

As vaccine development progresses, the rigor of documentation intensifies with greater scrutiny to details about materials, methods, criteria/specifications, and other parameters. This is particularly true for cGMP manufacture. For example, the most important documentation to support future clinical development relates to the genetic construct and host cells used as a basis for manufacturing. Any physical (e.g., source materials) or characterization information relating to the cells or genetic construct should be documented in a report that closely matches the information that would be required in the CMC section of an IND filing. The FDA eCTD application template contains a comprehensive list of CMC topics for inclusion in the IND. (https://www.fda.gov/media/150309/download). Human-origin or animal-origin materials used in the genetic construction or host cell handling carry risk of transmitting viruses or transmissible spongiform encephalopathy agents to eventual vaccine recipients. For these materials (e.g., serum or bovine trypsin), the supplier should provide certification of the country of origin, and the purchaser should record lot numbers and receipt dates. In cGMP manufacture, traceability of materials (such as key raw materials, analytical reagents, reference standards, control samples, etc.) is critical, thus the recording of Vendor, catalog number, lot numbers, and dates all become very important.

Developmental reports should contain detailed experimental results, discussion, abstract and summary that will provide information leading to confidence in the validity and repeatability of the experiments. Fundamental to cGMP manufacture is consistency and reproducibility of the manufactured vaccine including starting materials and methodology.

Likewise, it is strongly recommended that a technical report be prepared to document the preparation and characterization of materials used in GLP IND-enabling safety and toxicology studies, particularly if the

study is performed using non-GMP vaccine substance or vaccine product. The sponsor will need to justify the comparability of these non-GMP materials to vaccine materials prepared later under cGMP.

2.3 CHEMISTRY, MANUFACTURING AND CONTROLS (CMC) OVERVIEW

Chemistry, Manufacturing and Control (CMC) encompasses all of the activities required to produce and test the vaccine product in a form suitable for human clinical testing. Manufacturing and controls for vaccines are presented in the *Guidance for Industry - Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product* (January 1999). This document provides detailed guidelines for the manufacture of a vaccine product. In addition, FDA issued a draft guideline titled *Characterization and Qualification of Cell Substrates and Other Biological Starting Materials used in the production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases* (September 2006). This is intended to supplement recommendations provided by the following ICH documents*: Q5A, Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* and *Q5D, Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products*. It is recommended that these ICH documents, as updated, be used when a vaccine is to be manufactured.

The FDA document recognizes that the number of different cell substrates used in currently licensed vaccines is limited and that there is the need for development of new vaccines as new infectious diseases emerge or in consideration of agents for bioterrorism. Therefore, with limited experience with new cell substrates, FDA recommends manufacturers develop and apply the best technologies to ensure that they are safe. General issues that could affect safety and purity of vaccine products should be evaluated. Examples of such issues include karyotype and tumorigenic phenotype of the cell substrate, the identity and genetic stability of the cell substrate and virus seeds, and the requirement that the vaccine product be free of extraneous infectious microorganisms and potential oncogenic agents.

Qualification includes an assessment of the starting material to demonstrate it is safe for use in manufacturing a final product. This assessment includes:

- History and general characteristics of the cells
- Cell banking system
- Characterization of the cells through Quality Control (QC) testing.

Details regarding establishing viral seed banks or cell banks (characterization and qualification) are provided in the guidance documents referenced previously in this section.

3.0 MANUFACTURING

3.1 GOOD MANUFACTURING PRACTICES (cGMP)

Current Good Manufacturing Practices (cGMPs) are a system of control, monitoring and reporting designed to assure that a drug or vaccine product, whether investigational or commercial in nature, meets minimum FDA-prescribed quality requirements and is safe for its intended use. The full term ‘cGMP’ is typically used to describe a policy or broad implementation of quality standards. For example: “Clinical trial material must be manufactured under cGMP”, The shortened term GMP is usually used in reference to a specific activity or material. For example: “The first GMP lot of vaccine immunogen was used to manufacture the clinical trial material”.

cGMP standards are not prescriptive instructions on how to manufacture products, but rather are a series of performance-based requirements that must be met during the manufacturing process. cGMP is in place to assure that products are consistently produced and controlled according to FDA quality standards. In essence, when all documentation related to a single batch of product is reviewed, one should be able to reproduce that product from the documentation, if followed and the product is ensured to have the proper identity and purity. In general, each manufacturer involved in producing the vaccine bulk drug substance (DS) material (also known as bulk product) and final product must follow cGMP controls (detailed in 21CFR§(s) 210, 211 and 600 applicable to the specific manufacturing situation. The cGMP controls should be appropriate for the phase of clinical development. The FDA is moving to a “risk-based” regulatory approach, recognizing that the degree of cGMP compliance and control required for a

commercial product may not be reasonable for a Phase I study (see, for example, the FDA's *Guidance for Industry: CGMP for Phase 1 Investigational Drugs* [2008]). In keeping with the risk-based approach, aspects of cGMP related directly to product safety such as assurance of aseptic processing and product sterility remain fairly stringent and must be validated prior to manufacture of clinical trial material. The following areas of control apply to the manufacture of a Phase I investigational product.

1. Personnel should be experienced and trained for their assigned function.
2. Quality control functions should be established to define responsibilities (e.g., examination of materials used in production, approval of manufacturing and testing procedures, release or rejection of each batch of drug, investigation of unexpected events or results).
3. Adequate manufacturing and testing work areas and equipment should be available.
4. Environmental control of manufacturing and quality control test areas should be established.
5. Written procedures for acceptance/rejection of manufacturing materials, components, containers, and closures should be developed and available.
6. Written manufacturing and process control procedures, and test methods should be developed and available. Test methods should be qualified or validated appropriately for the phase of clinical development.
7. Laboratory controls and reference standards should be available for testing during manufacturing (e.g., source production materials, in-process material, packaging, bulk and final product) and for laboratory testing after production (e.g., identity, strength, potency, purity, as appropriate).
8. Real-time and accelerated stability testing of representative samples for quality and product characteristics should take place for a period of time of which the duration should be at least through the clinical trial period (i.e., the date of manufacture through the date of the last vaccine administration).
9. Packaging, labeling, and distribution control should have established written procedures.
10. Records documenting the manufacturing process and quality control testing and procedures should be kept.
11. Investigation and resolution of any testing or GMP compliance issues should take place before release of the clinical product. A written and preapproved policy should describe procedures for retesting in the event of an out-of-specification result or invalid test.

For new vaccine developers advancing an investigational product into Phase I clinical trials, manufacture of clinical trial material will usually be performed under contract by a CMO (contract manufacturing organization) under full cGMP control. The research investigator does not typically have the appropriate quality and documentation systems that are expected to conduct manufacturing-related work under cGMP. Note that the level of cGMP compliance is on a sliding scale and as clinical development advances from Phase I clinical testing to Phase III testing, the rigor and control systems required become more stringent. However, early in the development program the research investigator should be prepared to provide key information or reagents which may be needed to perform and support the manufacturing process. Common examples include analytical reagents, analytical methods, research-stage production protocols and samples of purified vaccine for use as initial reference material in development studies.

Several important regulatory guidance documents relate to the manufacture of vaccine products for clinical development and are referenced in the following sections. See Appendix A for additional information, with a focus on management of outsourced CMC (Chemistry, Manufacturing and Controls) activities in support of a vaccine development program.

3.2 MANUFACTURING TECHNOLOGY AND VACCINE BULK DRUG SUBSTANCE

3.2.1 General Considerations

Quality and purity of the vaccine bulk drug substance (DS; bulk material prior to final formulation and vialing) are related to proper control throughout the manufacturing process. Control is attained through the following:

- Appropriate quality and purity of the starting material, including the seed organisms and reagents
- In-process controls (testing) for intermediates
- Maintaining the process within parameters known to produce an acceptable product (these parameters will be reflected in formal process validation as a prerequisite to product licensure for general use)
- Testing and Quality Assurance (QA) release of bulk substance for further processing

Areas of manufacture used for producing cell banks and products should be controlled to prevent contamination or cross-contamination from other sources. QA-approved SOPs should be available to support the manufacture and testing of the vaccine.

The FDA guidance titled *Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product* (January 1999), provides a good overall description of the various methods used for production, including cell growth and harvesting, purification, and procedures to use to ensure containment and prevent contamination or cross-contamination.

Analytical testing is performed to show the identity, purity, potency, physical-chemical measurements, and stability of the vaccine bulk substance. During the research phase of the project and in preparing for cGMP manufacture, test batches should be produced to gain manufacturing experience and create reference materials for analytical development, formulation development and preliminary assessment of stability (ideally at GMP scale or 'pilot scale' using scale-down models which accurately reflect performance of the full-scale process. Test results obtained from these test batches will be used to develop specifications used for formal release of the GMP bulk DS and eventual clinical use. Impurity profiles should be assessed during process development for determination of lot-to-lot consistency; a subset of these such as host cell protein and host cell DNA levels, or chemical additives (residuals) used in the process, will need to be applied as release specifications. The vaccine bulk DS is typically the best place to perform required tests for adventitious agents and most of the potential process-derived impurities, as they would be present in higher concentration compared to the final product. This can allow addressing any results of concern before committing to final product manufacturing.

Reference standards representative of the planned GMP vaccine DS and final vaccine DP formulation should be prepared and thoroughly characterized. All data, including results of relevant *in vivo* and in vitro biological testing of the reference material, should be documented to ensure continued availability as the product develops over time.

It is often useful to manufacture an excess of bulk DS, which can then be used in future months or years to manufacture final vaccine product for a number of clinical trials or using different product concentrations or formulations. The quality of the DS substance after this hold step should be supported by long-term formal stability studies. Small scale samples should be prepared with the same container/closure materials, surface to volume ratio, and storage conditions as the actual released vaccine material.

3.2.2 Technologies for Vaccine Delivery

A variety of technologies have been used for manufacture of vaccines, and the feasible options have expanded considerably in recent years. The vaccine developer will need to adjust the production, quality, safety, and regulatory approaches to match their chosen technology; more novel approaches will involve greater regulatory scrutiny. In particular, the use of a novel cell line or vector in manufacturing, or a novel adjuvant, will require a good deal of supporting safety data and rationale for use. The main categories of vaccine technologies are listed here along with related considerations. These technologies may also be used in combination.

A. Killed Vaccines

A traditional, but straightforward approach to vaccination is to culture the target pathogen, inactivate by some method to avoid causing disease, and then administer. With this approach, the banking and production systems may involve handling of a pathogen requiring appropriate containment and safety measures (e.g., BSL2 or BSL3) prior to the inactivation step. The inactivation step itself will require

validation to avoid accidental transmission of disease, along with testing to determine residual amounts of inactivating agents in the final DS. If culture of the particular pathogen at scale is a novel activity, extra effort may be needed to develop a reliable manufacturing process and testing approach, and there may be fewer vendors able to support the project. Killed vaccines may also require use of an adjuvant to boost the immune response.

B. Subunit Vaccines

If immune response against one or more pathogen proteins is sufficient for protection, it is often advantageous to purify the antigen(s) in order to provide good product consistency and performance while enhancing safety. The protective antigen may depend on a particular glycosylation pattern or other post-translational modifications to maintain the correct structure; in this case the host cell line and characterization approach should reflect this feature. In other cases, an unmodified amino acid sequence may be sufficient, which allows a broader range of expression systems. Modern subunit vaccines are usually manufactured by recombinant expression of the antigen(s) of interest. However, older technologies, such as culturing the target pathogen on a suitable cell line or in embryonated chicken eggs followed by "splitting" (chemical disruption) and purification are still in use for some vaccines. For example, most currently marketed influenza vaccines are produced in this way. Adjuvants are frequently employed since purified protein subunit immunogens usually do not prime a potent immune response when administered on their own.

Protein antigen vaccines are amenable to a variety of detailed characterization techniques that have been developed for biological products, such as amino acid sequencing, native and reduced gel electrophoresis, Western blot, peptide mapping with LC/MS (liquid chromatography/mass spectrometry) identification of fragments, etc. Methods to assess three-dimensional structure are also routine. These methods provide good assurance of product quality, and product stability studies can often determine a correlation of potency loss with a particular mechanism such as aggregation, misfolding or chemical modification of particular amino acid residues. These activities add some expense but can provide assurance of quality and comparability when scaling up or changing manufacturing methods, without resorting to complex biological methods or even repeating clinical studies. If a recombinant DNA construct is used to manufacture a subunit vaccine, the construction method should be described in detail, a plasmid or vector map included, and the inserted gene should be completely sequenced. Early-stage investigators should fully document the construction of genetic elements for recombinant expression and ensure that no animal-derived components are used if the construct will be used for cGMP manufacturing of clinical trial material.

C. Live Vaccine (attenuated or vector)

A number of important vaccines are based on administration of a live pathogen that has been attenuated in some way, so that a protective immune response is achieved without causing disease. Vaccine development in this case will depend heavily on animal and clinical studies to demonstrate vaccine safety. Characterization of the vaccine should include direct assessment of the structural features leading to attenuation if this is known (e.g., deletion of a gene leading to virulence). If a vector is used, guidance documents describing the characterization of recombinant vectors should be followed.

It may not be possible to develop and qualify production methods that reliably purify the desired live virus away from other potential contaminating viruses. Unwanted viral contamination was found in polio vaccine early in its development, and even a recently approved vaccine (developed and characterized with modern methods) was found to have a xenotropic virus contaminant in some batches. For live vaccines, it will be particularly important to control and characterize sources of contamination, e.g., adventitious agent testing for the host cell line and viral seed stocks, and avoidance or tight control of animal-derived biological materials in the process such as serum and trypsin. Live virus vaccines usually do not include adjuvants.

D. Virus Like Particles

Virus-like particles (VLPs) are a subset of subunit vaccines which mimic the structure of a viral pathogen. VLPs lack the viral genes that allow replication or cause disease and can present antigens to the immune system in a format likely to generate a protective response. VLP's are typically based

on recombinant antigen expression and may use a variety of expression systems including bacteria, yeast, mammalian, or insect cells.

As with other recombinant vaccine approaches, the expression cell line should be characterized reflecting current guidance documents. Characterization methods for should include assessment of the VLP's structural features. If the construct has the ability to form a VLP either with or without the target antigen, then the proportion of antigen-containing VLP's should be characterized. As with live vaccines, it may be difficult to rely on separation or inactivation methods to reduce the risk of viral contamination arising from the host cell line or other routes. There may also be a possibility of carry-over of host cell protein or DNA due to entrainment or adsorption to the VLP's. Like other subunit vaccines, VLP vaccine formulations often include immune stimulating adjuvants.

E. Genetic Vaccines

Genetic vaccines generate a protective immune response by introducing genetic material to the vaccine subject, which then expresses the protective antigen *in vivo*. The primary technical approaches are plasmid DNA vaccines, mRNA vaccines, and viral vector vaccines. As with previously mentioned approaches involving recombinant expression, the genetic construct should be created and characterized following relevant guidelines. Developers may also be asked to characterize the biodistribution and persistence of the administered genetic material, in order to fully understand the duration of antigen delivery or any possibility of DNA or viral integration that could lead to cancer risk.

1. **Plasmid DNA.** DNA vaccines deliver naked plasmid DNA constructs expressing vaccine antigens of interest. Formulations often include the use of a liposomal lipid carrier or adjuvant in order to increase the efficiency of transfection and antigen expression in subject tissue. Another promising approach in clinical development involves electroporation. Testing of DNA plasmid for vaccine delivery is complex, and a variety of special characterization approaches have been developed to assess issues like plasmid fragmentation and supercoiling. Parental bacterial strains and production seed stocks for cGMP manufacturing (usually *E. coli*) must be thoroughly characterized and documented. Plasmid DNA vaccines sometimes include an adjuvant.
2. **Recombinant Viral Vector Vaccines.** Viral vector vaccines generally employ a virus known to be benign in humans. Vectors are typically modified to be replication-incompetent, or capable of only one or a limited number of replication cycles. Like other live vaccines, recombinant viral vector vaccines usually do not employ an adjuvant. Developers should consider the potential effect of native or vaccine-induced anti-vector immune responses on the efficacy of the vaccine, particularly if multiple doses are needed to accomplish a protective response. Anti-vector responses can greatly diminish the ability of the vectored vaccine to accomplish transfection and antigen expression. Examples of viral vectors commonly used for recombinant vaccine delivery include Vesicular Stomatitis Virus (VSV) and various types of adenovirus (Ad)). Certain considerations for use of recombinant viral vector vaccines may overlap with those already cited for live virus vaccines, notably the need to characterize viral persistence and biodistribution, as well as having a full understanding of the history and purity of the vector and the potential complications in purifying the vaccine virus away from contaminating viruses.
3. **mRNA Vaccines.** Use of *messenger* RNA (mRNA) for vaccine delivery is a relatively new technology which grew out of work on delivery of siRNA (small interfering RNA) therapeutics. Unlike plasmid DNA vaccines, which must enter the host cell nucleus to initiate antigen expression, mRNA vaccines need only pass across the plasma membrane and into the cytosol for expression. However, mRNA is highly susceptible to degradation *in vivo* and therefore must be delivered in a formulation which protects it from extracellular host nucleases but releases the mRNA for antigen expression upon uptake into host cells. This is usually accomplished by packaging the mRNA into the lumen of lipid nanoparticles (LNPs) composed of a polyethylene glycol (PEG) lipid, a phosphatidyl choline (PC) helper lipid, cholesterol and, most importantly, an ionizable lipid which mediates transit across the plasma membrane following uptake into acidified endocytic vesicles. There are two general mRNA design approaches for vaccine delivery. Standard mRNA delivery utilizes a single-stranded mRNA with 5' and 3' non-coding regions, a 5' cap and a 3' poly-A tail. Self-Amplifying mRNA (SAM) constructs incorporate the immunogen coding sequence into an mRNA virus replicon containing genes for intracellular

amplification of the mRNA, thus substantially reducing the required dose of mRNA. The mRNA component of mRNA-based vaccines is immunostimulatory, thus making mRNA vaccines "self-adjuvanting". Thus, heterologous adjuvants are not usually used.

A limiting factor for many government, academic and small biotech vaccine developers interested in mRNA vaccine delivery is restricted access to mRNA-LNP technologies due to intellectual property constraints. mRNA design – codon optimization and optimization of 5' and 3' non-coding regions – is critical to maximize expression of the antigen *in vivo* and promote strong immunogenicity. However, effective designs are usually patented or closely guarded information, leaving new mRNA vaccine developers to engage in lengthy design and optimization studies. Currently licensed mRNA vaccines also incorporate proprietary modified nucleosides such as n1-methyl pseudouridine to modulate the innate immune response against the mRNA. Finally, the compositions of ionizable lipids for use in the LNP delivery vehicle are proprietary. New vaccine developers interested in mRNA technology should be prepared to look for collaborations or partnerships with established mRNA delivery experts (academic laboratories or biotech companies) or seek out academics or companies that are willing to advise on and out-license the mRNA and LNP technologies on a fee-for-service basis.

F. Conjugate Vaccines

Conjugate vaccines are a type of subunit vaccine in which the antigen is a bacterial capsular polysaccharide. However, unlike protein antigens, purified polysaccharides in most cases generate only weak humoral immune responses due to the absence of helper T (T_H) epitopes. Therefore, immunogenicity is boosted by chemical conjugation of the polysaccharide to a protein providing T_H epitopes, such as an inactivated bacterial toxin. Like other subunit vaccines, most conjugate vaccines are formulated with an adjuvant to further boost the immune response. A number of important conjugate vaccines have been developed to protect against diseases caused by Haemophilus influenzae type B (Hib), pneumococcus, and meningitis bacteria. However, conjugate vaccines are technically complex. Scientists and vendors with directly relevant experience should be engaged in the development of new conjugate vaccines in order to avoid pitfalls related to production, testing/characterization, and formulation.

3.2.3 Vaccine Product

The final vaccine product is normally prepared by suspending the vaccine bulk in a formulation buffer usually composed of inactive ingredients. Special formulations may also be developed, and their complete composition must be defined. All ingredients used (composition and quantity) for producing the final vaccine product must be documented. If compendial excipients are used, citing a reference is an adequate substitute for testing. The composition and quantity of all adjuvants and preservatives added to the product should be defined; components that are critical for product quality or have potential safety issues should generally be tested in the final product in order to verify proper manufacturing.

The final vaccine product may be lyophilized or liquid stored under controlled conditions. The processing steps, container, closure, and storage conditions should be identified. The final testing of the product will involve methods that assure the identity, purity, strength and/or potency. Tests are performed on the final finished product that certifies it meets established specifications. Results of these tests are reported in a Certificate of Analysis (COA).

This description relates to injected vaccines administered subcutaneously, intravenously, or intradermally; other possibilities include oral and intranasal delivery. In addition to the needle and syringe method traditionally used for injected vaccines, there are many new devices that are approved or in development that will require additional vaccine-device compatibility studies (see Vaccine Delivery device section 3.2.3.2). This description relates to injected vaccines; other possibilities include oral and intranasal delivery. The developer should make sure that any processes or materials employed are compatible with the biological components of their vaccine. Oral vaccines will require control of microbial contamination similar to other oral pharmaceutical products, but this is much less stringent than injected vaccines.

3.2.3.1 Adjuvants and Vaccine Formulation

Adjuvants

Most subunit vaccines, including VLPs and polysaccharide conjugates, require co-formulation with an adjuvant to prime a strong, durable immune response. For many years aluminum-based adjuvants (alum) were the only clinically acceptable adjuvants for vaccine delivery. While having a strong regulatory history and excellent safety record, alum adjuvants often provide only modest stimulation of humoral immunity and poor stimulation of cellular immune responses. However, the last two decades have seen development of many potent new adjuvants with diverse and sophisticated mechanisms of action. The discovery of innate immune activation pathways and associated stimulators, in particular Toll-like receptor (TLR) ligands, has yielded a number of new adjuvants used in commercially licensed vaccines. These include adjuvants based on monophosphoryl lipid A (MPLA, a TLR4 agonist), CpG DNA (a TLR9 agonist), saponin-based immune stimulators and squalene-based emulsions. Many other new adjuvants are in various stages of research and clinical development.

Early-stage vaccine developers should be aware that effective adjuvant formulations rely not only on the active, stimulatory molecule but on the physico-chemical characteristics of the formulation, as well as interactions with the vaccine antigen(s). In many modern adjuvants the immunostimulatory species (e.g., MPLA) is incorporated into a particle-like structure such as a liposome, nanoparticle or emulsion which aids in trafficking to, and stimulation of, antigen presenting cells (APCs). In these cases, the particulate structure of the formulation is a critical quality attribute to be optimized and controlled. Co-formulation of immunostimulatory molecules with alum has also been employed to take advantage of the “depot effect” provided by the alum.

Most adjuvants other than alum can be filter sterilized, but some have lipid-like properties and may have a tendency to mix poorly or stick. A test for adjuvant concentration in the final product is generally required. In the case of an alum-adjuvanted vaccine, filter sterilization is performed before alum addition. Alum adsorption and subsequent steps should be performed using strict aseptic handling procedures to avoid contamination. Adjuvant quality, including limitations on particle size, is critical. If adsorption of the antigen to the adjuvant is required, the adsorption step should be performed under carefully documented conditions known to produce the desired degree of adsorption.

Adjuvants are defined as constituent materials in 21 CFR §610.15. These regulations state, “All ingredients…shall meet generally accepted standards of purity and quality” and that, “An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not adversely affect the safety or potency of the product.”

From a regulatory perspective, adjuvants are not considered active ingredients as defined in 21 CFR §210.3(b)(7) and vaccine adjuvants are not licensed separately. It is the adjuvanted vaccine antigen formulation that is tested in nonclinical and clinical trials and eventually licensed. There is a requirement that the adjuvanted vaccine formulation, as with any vaccine, must be both safe and effective, with its benefits outweighing the risks of adverse events. A WHO guideline published in 2013 describes the nonclinical, quality, pharmacological, toxicological, and other information needed to support initiation of clinical trials with a vaccine combined with a novel adjuvant.

Vaccine developers must provide a rationale for an adjuvant in the vaccine formulation. The “added benefit” of an adjuvant may be demonstrated as enhanced immune response, antigen-or dose sparing effect and/or increased breadth of immune response. Information to support the “added benefit” of the adjuvant may be derived from non-clinical studies, for example, *in vitro* assays and/or proof-of-concept studies in animal models conducted prior to the initiation of clinical trials or early phase clinical trials.

Early in clinical development (e.g., Phase I clinical trials), safety data may be derived by comparing the adjuvanted vaccine to a placebo or the unadjuvanted vaccine antigen, if feasible. Safety follow-up of human volunteers administered vaccine with novel adjuvant is typically longer than for nonadjuvanted vaccines (e.g., 12 months rather than 6 months) and includes specific inquiries regarding symptoms consistent with autoimmune and neuro-inflammatory diseases.

Adjuvants with a good safety record and strong regulatory history are preferred for smooth navigation of the regulatory process. Unfortunately, most adjuvants with preferred attributes, particularly those used in

licensed vaccines, are difficult to access due to intellectual property restrictions. Therefore, the early-stage vaccine developer is urged to establish access to a preferred adjuvant early in the development process through appropriate licensing agreements for Phase I/II development with an understanding of later licensing issues if the vaccine candidate progresses into Phase III and commercial development.

Formulation Development

Once a method for the vaccine bulk substance manufacture has been developed, the next major hurdle in a development program is often formulation development. The specific physicochemical properties of the bulk substance will dictate which formulation options are available. A vaccine used in clinical testing needs to have appropriate physical properties and stability. Formulation development is closely related to the type of vaccine drug substance (DS) under development (protein subunit, viral vector, etc.). As an example, a number of sophisticated characterization methods are available for subunit vaccines to guide formulation development (e.g., protein folding and conformation, assessment of protein crosslinks, determination of degradation pathways under accelerated stability testing) that may not be practical for a VLP or genetic vaccine.

The initial DS formulation is usually a minimal composition meant only to keep the material active under storage (usually frozen) until vialing. The final drug product (DP) formulation is generally prepared by thawing the bulk DS material, followed by the addition of excipients and diluents, as necessary, to achieve the target amounts or concentrations in the final vaccine product, and then filter sterilized. Adjuvant, if required, may be added at this stage. Note, however that for preparation of Phase I clinical trial material the immunogen and the adjuvant are often vialed separately, stored frozen at -80^{o}C and mixed immediately prior to administration ("bedside mixed"). This common strategy acknowledges the complexity and resource commitment required for developing stable antigen-adjuvant co-formulations and defers this activity until after Phase I results are obtained and the developer commits to advancing the candidate to Phase II.

In the case of pre-filled devices, the vaccine will be stored in the device or component of the device that will be used for delivering the vaccine. In some cases, additional formulation optimization/development may be needed to enable storage in the delivery device of choice (see section 3.2.3.2 Vaccine Delivery Device).

In the case of an alum-adjuvanted vaccine, filter sterilization is performed before alum addition; alum adsorption and subsequent steps should be performed using strict aseptic handling procedures to avoid contamination. Adjuvant quality, including limitations on particle size, is critical. The adjuvant adsorption step should be performed under carefully documented conditions known to produce the desired degree of adsorption.

Most adjuvants other than alum can be filter sterilized, but some have particulate or lipid-like properties and may tend to mix poorly or stick to surfaces used in processing. A test for adjuvant concentration in the final product is generally required. Adjuvants other than alum have not yet been approved for general use, and developers will typically be required to test their vaccine with and without the adjuvant in order to assess the benefits and risks associated with the adjuvant as opposed to the immunizing antigen.

3.2.3.2 Vaccine Delivery Devices

Most vaccines are administered by a traditional needle and syringe method and delivered either subcutaneously (SC), intramuscularly (IM), or intravenously (IV). However, these methods of delivery require trained providers, sharps disposals and can be limited in their reach due to several reasons including non-compliance related to needle-phobia (1). In addition to the SC, IM and IV route of administration, there are a few examples of vaccines (e.g., for Tuberculosis and Monkeypox) that are delivered intradermally (ID) using a special needle and technique called the Mantoux method; however, this method requires significant training for providers, and is fraught with challenges including imprecise delivery, local reactogenicity, and pain (2, 3). However, recently, there are a growing number and range of alternate devices (pre-filled with vaccine or filled on site) and alternate routes of delivery (e.g., intranasal, oral) that have the potential to enable broader delivery of vaccines with significant benefits.

If an alternate delivery device and/or delivery method is under consideration, sponsors are advised to initiate evaluations of devices for vaccine delivery at the non-clinical stage of development and integrate

alternate devices into the value proposition of their target product profile (TPP). Demonstration of at least comparable immunogenicity and safety relative to SOC (needle and syringe) in a relevant non-clinical disease model and/or in a PhI study is of key importance. Ultimately, proof of comparable immunogenicity with a new device and/or a new method of delivery is critical. Beyond this, demonstration of benefits at different stages of development that meet project needs as outlined in the TPP is also critical. For example, intradermal vaccination can lead to dose-sparing which could be evaluated in a Ph I study, and positive data could translate to lower product needs and potentially a more affordable product for global populations. In addition, social behavioral research can be conducted during Ph I development to evaluate acceptability of using an alternate device relative to SOC, from the perspective of health care providers, patients, and policy makers. More information on this topic can be found in *Human Factors Studies and Related Clinical Study Considerations in Combination Product Design and Development: Draft Guidance for Industry and FDA Staff, Safety and Performance Based Pathway: Guidance for Industry and FDA (September 2019),* and *Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process" Guidance for Industry and Food and Drug Administration Staff Document issued on: September 4, 2020.*

Evaluation of thermostability and vaccine integrity, as part of device-vaccine biocompatibility assessments, are typically one of the initial studies conducted preclinically. For example, some vaccines require ultralow temperatures for cold storage; however, re-formulation of such vaccines as solids into microneedle array patches (MAPs) can yield significant gains in thermostability. However, the extent of reformulation that may be needed to generate a thermostable vaccine as a MAP should also be considered in this risk-benefit assessment. Another study that is of importance for any novel device in development is assessment of delivery efficiency, which is done in a relevant animal model and if needed also in the clinic. This is typically conducted by the device developer or done in collaboration between the device developer and the sponsor. More on this topic can be found in *Format for Traditional and Abbreviated 510(k)s Guidance for Industry and Food and Drug Administration Staff (September 2019)* and *Guidance for Industry and FDA Staff: Early Development Considerations for Innovative Combination Products (September 2006).*

Alternative devices for intradermal or subcutaneous delivery include needle-free jet injectors that can rapidly deliver vaccines in a narrow, high-pressure liquid stream that penetrates through the skin. For example, ID and SC jet injectors from Pharmajet®, which have received clearances from stringent (4-7) regulatory agencies and prequalified by the World Health Organization (WHO), have been successfully evaluated in the clinic with most types of vaccines including live-viral, inactivated, nucleic acid and protein-based vaccines (8-17). Similarly, there are needle-free intranasal spray (or dropper) devices that are utilized to deliver some approved intranasal vaccines (e.g. for Influenza and COVID-19) (18-20). In addition, there are several active clinical studies ongoing with novel and approved nasal devices for intranasal delivery of vaccines for Influenza, COVID-19, RSV, Pertussis (21). In the case of vaccines delivered intranasally by nasal spray devices *in-vitro* characterization of the spray, including particle-size distribution and plume geometry, is recommended. Demonstration of comparability of spray characteristics between a novel device under development and an approved intranasal device is also suggested. Additional FDA guidance on this topic is described in *Guidance for Industry: Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action (April 2003).*

In addition to such needle-free devices, there is a rapidly growing industry of microneedle-based delivery devices, including microneedle array patches (MAPs) for intradermal delivery of vaccines (22-26) . The Vaccine Innovation Prioritization Strategy (VIPS) led by global health organizations UNICEF, BMGF, Gavi, WHO and PATH rated MAPs as “the most promising innovation to help address immunization programmatic challenges and improve coverage and equitable access to vaccines” (27). There are several MAPs in development that are designed with microneedles that are either hollow, coated, or dissolvable, and some have demonstrated benefits of enhanced thermostability of vaccines with equivalent if not superior immunogenicity, relative to standard of care, in the clinic (28-32). A good resource for guidance on MAPs and their utility for vaccines is the PATH Center of Excellence (33). This group aims to help define the regulatory pathway for MAPs to expedite clinical translation of the

technology for vaccines and essential medicines in LMICs[1].

3.2.3.3 Container and Closure, Sterile Fill

The vial and closure components for vaccine products should be suitable for long-term product contact with an injectable product that may be held for multiple years. The components should be washed, sterilized, and transported in controlled areas according to written procedures.

This material should then be dispensed into presterilized vials or prefilled delivery devices in a Class A, ISO 5 environment to avoid particulate or adventitious agent contamination. The room classification and filling procedure should be supported by microbial and particulate monitoring activities, and qualified to meet target levels both at rest and during operation. A sterile fill process validation must be conducted in advance of the GMP fill using sterile bacterial growth media with the same physical components as the GMP batch in order to demonstrate that the sterilization methods, environmental controls, and process steps will reliably produce a sterile product. Further information is available in FDA guidance document, *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practices* (September 2004).

3.2.3.4 Sampling and Control Testing

Sampling of drug product containers is performed by "skip testing"; in other words, units are randomly selected from the manufacturing procedure for sampling. The representative randomly chosen units should be checked for proper fill volume at the start of the filling run and then periodically, typically by weight differential vs. an empty container. Samples taken for quality and stability testing should also reflect a distribution of samples from various fill times. Vials or pre-filled devices should be inspected visually for defects, before and after labeling. An exact count of vials or pre-filled devices and approved labels should be maintained and documented, including those allocated for sampling. Samples allocated for testing purposes will probably exceed the numbers needed for the clinical studies themselves. Sample product vials should then be distributed to quality control test laboratories for the designated release, characterization, and stability studies.

Final product release testing should include repeat testing of many of the tests applied to the bulk drug substance for identity, purity, strength, and potency, as well as new tests relevant only to the final product vials. A typical testing approach for a protein-based vaccine is outlined below:

Purity/safety:

- Sterility
- Endotoxin
- Appearance
- pH
- Osmolality
- SDS-PAGE (native and reduced)
- Particle size determination (if alum-adjuvanted)
- HPLC (size exclusion or ion exchange, with conditions selected to isolate the desired active antigen from impurities)
- Freedom from adventitious agents that could reasonably be introduced during product manufacturing
- Acceptable levels of impurities derived from the adjuvant (if applicable)
- Acceptable levels of process-related and product-related impurities

Strength:

[1] Full guidance under development, anticipated publication date in 2026

- Content per vial (liquid fill volume)
- Total protein concentration
- Total adjuvant concentration (if applicable)
- Proportion of protein bound to the adjuvant (if applicable). Potency:
- Product specific biological assay. If no mechanistically relevant in vitro assay is available, an animal-based assay (e.g., in vivo relative potency) may be needed.

3.2.3.5 Assay Qualification, Verification and Validation

The performance of analytical methods used for product release testing must be rigorously demonstrated, identifying sources of error and characterizing critical performance parameters. Compendial (not product - specific) methods should be *verified* to show there is no interference from the buffer matrix. For early-stage vaccine development (Phase I), full *validation* of product-specific methods is not required. Rather, method *qualification* (sometimes referred to as 'pre-validation') is sufficient. Qualification demonstrates that the assay is scientifically sound, fit for purpose and addresses the following key parameters:

- **Accuracy:** The closeness of agreement between the expected and the observed value.
- **Precision (Repeatability)** Repeated sampling (same analyst, same day) of a defined, homogenous sample determines the degree of agreement among individual test results,
- **Specificity.** Demonstration that assay performance is unaffected by the possible matrices, excipients, degradants and impurities that may in the test sample.
- **Detection Limit.** The limit of detection (LOD) must be quantitated by spiking a control with a known quantity of analyte standard.
- **Quantification Limits**. These are defined as the lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) of the analyte that can be measured with an acceptable level of accuracy and precision; one must also define the acceptable signal-to- noise ratio of the analysis.
- **Linearity and Range**. The range in which the assay result is proportional to the analyte concentration defines the functional limits of the assay.
- **Robustness**. The effects of small but deliberate changes in the assay which provides an indication of reliability during normal operation (e.g., incubation times, concentration of critical reagents)
- For each method a qualification protocol should be prepared which includes acceptance criteria. During execution of a qualification protocol, an approved SOP or ATM must be followed. It is expected that the SOP or ATM will be revised post-qualification. Upon completion of qualification, a report must be written stating clearly if each parameter was met.

Regulatory requirements for assay validation are summarized in *Guidance for Industry: Bioanalytical Method Validation*, FDA CDER, May 2001 (http://www.fda.gov/cder/guidance/4252fnl.htm); USP <1225> Validation of Compendial Procedures; and *Validation of Analytical Procedures: Text and Methodology*, Q2(R1), ICH Harmonized Tripartite Guideline, November 2005. These documents can be used as reference as qualification will include similar key parameters.

3.2.3.6 Stability Determinations

Stability studies are performed to determine the usable product shelf life from the date of manufacture Testing at higher temperatures (accelerated stability) is typically performed in order to gain information on the nature of product degradation or potency loss over time, and directed studies are typically performed to further characterize the product including exposure of drug product to light, to physical manipulation (e.g. shaking or freeze/thaw cycling which could lead to product aggregation), and to temperature excursions outside of the intended storage range. If light sensitivity is observed, a darkened or opaque container maybe needed. Stability testing for an alum-adjuvanted vaccine should include a determination of the bound antigen, and analytical methods that reflect both alum-adsorbed and unabsorbed protein.

FDA has provided the following detailed guidance documents on stability:

- *Stability Testing of New Drug Substances and Products, (ICH Topic Q1A[R2]),* November 2003
- *Guidance for Industry: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* July 1996.

A generous excess of samples should be preserved as part of a GMP manufacturing batch because these will be helpful if retesting, new tests, or additional stability determinations are needed. A rough guideline is that a minimum of 400 vials of Phase I vaccine Drug Product should be reserved for release testing, stability testing and as retains for possible new tests or investigations.

4.0 GOOD LABORATORY PRACTICE (GLP) NON-CLINICAL TOXICOLOGY STUDIES

After clear evidence of immunogenicity and/or immune effectiveness data has been demonstrated, a critical component to vaccine development is the non-clinical toxicology testing of a candidate vaccine for safety. In general, one or more GLP nonclinical toxicology studies will be undertaken to demonstrate convincing safety prior to entry into human trials. The experimental design, selection of animal species, duration and output parameters are best presented to FDA to ensure that the executed study or studies will meet the agency's expectations. Thus, it is common practice to propose an experimental design in sufficient detail to gain input from the FDA. The most common pathway is to request a pre-IND meeting and submit a pre-IND package. One of the key components of the package is the design of proposed GLP non-clinical studies. A number of regulatory guidelines are available to provide general guidance of proposed study designs. These include but are not limited to the ICH's *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceutical; Guidance for Human Somatic Cell Therapy and Gene Therapy* (Topic S6, July 1997); and the World Health Organization's (WHO's) guidance on the preclinical evaluation of vaccines (2005).

In practice, pilot dose-range-finding safety studies in animals are conducted to provide useful toxicological information that will aid in determining the starting vaccine dose levels for the formal GLP IND-enabling toxicity studies. These pilot studies are generally not conducted under GLP. However, all definitive studies must be conducted in qualified GLP-compliant test facilities in accordance with the FDA's *Good Laboratory Practice for Nonclinical Laboratory Studies*, (21 CFR § 58). Good Laboratory Practices (GLP) is a system of control designed to assure the quality and integrity of data generated in non-clinical studies intended to support applications for research or marketing permits for products regulated by FDA. FDA GLP relates to *in vivo* animal studies and oversight of these studies is shared with United States Department of Agriculture (USDA). The intent for the GLPs is to ensure data integrity of the experiment performed. Areas of control under GLP include:

- Characterization, handling and storage of test articles, controls and reagents.
- Test facility management and equipment.
- Study conduct
- Storage of study records and electronic data capture systems.
- Standard operating procedures (SOPs)

In addition, adequate biosafety-level laboratories must be used to protect the personnel conducting these studies. The design of non-clinical study protocols for vaccines is product specific and is generally based on the design of the proposed clinical protocol using the planned dose(s) and dosing regimen as guidelines. In some cases, for vaccines that contain adjuvants, study designs may have to account for assessment of the safety profile of the adjuvant itself. On the other hand, alum-containing adjuvants used in some vaccines have been used in a large number of licensed vaccine products and therefore, it is likely that safety profile of these vaccines can be established by simply showing that that the vaccine is safe with or without the alum-absorption.

Although GMP manufactured vaccine material is the most suitable for the formal GLP IND-enabling toxicology studies, developers will often utilize vaccine material developed and released from a non-GMP demonstration run or engineering run. This can be risky if the engineering run process and testing have not been appropriately characterized and the material has higher levels of impurities than a GMP run.

Determination whether to utilize engineering run material must be based on the confidence in the reproducibility and consistency of the quality, purity, and potency of the vaccine material. The new vaccine developer should be prepared to demonstrate comparability between the engineering run material used for IND-enabling toxicology studies and the GMP clinical trial material. One advantage of using the engineering run material is that it can save time to complete the GLP IND-enabling toxicology studies while the GMP vaccine product is being manufactured in the same timeframe as execution of the toxicology studies.

4.1 ANIMAL MODEL SELECTION

An important consideration in the selection of animal models is their relevance to human use. For vaccine studies, the FDA currently recommends safety studies in one animal species as a prerequisite for the start of clinical investigation (https://cdn.who.int/media/docs/default-source/biologicals/vaccine-standardization/annex-1nonclinical.p31-63.pdf?sfvrsn=e87c28d8_3&download=true). However, because of possible species- or strain-specific differences, more than one animal model may need to be considered for some vaccine products. Rodent and nonrodent species are generally acceptable models for toxicity studies; however, specialized requirements may dictate the choice of species. In general, vaccines designed to elicit prophylactic immune responses against infectious disease are often monitored in rabbits. Rabbits are often considered an appropriate medium-sized nonrodent species of choice due to the vast amount of available historical data regarding the species. Such information is useful in assessing clinical pathology, immunogenic responses and the safety of vaccine products. In addition, since the immunogenicity and pharmacokinetics of vaccines do not directly scale on body mass or surface area, vaccine toxicity can be better evaluated in rabbits because their muscle mass can receive a volume equivalent to a full human clinical dose (e.g., 0.5 or 1 mL). If it is not clear whether or not the vaccine is immunogenic in rabbits, pilot studies to investigate dose ranges, dosing frequency, and immunogenicity should be performed before designing definitive toxicology studies and pre-IND meetings with the FDA.

The number of animals included in each dose group depends on the species selected. Unless the vaccine is specifically targeted for a particular sex, both sexes are generally included in the studies.

4.2 DOSE SELECTION AND PROTOCOL PLANNING

The dosing regimen, the number of inoculations, and the route of immunization of the pivotal GLP study are established based on the effectiveness profile of the candidate vaccine. Such information will also form the basis for planning the clinical protocol. As suggested in the previous section, pilot non-GLP acute single- dose range-finding studies can be conducted to characterize the systemic dose responses and local reactogenicity, monitor mortality, obtain gross observations, record changes in body weight, and generate clinical and macroscopic findings. Initial dose range-finding studies are conducted to provide useful information about a safe starting dose, as well as information about potential toxicities at the highest dose tested.

For studies evaluating more than a single dose, repeat doses are generally given on a schedule that mimics the proposed human regimen, although the schedule can be compressed. Typically, the number of immunizations in the animal study is the proposed number of clinical inoculations, plus one additional dose for an added margin of safety (N+1). The duration of the study will vary depending on the frequency, interval, and number of the doses administered. Accelerated schedules of vaccine immunizations are acceptable to the regulatory authorities; however, sufficient time must be permitted between immunizations to allow for animals to launch an adequate immune response and display any possible adverse reactions

Biological responses to vaccines are sensitive to the mode of delivery and therefore the same route of administration as the proposed clinical trial should be used in the GLP IND-enabling non-clinical studies.

4.3 TOXICOLOGICAL EVALUATIONS

Routine evaluations of the vaccine's safety during the course of the study are required. Some of the parameters that should be included in the GLP-compliant definitive safety study for a typical vaccine that is dosed using the subcutaneous route of administration are described below.

At a minimum, daily clinical or cage-side observation, injection site evaluation, weekly body weight, and weekly food and water consumption should be included in the study design. In addition, it is usually appropriate to include pre- and post-dosing body temperatures and pre- and post-dosing ophthalmologic

observations (the latter are particularly appropriate in studies of vaccines with adjuvants because of previous observations of adjuvant-associated uveitis).

Interim clinical pathology measurements should be considered for hematology, serum chemistry, serology, and urinalysis to identify toxicologic, hematopoietic, and immune function changes. These are particularly appropriate at early times following the first and last immunizations and at the end of the recovery period.

Parameters for clinical chemistry generally include:

- Alanine aminotransferase
- Alkaline phosphatase
- Aspartate aminotransferase
- Albumin
- Albumin-to-globulin (A/G) ratio
- Calcium
- Carbon dioxide
- Cholesterol
- Chloride
- Creatinine
- Globulin
- Glucose
- Lactate dehydrogenase
- Phosphorase
- Potassium
- Sodium
- Total bilirubin
- Total protein
- Triglycerides
- Urea nitrogen

Hematological parameters include:

- Erythrocyte count
- Hematocrit
- Hemoglobin
- Leukocytes
- Leukocyte differential
- Mean cell hemoglobin
- Mean cell hemoglobin concentration
- Mean cell volume
- Platelet volume and count
- Reticulocytes, and cellular morphology

Coagulation parameters include:

- Activated partial thromboplastin time
- Prothrombin time

Vaccine safety studies often incorporate immunogenicity evaluations such as seroconversion rates, antibody levels, and/or cell-mediated immune responses in the vaccinated animals.

Determining the local tolerance for reactogenicity is important if adverse effects due to either the vaccine product or the vaccine formulation occur. Visual inspection and recording of irritation at the immunization site (injection site or mucosal area) during the course of the study, followed by a microscopic histopathology examination of the immunization site, is highly desirable.

For acute toxicity evaluations, animals are generally euthanized 1 to 3 days after the last immunization. Reversibility of possible adverse effects is evaluated in tissues obtained from animals that are euthanized 2 to 4 weeks after the last immunization.

Gross observations are made, and organ weights are taken at necropsy. A suggested list of recommended tissues to be evaluated is included in the WHO *Guidelines on nonclinical evaluation of vaccines* (2005).

In addition to evaluating immune organs such as lymph nodes and associated lymphoid tissues, thymus, spleen, and bone marrow, particular attention should be paid to known target organs. Other organs to be evaluated in the control and high-dose animals include highly perfused organs and the reproductive organs.

Depending on the route of administration, additional organs may need to be examined. Target organs identified at the high dose should be evaluated for at the lower dose levels. If no target organs for toxicity are identified at the acute sacrifice in the high-dose animals, evaluation of the recovery sacrifice tissues may not prove necessary. Nevertheless, a full list of tissues should be collected and preserved, even

when only a select subset are examined histopathologically, to allow for potential future evaluation should safety issues subsequently arise. Biodistribution studies may be required in the case of viral vector-based and nucleic acid-based vaccines, and FDA should be consulted about such studies in the pre-IND meeting.

All study results should be checked by the scientific quality control/quality assurance staff to assure the integrity of the data. The quality assurance department audits the data and reports for compliance with the FDA's GLPs. The final report (or reports) of the GLP study is included in the IND document.

Alternatively, the FDA will review draft unaudited reports with the expectation that no major differences will be noted during the auditing process and that the final audited reports must be available upon request from the FDA within 120 days of the IND submission date.

If clinical trials are to include large populations of males and/or females of reproductive age, reproductive toxicity testing is also required prior to the late phase clinical trials. Reproductive toxicity studies are generally not required for Phase I trials unless the vaccine in question is specifically intended for the treatment of pregnant women.

4.4 SUGGESTED TOXICOLOGICAL STUDY DESIGNS

Suggestions for toxicological study designs concerning the vaccine are presented below.

4.4.1 Single Dose Range-finding Pilot Non-GLP Toxicology Study in Rabbits New Zealand White Rabbits, male (M) and female (F), n = 3 per sex

- Single dose administration
- Daily clinical observations and twice weekly body weights
- Clinical pathology (hematology, clinical chemistry) on Day 3 and/or Day 8
- Blood collected and processed to plasma Days 1, 3, and 8
- Gross necropsy on Day 8, with no tissues retained for histopathologic evaluation.
- Note that vaccine-specific antibody titers are usually not measured since 8 days post-primary immunization is too soon for the animals to mount a significant immune response.

4.4.2 Definitive GLP Safety Studies in Rabbits

Definitive, IND-directed toxicology studies are required to demonstrate the safety of a drug or vaccine before initiation of human trials. All the studies outlined below are required to be conducted under full GLP compliance. These GLP studies must include analytical support to confirm purity, ID, stability, homogeneity, and concentration of dose formulations. There is no official FDA guidance on non-clinical safety studies for prophylactic vaccines, but FDA follows WHO guidelines on preclinical development of vaccines. https://cdn.who.int/media/docs/default-source/biologicals/vaccine-standardization/annex-1nonclinical.p31-63.pdf?sfvrsn=e87c28d8_3&download=true

A description of a typical toxicity study in rabbits is shown below:

- 10 M/10 F per dose group are used for the main study, with a control.
- The route of administration is modeled after the intended route in the clinic.
- If feasible, the highest dose (in absolute terms) to be used in the proposed clinical trial should be evaluated in the animal model. However, the dose is sometimes limited by the total volume that can be administered in a single injection, and guidelines on animal welfare should be followed. In such cases, the total volume may be administered at more than one site using the same route of administration. Alternatively, a dose that exceeds the human dose on a mg/kg basis and that induces an immune response in the animal model may be used. In such cases, the factor between human and animal dose should be justified. The number of doses administered to the test animals should be equal to or more than the number of doses proposed in humans. Administration of the number of planned clinical doses plus one extra (n+1) is a common practice to provide an extra safety margin. To better simulate the proposed clinical usage, vaccine doses should be given at defined time intervals rather than as daily doses; the dosing interval used in

the toxicity study may be shorter (e.g., an interval of 2–3 weeks) than the proposed interval in clinical trials in humans.

- Detailed physical examinations are performed the day before each immunization, 2–4 hr. after each immunization, twice weekly thereafter, and at sacrifice.
- Body weight is measured before each dose, weekly thereafter, and at sacrifice.
- An ophthalmological examination is conducted pre-study and before necropsy.
- Local irritation/reactivity at the injection site is evaluated by using a standardized scoring scale before each injection and at 24, 48, and 72 hr. (daily for 3 days) after each injection, or until any irritation disappears.
- Clinical pathology (serum chemistry, hematology) is performed 3 days following each immunization and 14 days after the last immunization.
- Serum samples can also be collected at defined times to monitor for immunogenicity.
- 5 M/5 F per group are necropsied 3 days after the last immunization, and 5 M/5 F per group are necropsied 14 days after the last immunization to evaluate the reversibility of any potential toxicological endpoints.
- Organ weights and weight ratios are determined at necropsy.
- A histopathologic evaluation of high dose and control tissues is conducted; target organs are evaluated for the mid- and low-dose groups.
- Urinalysis is conducted before necropsy.

5.0 GOOD CLINICAL PRACTICE AND THE CLINICAL TRIAL CONCEPT

Good clinical practice (GCP) is a set of internationally recognized ethical and scientific quality requirements that must be followed when designing, conducting, recording and reporting clinical trials that involve people. The relevant information may be found through the links listed below.

(https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf; https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/good-clinical-practice)

Clinical trials are conducted to allow human safety and efficacy data to be generated that can lead to product approval by regulatory authorities. The trials take place once adequate nonclinical safety, immunogenicity and effectiveness information has been obtained, as discussed above, and must support the clinical trial approach. Design and execution of clinical studies should follow the ICH guideline, E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) (March 2018). The FDA Web site: http://www.fda.gov/ScienceResearch/SpecialTopics/RunningClinicalTrials/ucm155713.htm can also be referenced. Clinical trial designs depend on the development stage of the vaccine product and fall outside the scope of this PDP. As the focus of this document is to advance to Phase I clinical trial testing, parameters for Phase II and III considerations will not be discussed.

Initial factors to consider when designing the clinical protocol are listed below. These components are not all inclusive but can impact the design of the protocol.

- Open label, Single blind vs double blind design, etc.
- Method of vaccine of delivery, including device(s) used.
- Number of doses of vaccine to be studied based on pre-clinical study results.
- Will a booster dose be incorporated?
- Timing of booster (if used) after the priming vaccination
- Length of a follow-up period to be employed
- Any special eligibility requirements based on the vaccine design (e.g., attenuated viral vectors require consideration for extent, route, and duration of shedding, are immune-competent naïve participants required)
- Use of oversight committees for safety
- Use of toxicity grading scale
- Reporting period for reactogenic events after vaccination

after the last vaccine dose
- Number of participants to be enrolled.
- Duration of study participation.
- Age group to be studied for the initial results
- Eligibility criteria, (including prohibited medications effecting immune system and timing of licensed vaccinations permitted during study participation if needed)
- Number of participants to be enrolled.
- Duration of study participation.
- Reporting period for adverse events after vaccination
- Effective pause rules for participant safety
- Initiation of contraception prior to vaccination and duration of contraception use after vaccination
- How will pregnancy be followed?
- Follow-up for pregnant partners of participants
- Length of time for the infant being followed
- Use of oversight committees for safety
- Timing of clinical laboratory safety data aft
- Timing of collection of immunogenicity data after vaccination
- Any special clinical testing needed for immunogenic data collection (e.g., plasmapheresis, leukapheresis, etc.)
- Qualification or validation of the immunogenicity assays to be used

Other factors after the above have been considered are the following:
- Do all study visits need to be in clinic visits?
- Effects of seasons on recruitment that impacts timing the start of study
- Should adverse events of special interest be identified,
- Where to execute the study (e.g., country)?
- Do all study visits need to be in clinic visits?
- Should adverse events of special interest be identified
- Will an academic site, commercial site, or a combination be needed to execute the study?

Each clinical protocol design will undergo regulatory scrutiny and, therefore, it is beneficial to initiate communications with FDA clinical representatives early in the development process to be sure that the study design meets the FDA's expectation and supports the product's development stage. Clinical trials are conducted in a series of steps designated as phases. Initially, clinical studies enroll immunocompetent, healthy volunteers, volunteers in good general health, and/or patients in small clinical trials that are followed by larger scale studies. Testing of clinical trial samples which are not standard medical tests may be performed following the DAIDS guidance Good Clinical Laboratory Practices Guidelines (August 2021) found at the NIH website: https://www.niaid.nih.gov/research/daids-clinical-research-laboratory-specimens-management

Phase I trials are the first to be conducted in human subjects. The primary objective is to evaluate safety and reactogenicity while the secondary objective is to evaluate the type and extent of the immune response, usually in healthy normal volunteers, or volunteers in good general health. The size of the study population (20-100) may limit the ability to create a statistically powered study. Phase I clinical studies can also be a dose-ranging (dose-escalation) study to determine the appropriate dose for vaccine use. Once initial safety is confirmed through Phase I results, the candidate vaccine is normally evaluated in further trials (Phases II–IV).

Phase II trials are designed to assess the best effective vaccine dose with an acceptable safety profile, vaccine preparation, and administration schedule of the vaccine. The resulting data would then help design the Phase III trials. A Phase II needs to have a defined sample size that is statistically powered because a clinically useful outcome for safety and immunogenicity is needed. Safety continues to be assessed as in Phase I trials. Phase II trials have a greater number of participants (e.g., 100-400) compared to Phase I. Phase II trials continue to assess which vaccine does is to be used in the next phase. The eligibility criteria may be broader and include groups of people from diverse backgrounds to ensure representation across different populations. Results of a Phase II clinical trial frequently terminates further development when there is no clear benefit demonstrated or serious/severe adverse events are seen.

Phase III trials are randomized-controlled multicenter trials performed in a larger patient population which evaluate the efficacy and safety of the final vaccine preparation. Efficacy is usually based on how often the diseases occurs but other endpoints such as severity, or possibly the infection incidence could be used. A marker or correlate of protection may be established. Most Phase III studies are designed as randomized, double-blind, and placebo-controlled to avoid the potential for evaluation bias. The study design would represent the typical use of the vaccine in the general population. Statistically meeting the study's primary objective is the major goal and the prime consideration for obtaining FDA approval. FDA may not approve the vaccine if the statistical data is not compelling or may require an additional Phase III study to be performed. Therefore, the population size of a Phase III study is greater than a Phase II study, e.g., 1000 participants or more.

Phase IV trials are safety surveillance studies that may be required by regulatory authorities following a product's approval. They may also assess effectiveness for direct or indirect disease prevention in a real-world setting.

6.0 REGULATORY CONSIDERATIONS

In the US, the Center for Biologics Evaluation and Research (CBER) of the FDA has authority for the regulation of prophylactic vaccines and other biological products. Its legal authority derives primarily from Section 351 of the Public Health Services Act (PHS Act) and certain sections of the Federal Food Drug and Cosmetic Act (FD & C Act). The statutes of the PHS Act are implemented through regulations codified in the Code of Federal Regulations (CFR) which is published annually. Regulations that are specifically applicable to vaccines and other biological products are contained in Title 21 of the CFR, parts 600 through 680 (https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-600). To protect human subjects enrolled in clinical trials, FDA has issued regulations that govern Investigational New Drug (IND) products, and these are contained in 21 CFR §312. (https://www.ecfr.gov/current/title-21/chapter-I/subchapter-D/part-312) 21 CFR 312.21(a) describes phase I investigational trials. The purpose of Phase I prophylactic vaccine clinical trials is to evaluate the preliminary safety and immunogenicity of the investigational product. These trials are typically small in size (20 to 80 patients), and the volunteers are most frequently healthy adult subjects. 21 CFR 312.23(a)(7) specifies that sponsors must submit chemistry, manufacturing and control (CMC) information on a drug or biological product as part of an IND application. 21 CFR 312 (a)(8) specifies the pharmacology and toxicology information that should be part of an IND application.

In addition, sponsors/manufacturers must also comply with regulations for Current Good Manufacturing Practices (cGMPs) (parts 210 and 211). However, certain requirements in part 211 pertain to the commercial manufacture of products and thus, FDA realized that investigational drugs used for Phase I clinical trials would need to be exempted from complying with *all* requirements outlined in parts 210 and 211. The Guidance for Industry entitled " cGMP for Phase 1 Investigational Drugs," (https://www.federalregister.gov/documents/2008/07/15/E8-16002/guidance-for-industry-current-good-manufacturing-practice-for-phase-1-investigational-drugs) (September 2008) describes approaches manufacturers may use to implement manufacturing controls that are appropriate for the Phase I clinical trial stage of development. It states that compliance with cGMP for Phase I investigational drugs manufacture will be mostly obtained through well-defined, written procedures, adequately controlled equipment and manufacturing environment and accurately and consistently recorded data from manufacturing and testing.

Guidance documents issued by the FDA regarding manufacture, testing and clinical evaluation of vaccines and other biologicals do not have the force of law but are intended to provide recommendations that are current with areas of rapidly progressing science, and for specifying a degree of detail beyond what is included in the regulations. These documents are available at CBER's website. (https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/vaccine-and-related-biological-product-guidances).

In addition to guidance documents issued by the US FDA, the International Council on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) has developed guidelines and technical requirements for the development, approval, and safety monitoring of medicines. ICH regulatory members adopt these guidelines and are expected to implement them. These documents are

available at https://www.ich.org/page/ich-guidelines. The World Health Organization (WHO) has also developed guidelines on the non-clinical and clinical development of vaccines. Two of these guidelines, i.e., the "WHO guidelines on non-clinical evaluation of vaccines," https://www.who.int/publications/m/item/nonclinical-evaluation-of-vaccines-annex-1-trs-no-927 and the "WHO guidelines on the non-clinical evaluation of vaccine adjuvants and adjuvanted vaccines" https://www.who.int/publications/m/item/nonclinical-evaluation-of-vaccine-adjuvants-and-adjuvanted-vaccines-annex-2-trs-no-987 provide important information with regard to non-clinical studies to support proceeding of the vaccine candidate to Phase I clinical trials.

The FDA encourages meetings with sponsors to guide the evaluation of the vaccine through various development stages and to help resolve scientific issues concerning the product. Detailed information on the conduct of regulatory meetings is described in 21 CFR §312.47 and in relevant guidance documents. During these meetings, FDA personnel takes official meeting minutes, and these are provided to the sponsor. They serve as a permanent record of any agreements reached. There are different types of meetings; the meeting most relevant to sponsors interested in bringing an investigational product into the clinic is the pre-IND meeting.

6.1 THE PRE- IND MEETING

In preparation of an IND submission, it is strongly recommended that that the new vaccine developer hold a pre-IND meeting with the FDA. The pre-IND meeting serves as a forum for early communication between the sponsor and the FDA and is a good opportunity for FDA to indicate the Agency's expectations regarding data and information to be included in an IND application, including non-clinical IND-enabling studies, clinical study design, manufacturing processes, methods and any data requirements that require resolution prior to the initiation of clinical trials.

The Pre-IND meeting is conducted with representatives from the appropriate FDA Division. The main purpose of this meeting is to ask FDA representatives specific questions concerning the vaccine design and development, the non-clinical study approach, the manufacturing and controls used in production, analytical testing, and the adequacy of the proposed first in human clinical study. These pre-IND questions are to solicit FDA feedback to ensure the information provided in the IND will meet FDA expectation, which will allow the clinical trial to go forward without delay. The request for the meeting should be accompanied by a background document that summarizes information available for the vaccine, and administrative details, including the proposed meeting agenda, the list of specific questions, expected outcome from the meeting, the names of attendees with title and affiliation, and the names of requested FDA representatives, if known. The request for a proposed date and time are included for FDA's consideration. Additional information can be found in the Guidance for Industry entitled "Formal meetings between the FDA and sponsor or applicants of PDUFA products" available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/formal-meetings-between-fda-and-sponsors-or-applicants-pdufa-products-guidance-industry.

Included with the meeting request or no less than 30 days prior to a scheduled meeting, a Pre-IND briefing package is sent to the designated FDA reviewing office. The briefing document should include the vaccine development background and the rationale for the proposed investigation clinical investigation. Information regarding the manufacture and control processes (including flowcharts), animal and/or *in vitro* findings resulting from early investigations, the proposed non-clinical safety testing, and the design of the proposed Phase I clinical trial(s) should be included. Any previous human experience with the vaccine or safety data from a similar vaccine construct should also be included described.

The FDA will generally respond within 14 days after receiving a meeting request, and if the request is honored, FDA will schedule the meeting to take place within 60 days of receiving the written request.

Prior to the scheduled meeting date, FDA will provide a written response to the Pre-IND questions and opines on the data and information included in the briefing package. Based on the FDA's review and comments, it is not unusual for the proposed non-human animal safety studies or the clinical study protocol to undergo modification. Therefore, this is one of the main reasons not to start the non-clinical toxicology studies until after the pre-IND meeting to avoid the need to repeat a study based on comments and requirements for FDA acceptance. Required GLP non-clinical work can be initiated as soon as the GMP or GMP-like manufactured product is available. **INTERACT** or an **IN**itial **T**argeted **E**ngagement

for **R**egulatory **A**dvice on **C**BER/CDER Produc**T**s refers to a meeting at a time early in product development. The appropriate timing for an INTERACT is when a sponsor has an investigational product to be evaluated in a clinical study and has performed preliminary non-clinical proof-of-concept studies with the intended investigational product, but safety toxicology studies have not been performed. Unlike pre-IND meetings, there is no Prescription Drug User Fee Act (PDUFA) mandated date for FDA to schedule the INTERACT meeting. Of note, INTERACT meetings are uncommon for prophylactic vaccines. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

6.2 IND SUBMISSION

To be able to conduct first-in-human clinical trials with the vaccine candidate, a sponsor must obtain permission from the FDA through submission of an IND application. The IND Application submitted to the FDA should provide all components in the development of the candidate vaccine. The required content and format are described in detail in 21 CFR 312. In summary, the contents include:

- An introductory statement that presents the vaccine background, structure, scientific rationale, and all preliminary efficacy data.
- The general investigational plan for the drug/vaccine that describes the proposed Phase I safety clinical study, risks, and benefits, and an outline of a future investigational approach.
- An investigator brochure that provides guidance to the clinical investigator concerning essential facts regarding the IND candidate for use in the clinical trial.
- The clinical study protocol for the investigation that has been designed on the basis of ICH *Good Clinical Practice: Consolidated Guidance* (Topic E6, April 1996).
- The chemistry, manufacturing, and controls section that identifies the manufacturer; provides a process description with flow diagram(s) of the bulk vaccine and final formulated vaccine product; and identifies control test procedures, release acceptance criteria and specifications, final vaccine content, container and closure, labeling/packaging, and stability of the vaccine over time.
- The pharmacology and toxicology section that sets forth animal studies that provide all available information concerning the vaccine's effects, mechanisms of action, and biodistribution, as well as its safety profile when given at the dose level and by the mode of administration proposed for the clinical trial.
- A description of previous human experience with the vaccine candidate or any similar product, class, or configuration, with references to studies, regulatory submissions, and publications.

The IND includes the form FDA 1571, which identifies the Sponsor and provides the particulars of the IND submission. Form FDA 3674, a certification required by 42 U.S.C. § 282(j), Section 402(j) Public Health Service (PHS) Act of Title VIII of the Food and Drug Administration Amendments Act (2007), must also accompany the IND application. Form FDA 3674 serves to certify compliance with the requirements found at http://www.fda.gov/RegulatoryInformation/Guidances/ucm125335.htm that is intended to ensure the public has access to information about certain clinical trials.

The FDA has 30 days to review the original IND application and determine whether study participants will be exposed to any unacceptable risks. If the Sponsor of the IND does not hear from the FDA within the 30-day time frame, the trial may begin.

Prior to initiation of the study, a Form FDA 1572 must be sent to the FDA providing information regarding the clinical trial site(s) and the investigator(s). A Form FDA 1572 must be completed for each trial site and sent by the Sponsor to the FDA under the IND reference number before the clinical trial begins at the designated trial site.

A signed informed consent must be obtained from each study participant approval for the study must be obtained from a local institutional review board.

Following the initial IND submission, maintenance of the IND application is performed through amendments, safety reports, and annual reports. Each additional submission must be accompanied by a

completed Form FDA 1571 that identifies the content of the submission, the FDA assigned IND reference number and a consecutive Serial number sequence.

The IND information and data consist of approximately 6–10 volumes, identified as Serial Number 0000. The IND includes the form FDA 1571, which identifies the Sponsor and provides the particulars of the IND submission. Form FDA 3674, a certification required by 42 U.S.C. § 282(j), Section 402(j) Public Health Service (PHS) Act of Title VIII of the Food and Drug Administration Amendments Act (2007), must also accompany the IND application. Form FDA 3674 serves to certify compliance with the requirements found at http://www.fda.gov/RegulatoryInformation/Guidances/ucm125335.htm that is intended to ensure the public has access to information about certain clinical trials.

6.3 VACCINE APPROVAL PATHWAYS: TRADITIONAL, ACCELERATED AND ANIMAL RULE

As described above, vaccines are licensed based on data derived from adequate clinical trials showing that the products are safe and effective. However, licensure does not necessarily require demonstration of efficacy in a clinical trial using a clinical disease endpoint in humans, which is referred to as 'traditional' approval. In the US, products for serious or life-threatening illnesses providing meaningful benefit over existing treatments can be approved using the accelerated approval provisions, for which approval would rely on adequate and well controlled clinical trials establishing an effect of the product on a well-established surrogate endpoint (e.g., immune response) that is reasonably likely to predict clinical benefit. For example, most influenza vaccines are approved based on elicitation of neutralizing antibodies, a well-established correlate of protection against disease. In another alternative pathway the US regulatory provision known as the Animal Rule can be used when human efficacy trials are not ethical or feasible and when the accelerated approval provisions cannot be used. Following safety and immunogenicity studies conducted in humans, and under specific conditions supporting the conclusion that a product is reasonably likely to produce a clinical benefit, the Animal Rule allows adequate and well controlled studies in animals to provide evidence of efficacy of the product. The Animal Rule pathway requires extensive characterization of the animal challenge model and close coordination with FDA throughout the Clinical process. New developers considering advancement of a vaccine candidate through the Animal Rule are urged to engage an expert regulatory consultant for guidance through the process.

7.0 TIMELINE AND COST ESTIMATES for NON-CLINICAL and CLINICAL DEVELOPMENT

Cost and timeline planning for a vaccine development campaign are critical activities for the developer, funders and other program stakeholders. The developer should be prepared to present and defend high-level cost and time estimates backed up by detailed proposals tailored for each specific activity. The cost estimates and timeline presented in Table 2 and Figure 3 below are examples based on Phase I development of a recombinant protein subunit vaccine formulated with adjuvant and delivered by SOC needle/ IM. It is assumed that most – if not all – of these non-Clinical and Clinical development activities will be performed by contractors (CDMOs, non-Clinical and Clinical CROs). Time and cost estimates are not made for POC immunogenicity and animal model challenge studies as these are expected to be performed by the research investigator's laboratory. Costs for adjuvant access (purchase and/or licensing) are also not provided due to the wide cost range depending on the chosen strategy. Key variables to take into consideration for both timing and cost include but are not limited to the expression system used to manufacture the immunogen (prokaryotic or eukaryotic); adjuvant access; and the study design, duration and number of subjects enrolled in the Phase I clinical trial (Table 2 assumes n=60).

Table 2. Cost estimates for non-Clinical and Phase I Clinical development for a protein subunit vaccine

Activity	Estimated Cost
Non-clinical POC immunogenicity and animal challenge studies	Widely Variable
Cell Line Development (RCB)	$350,000 – 400,000
Master Cell Banking (MCB)	$250,000 – 350,000
Process, Analytical & Formulation Development, non-GMP Demonstration Run	$1,000,000 – 2,000,000
Viral clearance study	$200,000 – 300,000
cGMP Manufacturing (DS)	$1,500,000 – 2,000,000

cGMP Manufacturing (DP)	$240,000 – 300,000
Rabbit GLP Toxicology Study	$600,000 – 900,000
Adjuvant access/licensing	TBD
Phase I Clinical Trial	$5,200,000 – 7,200,000
Total	**$9,350,000 – 11,350,000**

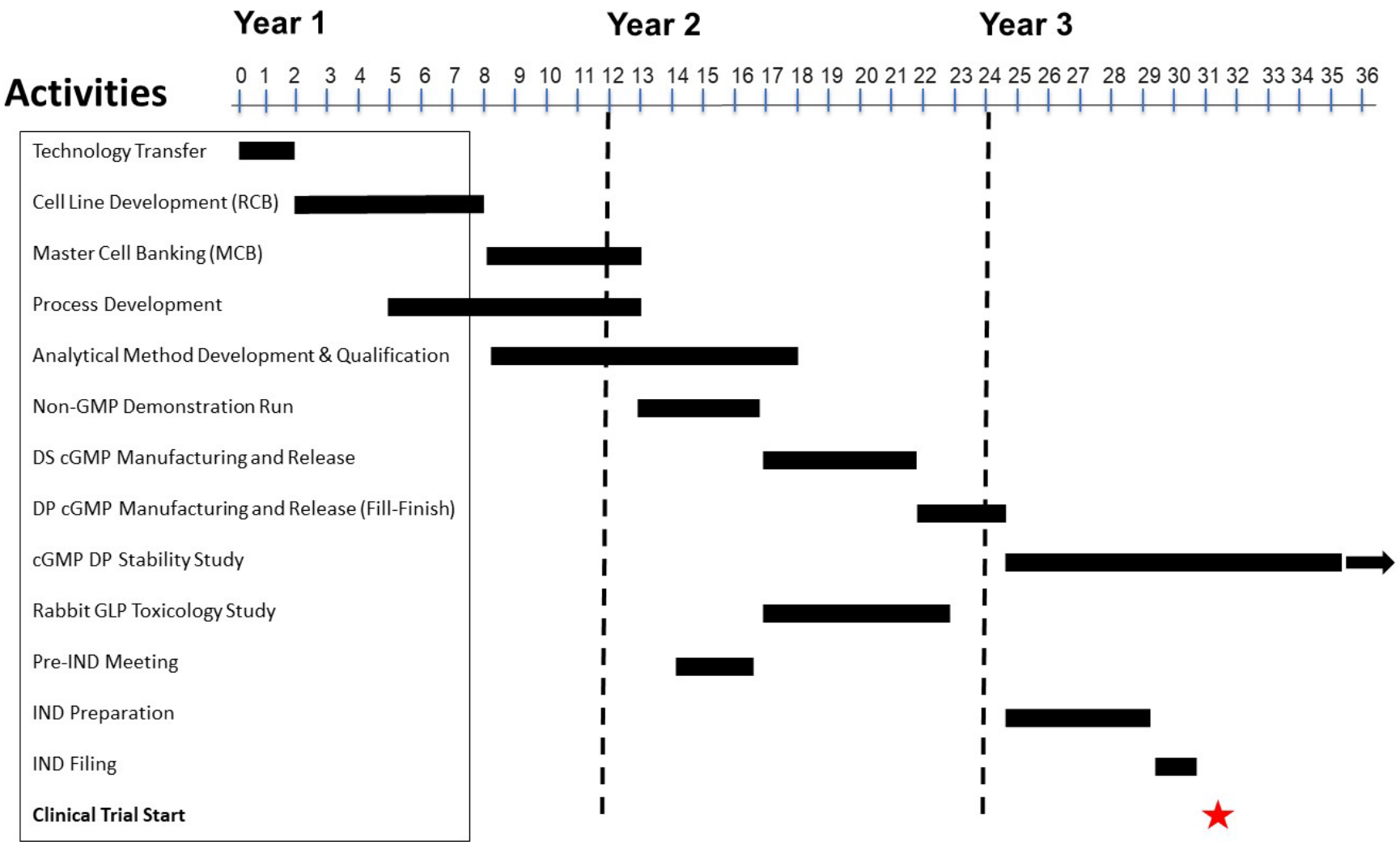

Figure 3. Development Timeline for a Recombinant Protein Vaccine Immunogen Manufactured Using a Mammalian Cell Line. This figure illustrates the major activities and associated timings required from technology transfer through clinical trial start. It is assumed that all development activities will be performed by contract development /manufacturing organizations (CDMOs) and contract research organizations (CROs). The individual activities are not meant to be all-inclusive, but to illustrate the breadth and timeframe of a non-clinical development project. Furthermore, the figure

uses a recombinant protein vaccine immunogen as an example. Specific activities and timings may vary for other vaccine delivery modalities.

8.0 REGULATORY AGENCY GUIDANCE DOCUMENTS CITED IN THE TEXT

DAIDS *Good Clinical Laboratory Practice Guidelines* (August 2021)

FDA Good Manufacturing Practices (21 CFR§(s) 210, 211 and 600

FDA. *Good Laboratory Practice for Nonclinical Laboratory Studies* (21 CFR § 58), current June 2023.

FDA. *Guidance for Industry: CGMP for Phase 1 Investigational Drugs*. 2008.

FDA. *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practices*. October 2004.

FDA. *Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and*

Therapeutic Vaccines for Infectious Disease Indications (Feb 2006)

FDA *Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule (February 2009).*

FDA *Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications February 2010.*

FDA *Guidance for Industry: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products July 1996 (ICH Q5C).*

FDA *Principles of Premarket pathways for Combination Products: Guidance for Industry and FDA Staff (Jan 2022)*

FDA *Safety and Performance Based Pathway: Guidance for Industry and FDA (September 2019)*

FDA *Bridging for Drug-Device and Biologic-Device Combination Products Guidance for Industry: Draft Guidance (December 2019)* FDA

FDA *Requesting FDA feedback on Combination Products: Guidance for Industry and FDA Staff (December 2020)*

FDA *Format for Traditional and Abbreviated 510(k)s Guidance for Industry and Food and Drug Administration Staff (September 2019)*

FDA *Guidance for Industry and FDA Staff: Early Development Considerations for Innovative Combination Products (September 2006)*

FDA *Human Factors Studies and Related Clinical Study Considerations in Combination Product Design and Development: Draft Guidance for Industry and FDA Staff*

FDA *Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process" Guidance for Industry and Food and Drug Administration Staff Document issued on: September 4, 2020*

ICH. *Clinical Investigation of Medicinal Products in the Pediatric Population* (Topic E11). August 2017.

ICH. *Derivation and Characterization of Cell Substrates used for Production of Biotechnological/Biological Products* (Topic Q5D). ICH July 1997, FDA September 1998.

ICH. *Good Clinical Practice: Consolidated Guidance* (Topic E6) ICH November 2016, FDA March 2018.

ICH. *Impurities in New Drug Products* (Topic Q3B[R2]). ICH June 2006, FDA July 2006.

ICH. *Impurities in New Drug Substances* (Topic Q3A[R]). ICH October 2006, FDA June 2008.

ICH. *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceutical; Guidance for Human Somatic Cell Therapy and Gene Therapy* (Topic S6). ICH June 2011, FDA May 2012.

ICH. *Quality of Biotechnological Products: Analysis of Expression Construct in Cells used for Production of rDNA Derived Protein Products* (Topic Q5B). February 1996.

ICH. *Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (Topic Q5C). ICH November 1995, FDA July 1996.

ICH. *Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (Topic Q6B). ICH March 1999, FDA August 1999.

ICH. *Stability Testing of New Drug Substances and Products* (Topic Q1A[R2]). ICH February 2003, FDA November 2003.

WHO *WHO good manufacturing practices for biological products Annex 3TRS 996 201*

APPENDIX A

cGMP MANUFACTURING CONSIDERATIONS AND RISK MANAGEMENT

INTRODUCTION

The activities leading to the initiation of clinical testing for a novel vaccine will typically involve substantial cash commitments and the efforts of dozens of people, some within the entity responsible for developing the vaccine and many who work for external organizations. The manufacture of nonclinical and clinical materials to support the testing of a candidate vaccine will demand a significant proportion of the project's resources. This section provides some considerations for manufacturing and testing in a manner that will achieve regulatory approvals, and meet the project's goals for assessing the efficacy and safety of the candidate.

PROJECT GOALS

The regulatory system for drug development is generally applied to product candidates that are developed from the start with the intent of eventual commercialization. While many clinical trials are conducted by organizations with experience in developing and marketing commercial products, this product development plan assumes that the sponsor does not have the internal experience or capabilities needed to develop commercial products and will rely heavily on external resources to proceed with clinical testing and prepare for eventual commercialization.

Vaccine development is a challenging enterprise, and even experienced manufacturers with substantial resources routinely experience unexpected technical and regulatory setbacks (unrelated to the safety or efficacy of the product) along the path to commercialization. For an inexperienced developer with limited funding, similar setbacks can result in the termination of the project.

Decisions related to manufacturing and testing will have a major impact on the risk of technical or regulatory setbacks, and the prospects for further development of the product in the event of a successful trial. The lowest overall time to market and development cost can be achieved by investing heavily in the project in the early stages of product development, but this approach is rarely taken because the investment is wasted if the product does not prove to be safe or effective. The goal of the early-stage developer should be to deploy the available funding efficiently to obtain the clinical data that will attract further support for the project. This is best accomplished by minimizing technical and regulatory risk while preparing for the clinical study, while also creating a firm foundation for further clinical development- in other words avoiding the need for any 'do-overs' to proceed through the next stages of clinical development.

Regulatory guidance documents for CMC activities and compliance should be used as the basis for project plans, and any deficiencies relative to guidance should be addressed by data support (when possible), a strong rationale, and direct communication with FDA when needed. These considerations connect directly to CMO choice, as the best vendors from a cost or availability standpoint may have deficiencies in other respects that will be difficult for the IND sponsor to defend.

PROJECT STRUCTURE AND RESPONSIBILITIES

In general, the development of a candidate vaccine for testing in humans will require the sustained and coordinated efforts of individuals with expertise in a variety of disciplines. In pharmaceutical and biotechnology firms pursuing product development, projects are typically organized in a project team format with clearly defined roles and responsibilities to address the hundreds of required tasks. In a typical project team structure, all of the necessary functions/scope should be represented; qualified individuals may have responsibility for multiple functions. Many project tasks, including CMO-related activities, fall cleanly into one of these categories and can be assigned to the responsible individual. Other tasks require close coordination among several disciplines and are best addressed at the Project Team level.

Typical Vaccine Development Project Team Structure:

- Team Leader: accountable to IND holder or funding organization for overall project performance. The IND holder is the entity responsible for developing the product, from a legal (liability) and regulatory standpoint. This may be the Principal Investigator for grant-supported projects.
- Project Manager: details, coordinates and tracks all the tasks required to accomplish the project, and budget allocations. Documents project-relevant information and decisions.
- Research: represents the science underlying the product candidate, including mechanism of action
- Clinical Development: responsible for clinical trial protocol and activities
- Nonclinical/Tox: responsible for safety studies to support the IND
- Technical Development: responsible for process development, analytical test method development, formulation and packaging
- GMP Manufacturing: manage outsourced activities to assure supply of clinical product
- Quality: Quality Control ensures appropriate testing activities for CMC materials (usually outsourced); Quality Assurance activities ensure cGMP system compliance for release of materials intended for human use, and compliance with GLP and good clinical practices for nonclinical and clinical testing
- Regulatory: responsible for information exchange with regulatory agencies and obtaining necessary approvals
- The team should also have access to legal support including assessment and negotiation of intellectual property rights, and a clear understanding of negotiating and approval authority to execute contracts on behalf of the project and sponsor.

CHOOSING A CMO

With the high cost, specialized staff and facilities required for cGMP manufacturing of biological products, most academic, government and small biotech vaccine developers will choose to have GMP clinical trial materials manufactured by a commercial contract manufacturing organization (CMO). The project areas most directly involved in CMO selection and management, in rough order of priority, will be Manufacturing, Quality, Technical Development, Regulatory, and Project Management. If current employees are not available to manage these responsibilities, the sponsor will need to identify and secure the needed expertise, e.g., by hiring experienced consultants. While it is helpful if a CMO has directly relevant experience, the project team members will need to actively monitor and manage the CMO activities, make decisions on behalf of the sponsor, and take responsibility for the CMOs efforts and results. *The responsibility for demonstrating overall cGMP compliance to FDA rests with the IND holder, not the CMO*.

Capabilities

The selection of a CMO needs to reflect the match of the full project scope to the vendor's capabilities. The project will be much easier to manage if a 'one stop shopping' approach can be taken so as to minimize technology transfers between contractors. CMOs serving the biotechnology industry will generally have the capability to manage the manufacturing steps starting with a released cell or virus bank and proceeding through bulk drug substance (API) and including analytical development and cGMP-compliant testing. Particular CMOs may have additional important capabilities, for example:

- Cell line development
- Cell banking
- Process development and analytical method development
- Formulation and lyophilization
- Sterile filling into the final drug product container

- Packaging and labeling suitable for clinical trials
- Long term storage of cell banks, drug substance, drug product
- Stability studies

CMOs with multiple capabilities can offer important benefits, as this can minimize the need for technology transfer, controlled shipping of materials, coordination of schedules and communications among the sponsor and CMOs, etc. In the case of subunit vaccines manufactured using an *E. coli* or mammalian cell host, many vendors have the capability to perform the various technical development and manufacturing activities; it should be possible to obtain competing bids from multiple qualified firms.

CONSIDERATIONS

Types of CMOs

Most early-phase biological products developed by small firms and nonprofit entities are produced by CMOs, because of the significant time and investment needed to establish cGMP manufacturing capabilities. An important consideration for selection is the financial stability of the CMO.

A dedicated CMO is a firm that relies on its manufacturing operations to make a profit. These firms are typically configured well to identify and support client needs and may have a substantial record of client successes to provide confidence in future performance. Their client base is typically oriented more towards commercial firms with projects that could lead to increasing repeat business or eventual commercial manufacture. These firms tend to be more expensive and aim for a relatively higher level of service and performance. They may also be better configured to support a broad range of required activities.

A number of firms that are primarily focused on developing their own products will also provide contract manufacturing services. This can be a sensible approach for companies to support the expense of maintaining cGMP capabilities if their own products do not fully utilize the facility. There are some notable successes with this approach, but also some important pitfalls. Clients should expect the developer/CMO to protect the interests of their own projects above client projects, which could impact scheduling and resourcing.

Financial stability is especially important; biotech firms in financial trouble may offer CMO services as a temporary measure to obtain revenue. Finally, the company may not have the 'service mindset' that will lead to an effective collaboration. A good CMO should have project managers and team leaders who are flexible and responsive to client needs and communicate well; developer/CMOs may also underestimate the need for business, legal and regulatory support.

An increasing number of cGMP manufacturing operations have been established with direct support from academic, federal or state agencies. These are often non-profit and may be associated with a university or medical center. These groups tend to have more academic/government clients and may make more use of niche or leading-edge technologies. Historically they have had more issues with staff qualifications, cGMP compliance and regulatory support. Clients should be diligent in asking about the CMO's experience base and checking references. In the section that follows a variety of questions and issues are presented that should be addressed during the selection of a CMO.

Experience Base, References, and RFPs

The best predictor of performance by a CMO is its past performance on similar projects. The CMO should provide details such as its history, the number of batches they've produced for human use and for which clinical stages, the number of batches making your particular type of product or using the same cell line, etc. The following suggestions relate to vendor experience:

- Ask to see the vendor's organization chart and CVs for key staff, including the points of contact for your project.
- Ask for direct references from firms who have hired the vendor, ideally for a project like yours. Have them describe their largest clients and types of projects.
- Ask about the vendor's capacity for process development. Small scale and full-scale development

batches will be useful to establish the process performance and guide the setting of product specifications. Reference checking should reflect any planned development work, and any major categories for GMP work (e.g., aseptic filling).

- Write a formal request for proposal (RFP) describing in detail the technical scope of work and contractual requirements of the project. The RFP can be distributed to multiple candidate CMOs to elicit competitive project proposals.

Terms and Price Negotiation

If the contracted work involves familiar cell lines and production methods, it will be reasonable to obtain project/price quotes from several vendors. Considerations for the negotiation include:

- Does the project quoted fully describe the scope and responsible parties for all the required activities?
- Is the vendor employing any patented or trade-secret technology, e.g., the expression system or host cell line? If the project progresses later by switching to another vendor or partner, what are the terms for continuing to use the technology? If the project has a development component, who has the rights to any inventions?
- What up-front payments are needed to reserve the manufacturing capacity? If the project is delayed on the client side (e.g., due to regulatory delay or problematic test results), are these payments forfeited? The client may have a forfeiture rate that increases as the project start date approaches.
- How are disputes resolved?
- How is activity or project success defined? If the manufactured batch cannot be released due to a product testing failure or a GMP compliance issue, what payments are still due to the vendor? Is the CMO obligated to repeat the contracted batch if they are responsible for the batch failure?

Quality and Regulatory Affairs

Other than technical performance of the project activities, achieving cGMP compliance can be the most challenging aspect of a contracted project. A key component of the contract is the Quality Agreement. This should describe in detail the responsibilities of each party in approving, testing and releasing the product or drug substance. It should define what types of incidents and investigations will result in notification to the client, and their involvement in any resolution. The Quality Agreement should be reviewed and signed by the individual who will be responsible for Quality Assurance decisions on behalf of the sponsor. The scope of the audit should be clearly defined according to the SOW for that particular CMO, and activities required. It is also advisable to have a CMC SME on the quality audit to ensure all technical questions can be addressed.

The Quality agreement should be drafted after the audit is performed and the audit report has been released. Any findings that are of a concern can be noted in the agreement and mitigating actions, if required, can be agreed upon.

- The sponsor should conduct a formal cGMP audit of the selected CMO. Many early-stage vaccine developers will engage expert Quality consultants to conduct the audit. While expensive (up to several days of effort), a full audit is an essential aspect of assuring that the CMOs quality and technical systems are suitable to achieve the sponsor's project goals.
- Has the vendor had any formal inspections by federal or state regulators? Any warnings, shutdowns, disbarments of staff?
- Audit record: have the vendor describe their history of client audits and mention any key or recurring findings, and remedial measures.
- If a 'conditional release' approach is needed (e.g., the drug substance will be shipped for further manufacturing before all test results are in hand), this arrangement should be made clear.
- Does the vendor have a Biologics Master File describing any aspects of their process or

operations?

- Regarding the execution of a batch, the agreement should define the degree of sponsor Quality participation in assessing process and cGMP deviations.

Some of the most important issues concerning CMOs relate to their operations as a multiproduct facility. cGMP regulations require the sponsor to assure that their products are not 'adulterated', containing any unintended substances. In the case of a multiproduct facility this creates the need to maintain and document procedures to prevent cross-contamination. If the facility works with live viruses or spore forming organisms these issues will require special attention.

One aspect of product segregation is line clearance (also referred to as "product change-over"); the CMO should have procedures for clearing the processing areas of all materials related to the previous project, fully cleaning and decontaminating the equipment and facility, and then introducing the equipment and materials needed to initiate the next batch.

It is important to demonstrate control over the manufacturing facility and environment. Cleaning and decontamination are key activities and sometimes problematic. The following points should be addressed:

- The CMO should provide a floor plan illustrating room/corridor classification, air handling and zone/suite segregation, gowning and airlocks, and material and personnel flows through the facility. The area classification relates to the cleanliness of air in the area; this is critical for those steps that involve open containers, or preparation of an injectable final product.
- The vendor should describe recent environmental monitoring data for the areas that will be used for manufacturing.
- If aseptic filling of an injectable product is planned, have the vendor describe their approach to qualification of the filling procedure. In general, one or more fills of sterile media should be performed under conditions simulating the GMP fill, to ensure that the filling process is capable of maintaining sterility.
- Review the areas and approach for quarantine/testing/release of raw materials, and controlled storage of cell and virus banks, processing intermediates and products. What labeling and segregation procedures are in place to avoid mix-ups or use of unapproved or expired materials?
- The CMO should disclose the types of host cells and viruses that have been used in the facility. The CMO should disclose any presence of antibiotics in the facility or use in the process, as there are specific regulatory concerns regarding antibiotic contamination.
- Any equipment that is shared use and also in contact with the product should have a qualified cleaning procedure that is demonstrated to remove traces of the previous product. A nonspecific test for cleaning performance such as Total Organic Carbon is often sufficient. If toxic substances or live virus have been in contact with the equipment, more stringent cleaning and detection methods may be needed to demonstrate lack of adulteration. Equipment that is often shared includes glass or stainless steel bioreactor equipment, chromatography columns and tubing, filling needles and transfer lines for aseptic filling, and lyophilization equipment.

Location

CMO activities are generally conducted at sites remote from the sponsor location. The CMO will generally have communication and operational approaches to support a successful project, but sponsors should consider the following:

- If the CMO is in a different country, expenses will be increased due to costs for controlled shipping of samples, reagents and products. Special care should be taken to ensure that irreplaceable materials or products don't thaw if delayed by customs or air travel disruptions. This is a greater risk if the materials are labeled as biohazards.
- Engagement of a foreign CMO for product development work funded by the US Government may be complicated by federal contracting rules. Developers in this situation should consult the US government agency supporting the work for input on the rules and approval mechanism, if needed.

- Travel budgets should be adequate for vendor assessment and for occasional face-to-face meetings with key sponsor staff. If the sponsor is more than a few time zones different from the CMO, communication logistics will take some extra effort.
- Import and export of biological materials may be subject to review or permit requirement by USDA, FDA or Commerce Department, and like agencies in the other country; substantial delays are possible. On the financial side, exchange rate considerations introduce an element of budget risk.
- Many aspects of drug manufacture are harmonized between FDA and non-US regulatory agencies via the ICH process (http://www.ich.org/cache/compo/276-254-1.html), but the sponsor needs to ensure that they can represent the activities as compliant with US cGMP. The CMO should explain how they will support client needs for communication with regulatory agencies and preparation of regulatory filings.

Communications and Visibility

Related to the Quality Agreement described earlier, the sponsor should have reasonable access to information regarding the actual manufacturing process and execution. Ideally, the CMO will work with the sponsor to develop a detailed manufacturing batch record that can be approved by the sponsor as well as the CMO prior to manufacturing. After manufacturing, the sponsor should be given a copy of the primary executed batch records for manufacturing. The scope of this request should be reasonable; summary data or results may be reasonable for items like environmental monitoring, equipment calibration or component sterilization data. The full batch record should be shared with the sponsor/IND holder. It is recommended to be done after the QA technical sign off by the QA of the CMO, once all deviations and activities have been completed. A thorough review of the batch should be done and, to build a sponsor dossier, signed off by the internal QA. This would form the basis of the Clinical release of IP to the clinics.

If resources permit and the durations of key processing steps are relatively short, the sponsor should consider a 'man-in-plant' approach.

Metrics and Criteria

Performance metrics and criteria are at the core of the sponsor/CMO partnership. A successful project requires a foundation based on sound contracts, structures and plans, but also a 'partnering' mindset on both sides to work through issues and achieve the best outcomes for all. Biologics manufacturing is complex and nearly every project will involve some degree of cGMP deviation, unexpected process yields or test result, etc. As the contract between the parties will serve as the formal basis for resolving any disputes, it should reflect the following:

- Did the sponsor provide a process and test procedures that have already been demonstrated to produce a product within the agreed parameters, or is the batch produced on a 'best efforts' basis, e.g., as the first attempt to make the product in this way? If the process uses equipment or a production scale that is different from prior sponsor experience (as is typical), the expectations and contractual outcomes should be defined carefully. The sponsor may or may not be willing to produce a certain amount of material for a given fee, depending on their comfort level with the prior experience.
- In general, the CMO costs and business plan are based on utilization rates for the facility and staff, and they will seek payment for this utilization regardless of the 'yield' outcome for the sponsor. Adjustment of the contract terms after a non-GMP batch at full scale can be a useful mechanism to set reasonable expectations for yield performance of the GMP lot. This non-GMP material may be suitable for use in nonclinical safety studies if comparability to the GMP process and product can be demonstrated.
- The CMO should provide a Certificate of cGMP Compliance once their Quality department has reviewed the batch record, environmental monitoring data and test results. The contract should ideally have provisions for partial or full refund or repeat of the production effort if the CMO cannot assert regulatory compliance due to its own deficiencies.

In summary, contract manufacturing organizations provide an efficient way to provide cGMP clinical product for a large number of early-stage projects without the capital investments and hiring costs that

would be required to set up ‘in-house’ production at each sponsor site. The sponsor/CMO relationship can be mutually rewarding if based on sound organization and quality agreements as well as attention to the technical execution of the project activities.

APPENDIX B
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