Good Practice Guide

Development of Investigational Therapeutic Biological Products



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We Dedicate this Good Practice Guide in Loving Memory of Max Seales Yonker (1963 – 2005)



Max joined ISPE in March of 1996 and was honored to be elected to the ISPE International Board of Directors in 2002. Max was a leader in the ISPE Clinical Materials Committee and the ISPE San Francisco/Bay Area Chapter.

She was and will continue to be an inspiration to us all, for her tremendous contribution and passion in the area of investigational therapeutic biological products, from which the idea of this Guide originated from. She spent the last several years of her career participating in the quest for cancer's cure. We celebrate the life of a spectacular woman whose combined brilliance, compassion, wit, and the warrior spirit has touched many of our lives.

Michael Ku
Past Chair (2005)
ISPE Clinical Materials Committee

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1 Introduction

1.1 Overview

With the rapid growth in the biopharmaceutical industry over the past two decades, the number of newly approved biological products has dramatically increased. In 2005, a record 21 biological products received US Food and Drug Administration (FDA) approvals, including therapies for the treatment of rheumatoid arthritis, diabetes, cancer, and rare genetic disorders.

The increasing number of approved biological products on the global market represents the ability of many biopharmaceutical companies to overcome major challenges, especially during the critical process development and manufacturing stages of their product development.

This Guide will consider the major issues that will confront a biopharmaceutical company in moving therapeutic biological products from the laboratory, to the clinic, and beyond.

1.2 Purpose

This ISPE Good Practice Guide (GPG) is intended to provide readers with an understanding of issues surrounding product and process development, manufacturing, investigational product supply chain management, Quality Control/Quality Assurance, and global regulatory requirements for biopharmaceuticals.

The intended audience for this Guide includes the following disciplines:

- Clinical Supply/Clinical Trial Materials
- Clinical Research
- Manufacturing
- Project Management
- Quality Assurance/Quality Control
- Regulatory Affairs
- Research and Development

1.3 Scope

This Guide specifically addresses the methods and challenges surrounding **recombinant therapeutic biological product** development, including considerations during its use in clinical trials. It focuses on:

- Project Planning/Management
- Preclinical/Clinical Phases
- Comparability and Bridging Studies
- Active Pharmaceutical Ingredient (API)/Drug Substance (DS)/Drug Product (DP)/Placebo Process Development

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- Manufacturing of DS/DP
- Process Validation
- Supply Chain Management of Biological Investigational Products for Clinical Trials
- Quality Control/Quality Assurance Considerations
- Global Regulatory Strategies

The regulations and guidelines from the US, EU, Canada, Japan, and ICH have been considered in the development of this Guide.

While not within the scope of this Guide, it is recognized that the development of other biologics (e.g., cellular and gene therapy) may require additional considerations for their successful product development from the laboratory to the clinic.

1.4 Benefits

This Guide is intended to provide industry professionals with critical information and planning tools in developing therapeutic biological products through preclinical and clinical development phases. It is intended to enable the effective selection of development priorities, and ultimately, the efficient use of time and resources.

1.5 Objective

This ISPE GPG is intended to provide insights on good practice for successfully developing therapeutic biological products.

1.6 Structure

This ISPE GPG: Development of Investigational Therapeutic Biological Products contains six main sections:

- Definition and types of Biological Products
- Product Development
- Process Development and Manufacturing Considerations
- Supply Chain Management of Biological Investigational Products for Clinical Trials
- Quality Control and Quality Assurance Considerations
- Global Regulatory Strategies

These subjects are considered in the main sections of the Guide. Appendices provide additional material and guidance to assist in the identification of regulations, issues, and topics that also should be considered.

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1.7 Key Concepts

The key concepts and principles involved in the development of therapeutic biological products are:

- Utilization of Project Management and Planning Tools (e.g., Product Target Profiles, Timeline, Clinical Supply Plans, and Manufacturing Process Plans)
- Process Development and Manufacturing
  - Expression System Development/Cell Line Development (e.g., bacterial, yeast, and mammalian cells)
  - Product Expression (Cell Culture/Fermentation)
  - Product Isolation/Recovery and Purification
- Drug Product (DP) Process Development
  - Formulation/DP/Placebo Manufacture and Stability
- Manufacture of Drug Substance (DS)/DP
  - Raw Material and Facility Considerations
- Process Validation Studies
- Supply Chain Management of Biological Investigational Products for Clinical Trials
  - Safety
  - Supply Limitations
- Blinding
  - Sampling
- Analytical Testing and Product Release Considerations
  - Stability and Shelf Life
  - Storage and Handling
  - Labeling
- Shipping
- Quality Control and Quality Assurance Considerations
  - Environmental Monitoring and Control Systems
  - Equipment Qualification Systems
  - Analytical Methods Qualification/Validation Strategies
  - Document Generation, Review, and Approval Systems

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- DS and DP Release Considerations
- Product Quality and Stability Requirements

- Global Regulatory Strategies
  - US, EU, Canada, Japan, ICH, Importation/Exportation

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2 Definition and Types of Biologics

Currently, within the field of biotechnology, terminology and definitions are not standardized on how to describe biological products. Within North America, Europe, and Japan, publications such as the United State Pharmacopeia, European Pharmacopeia, ICH guidelines, FDA, and CPMP guidelines refer to biological products, among others, as:

- Biotechnology Derived Articles
- Biotechnological/Biological Products
- r-DNA Derived Protein Products
- Medicinal Products Derived from r-DNA technology
- Biotechnology Derived Products
- Bioproducts
- Biotherapeutics
- Biopharmaceuticals
- Biological Medicinal Products

Biologics or Biological Products are products that, generally, are manufactured under license in accordance with the regulatory requirements of the local Regulatory Authority. These local Regulatory Authorities have responsibility, including pre-market review, over the following categories of therapeutic biological products:

- Monoclonal antibodies for *in vivo* use in the prevention or treatment of disease
- Proteins intended for therapeutic use, including cytokines (e.g., interferons), enzymes (e.g., thrombolytics), and other novel proteins. Therapeutic proteins derived from plants, animals, or microorganisms and recombinant versions of these products are included in this category.
- Immunomodulators (non-vaccine and non-allergenic products) intended to treat disease by inhibiting or modifying a pre-existing immune response
- Growth factors, cytokines, and monoclonal antibodies intended to mobilize, stimulate, decrease, or otherwise alter the production of hematopoietic cells *in vivo*

Combination products comprise a biological product component with a device and/or drug component. This ISPE Good Practice Guide will focus on the above categories of biologics and will to refer to them using the term Therapeutic Biological Products (TBP). Additional categories of biologic products are listed below. They are covered by the Center for Biologics Evaluation and Research (CBER) and include:

- Cellular products, including products composed of human, bacterial, or animal cells (such as pancreatic islet cells for transplantation), or from physical parts of those cells (such as whole cells, cell fragments, or other components intended for use as preventative or therapeutic vaccines)
- Vaccines (products intended to induce or increase an antigen specific immune response for prophylactic or therapeutic immunization, regardless of the composition or method of manufacture)

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- Allergenic extracts used for the diagnosis and treatment of allergic diseases and allergen patch tests
- Antitoxins, antivenins, and venoms
- Blood, blood components, plasma derived products (e.g., albumin, immunoglobulins, clotting factors, fibrin sealants, proteinase inhibitors), including recombinant and transgenic versions of plasma derivatives (e.g., clotting factors), blood substitutes, plasma volume expanders, human or animal polyclonal antibody preparations, including radiolabeled or conjugated forms, and certain fibrinolytics such as plasma-derived plasmin, and red cell reagents

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3 Biopharmaceutical Product Development for Recombinant Therapeutic Bioproducts

3.1 Project Management and Planning

Therapeutic Biological Products (TBP) clinical development requires focused emphasis on creative problem solving accompanied by transparent risk management and planning strategies. This challenging combination is prompted by the inherent characteristics of TBP development programs that generally distinguish them from more conventional small molecule development in the following ways:

- Relatively longer timelines for critical path activities, especially those concerning process development and manufacturing
- Costs associated with making toxicology and clinical trial materials
- Alternatives to conventional non-clinical approaches, necessitated by potential species – specific pharmacologic activities of the therapeutic protein being developed
- Making more at-risk investments to support parallel processes
- Creating/using novel routes, formulations, and schedules of dosing
- Need for real-time stability testing due to lack of predictive models
- Requirement for compatibility studies as process and formulations evolve during stages of development
- Need for unique or specialized formulations and associated analytical technologies
- Designing clinical programs that support early strategies for mitigating risk, such as those that provide “Proof of Mechanism” or “Proof of Principle” (POP) results, given the final ‘commercializable’ form

Perhaps the greatest driver for these strategic considerations is the significant portion of total project cost that is directly related to process development and manufacturing components. For example, Figure 3.1 illustrates that more than 50% of a typical bioproduct development program budget through clinical Phase 2a is directed toward Chemistry, Manufacturing, and Controls (CMC) activities, with half of this cost directed to manufacturing the bulk drug substance (Figure 3.2).

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Figure 3.1: Percent of Overall Development Costs through Phase 2a by Key Functional Area

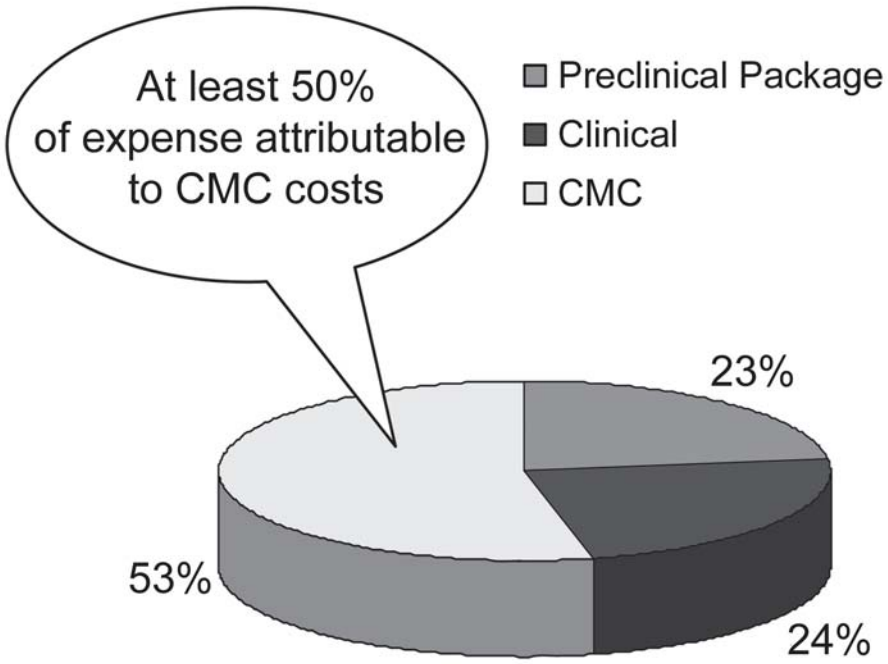
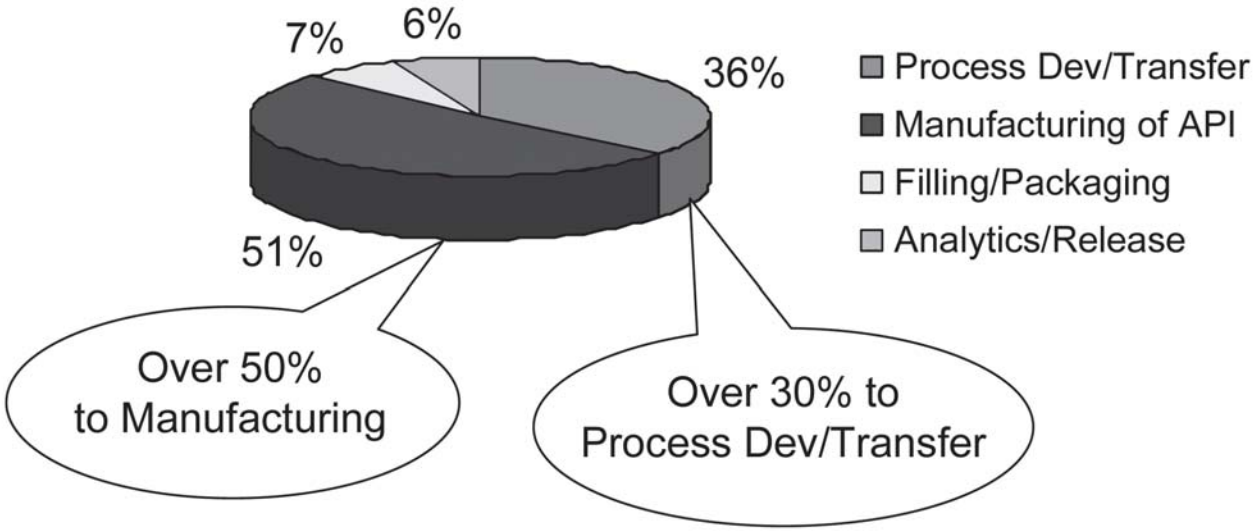


Figure 3.2: CMC Cost Breakdown by Percentage



Thus, the complexity of TBP development demands cross functional strategic and tactical involvement from the very earliest planning stages, and benefits from the use of four simple planning tools is recommended.

3.1.1 Project Planning Tools

1. Product Target Profile (PTP)

The PTP is typically an abbreviated summary of the minimum required attributes of the product that is being developed, prefaced with a statement of the intended indication and approvable endpoint(s). This document can be considered as an abstracted form of a package insert, and serves to focus all cross functional efforts on an agreed common target. An example of a simplified PTP is shown in Figure 3.3.

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Figure 3.3: Product Target Profile

Product: Widgets

Indication: For reduction of XXXX

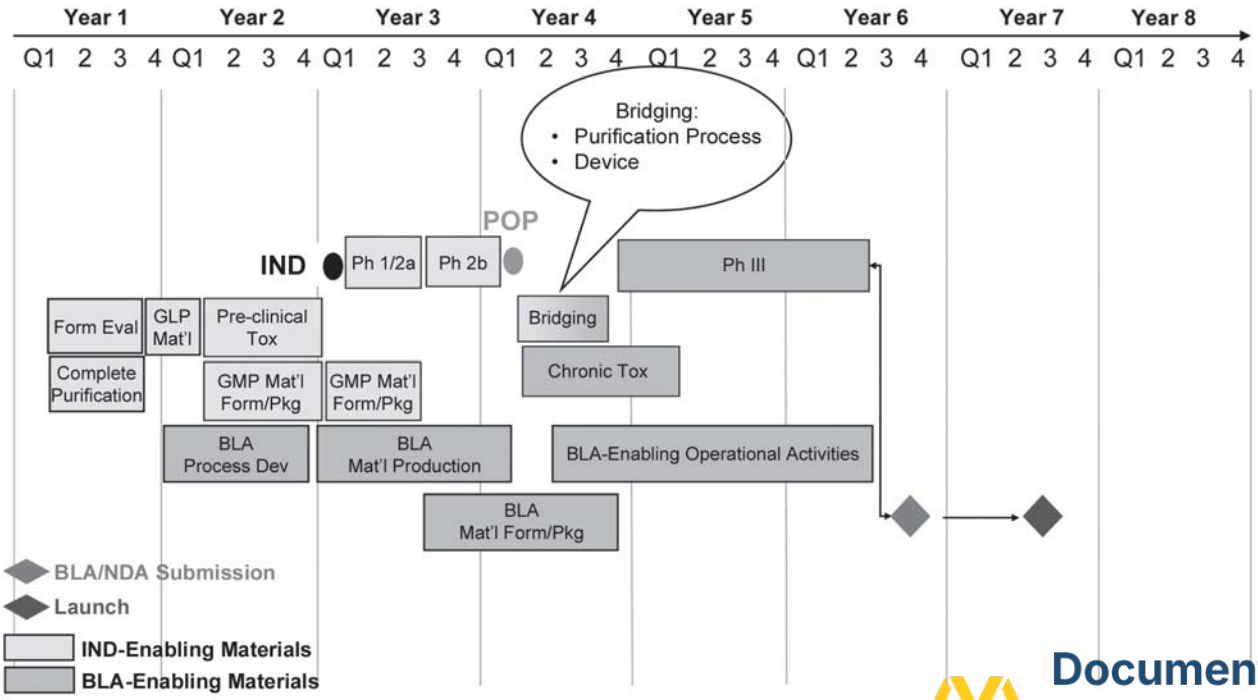
Target Launch: 2008

Attribute	Minimum	Phase
Pharmacology	Positive in X predictive animal model	Preclinical
Efficacy (USP)	40% reduction vs. Gidgets	Surrogate evidence, Phase 2; approvable endpoint Phase 3
Safety/Toler.	Comp. to Gidgets	Phase 1, 2, 3
Dosing	Once daily, s.c. inj.	Phase 1, 2, 3
Formulation	Liquid, 2-8°C	Phase 1, 2, 3
Device	Prefilled Syringe Prefilled needle-free device	Phase 1, 2 Phase 3

2. Project Timeline

The PTP is typically accompanied by a summary timeline that graphically illustrates the interplay of all relevant cross functional areas at a high level. The components of this timeline are extracted directly from the PTP and are arrayed such that the timing of collaborations, transfers, and handoffs between functions are clearly visible. In particular, the nature of the TBP that is manufactured in support of each development stage should be described. An example is given in Figure 3.4.

Figure 3.4: Widget Clinical Development Overview (coded by CMC stage)



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3. Clinical Supply Profile (CSP)

Figure 3.5 shows a summary of a Clinical Supply Profile, which is a natural extension of the PTP and the project timelines shown above. This tool is essential to ensure that enough high-quality investigational product is available and accessible in a timely manner to support clinical trials as well as the requisite supporting stability and release testing programs. Frequently, clinical materials are in limited supply, and careful planning is necessary to avoid waste and unnecessary excess inventory. A chart such as the one below serves as a useful reference to manufacturers to gauge how many grams of material and final container product lots will be needed and by when they will be needed.

Figure 3.5: Clinical Supply Profile Overview

Product:	Widgets
Trial/Location:	Phase 1 safety study/USA
Shipment Date:	11 April 20XX
Delivery Date:	12 April 20XX
Study Start:	19 April 20XX

Attribute	Comments
Dosage Form	Liquid for once daily s.c. injection
Comparator Required?	No
Number of Arms	4; 1 Placebo, 3 Active
Patients per Arm	5
Vials Needed	10 Placebo; 20 Active
Fill Size	1 ml Placebo; 1 ml active @ 10 mg/ml
Placebo	Behicle
Diluent	Commercial phys. saline, 35 x 10 ml vials
Syringes	50 x 1 cc tuberculin
Storage	2-8°C (for up to X weeks)
Blinding Needed?	No: on site pharmacist

4. Manufacturing Process Profile (MPP)

During biopharmaceutical development, it is essential to capture a general history of how the manufacturing and analytical processes have evolved, along with a record of the key process/product pedigrees. The MPP (see Figure 3.6) can serve as a platform for regulatory submissions concerning CMC issues, and for technology transfer activities to contract manufacturing organizations. It should be considered a living document that is updated as information about the product characteristics is generated.

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Table 3.6: Essential Components of a Manufacturing Process Profile

Intended point(s) of use (US? Europe?)
Schematic diagram of all process steps
Sampling strategy/handling
Which assays are performed when
Definition of hold steps
Definition of release criteria
Description of stability-indicating assays and criteria
Description of storage and handling conditions
Consideration of options/alternatives for special needs of project (limiting amounts of recombinant protein product)
TSE risk evaluation
Process/Product pedigree and disposition

3.2 Development Phases

3.2.1 Preclinical Phases

The tools described in this section serve as a solid foundation against which to "map" the required preclinical and clinical phases of biopharmaceutical drug development. The first of these two stages concerns the demonstration in animal species of the biological efficacy, the safety and toxicology profiles, and the pharmacodynamic properties of the drug in question. These studies are planned in an ascending order of stringency, ultimately resulting in a high quality data package that is submitted to regulatory authorities in support of the subsequent clinical development program.

3.2.1.1 Preclinical Modeling (Proof of Mechanism or Pharmacology)

Human-based biopharmaceuticals may have limited pharmacologic activity in non-human systems. In some cases, when performing animal pharmacology experiments with biological molecules only certain animal species can serve as appropriate models. This testing may require the use of special genetically modified animals in which human-derived proteins can be tested in an otherwise foreign background. Another approach some programs rely on is the use of surrogate *in vitro* or *ex vivo* endpoints (using human or primate cell systems) to validate the mechanism of action. Significant immunological reactions also may limit the usefulness of some animal systems, particularly in repeat dosing experiments. In addition, the pharmacology phase of preclinical programs yields essential guidance for defining those bioanalytical tests that will need to be conducted in the clinical phases of development.

In general, materials for use in these experimental stages can be research grade, as long as they have acceptable level of endotoxin and can be demonstrated to be stable, intact, and active under the conditions of use.

3.2.1.2 Preclinical Toxicology (Pilot Studies; Non-GLP)

Pilot toxicology studies often are performed in advance of pivotal GLP programs for the purpose of confirming that the targeted dose(s) to be carried forward into the regulated portion of development are appropriate and are unlikely to result in unexpected toxicity or tolerability issues. Pilot studies of this type also are useful as another risk mitigation step to confirm dosing in further studies, thereby reducing material requirements and potential wastage of costly clinical GLP grade drug substance/product.

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Pilot studies need not be performed with GLP material, but the material that is used should have associated documentation as to its quality and should be representative of the material that will be used in pivotal studies.

3.2.1.3 Regulatory Toxicology (Pivotal GLP Studies)

Pivotal toxicology (and other regulated studies) is conducted to demonstrate the safety, tolerability, and pharmacokinetic attributes of the biopharmaceutical using multiples of the intended clinical dose, route, and schedule of administration usually determined from the pilot toxicology studies. Typically, pivotal studies are conducted in two different animal species and usually require significantly more material than the clinical studies they are designed to support, at least in the early stages of development. Toxicology studies often last much longer than anticipated because of the way in which the data are collected and processed. Toxicology studies have an in-life portion during which animals are dosed with drug or placebo, followed by necropsy and/or a period of recovery, followed by necropsy. Tissues are then prepared for sectioning and histopathological evaluation. The period following necropsy can extend for between three and four months, depending on the size and complexity of the study. The results should be reviewed by the appropriate quality group, and the report should be included in regulatory submissions. Therefore, a 28 day (in life) toxicology study may actually take seven months from start to receipt of a draft report.

Over the lifespan of a clinical development program, several toxicology studies likely will be required with the goal of evaluating the safety of the drug over progressively longer administration times. One obstacle faced by developers of biopharmaceuticals is the potential for antibodies against the human drug to develop in the animal species upon continued administration. The impact of these findings is dealt with on a case-by-case basis, but may result in the need to confine such studies to non-human primate species.

Materials for pivotal studies should be representative of those that will be used in the clinical program, and should have appropriate documentation regarding testing results, performed under GLP conditions and procedures with analytical assays qualified for this stage of development. Sometimes the material used for regulated preclinical studies is made as one lot of a larger manufacturing campaign from which the subsequent clinical material is also derived.

3.2.2 Clinical Phases

Clinical development is divided, in general, into three segments that assess safety and efficacy of the biopharmaceutical product. Each phase is designed to balance the risk of continued development with the potential benefit to patients, as well as identify any potential long term risks. A product that proves to be both safe and effective will be submitted to the regulatory agencies for marketing approval.

3.2.2.1 Phase 1

Clinical Phase 1 studies are designed to test the safety and tolerability profile of a drug in a very controlled, stepwise approach, usually starting at a single dose that is below the preclinically-determined No Adverse Effect Level. These “first in man” studies are generally carried out in healthy volunteers, but there are exceptions in which Phase 1 studies employ patients with the targeted disorder. Following safe completion of the single dose administration, subjects may then enter a second stage in which they receive repeated doses over several days.

The data from the Phase 1 study or studies is used to determine the dosages and dose frequency to be studied in the Phase 2 studies. It also may be used to determine preliminary efficacy endpoints that may be used as potential endpoints for further studies.

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3.2.2.2 Phase 2

Historically, Phase 2 studies are conducted to evaluate the efficacy of various doses of a drug; these studies are often referred to as “Dose Ranging Studies.” The Phase 2 studies usually have multiple arms, one of which maybe a placebo or comparator, the others being different dosages of the drug, from potentially subtherapeutic to the maximum effective dose identified in the Phase 1 trial(s). An important objective of such trials is to identify the dose that will be carried forward into the larger Phase 3 trials pending successful impact on a predetermined primary endpoint(s).

Phase 2 studies also are used to determine the suitability of potential endpoints for the Phase 3 studies. The importance of choosing the correct primary endpoint in a Phase 3 trial is the key to the approval of a new drug. Regulatory agencies are increasingly looking for endpoints that have a clinical outcome (i.e., patients can live longer, walk further, have less seizures, etc.) versus so called surrogate outcomes (i.e., reduction in the levels of urinary substrates excreted by a patient, plasma levels of a target protein are decreased, etc.) on which to base their approval. The Phase 2 studies can be used to demonstrate which outcomes have the potential to be used as the Phase 3 primary or secondary endpoints. They also can be used to identify potential alternative indications of a product.

A new variation on Phase 2 strategy is emerging with the proliferation of new biopharmaceuticals with novel mechanisms of action. Increasingly, Phase 2a or Phase 1/2 studies or “Proof of Concept” studies are being designed to validate both the efficacy of the drug and the relevance of the mechanism of action in the disease of interest. Therefore, Proof of Concept studies are characterized by administering the drug at the highest dose and/or using the most aggressive schedule that has been shown to be safe and tolerable in Clinical Phase 1 and Preclinical Toxicology, as compared with placebo (and/or comparator). The intent is to provide an early exit point to eliminate the biopharmaceutical candidate from further resource intensive development if it does not perform, even under maximized conditions. The advantage of this strategy is in the risk reduction it provides for choosing between future development opportunities.

Frequently, the Proof of Concept strategy results in the need for subsequent Phase 2 study(s) (Phase 2b) that will provide the more conventional dose ranging information with which to enter Phase 3.

However, in general the Investigational Product used to support the Phase 2 studies should be designed to be reflective of the product used in the Phase 3 studies. Any manipulations of the product should be kept to a minimum to ensure consistency throughout the studies. However, the Phase 2 studies provide an excellent opportunity to “field test” and refine the formulation, handling, preparation, and administration procedures prior to the Phase 3 studies.

3.2.2.3 Phase 3

The Phase 3 trials are designed to be the definitive test of the safety and efficacy of the new drug at the optimal dose and frequency as determined by the Phase 2 program in the population the drug is intended to treat. These trials are usually randomized, double blind, placebo (where possible), or comparator controlled studies that seek to show a statistically significant difference in a predetermined primary endpoint that has been deemed ‘approvable’ by the relevant regulatory agencies. The status of “approvable” is dependent in a large part on the outcome of the endpoints that were refined in the Phase 2 trials. Phase 3 trials can involve more than 1000 patients due to the requirement for demonstrating safety in a larger patient base, more reflective of the general population.

For new drugs used to treat orphan diseases and new drugs used to treat cancer and HIV, approval may be granted with smaller Phase 3 trials. This may be due to the rarity of patients, as in orphan diseases, or the necessity to bring new therapies to the market more rapidly. Approvals granted in these accelerated cases often come with significant post marketing commitments to confirm the safety and efficacy of the drug when exposed to larger patient numbers.

The Investigational Product used to support the Phase 3 trials preferably are produced in the facility and at the scale intended to support the launch of the commercial product. With minor differences, the procedures for the handling, storage, preparation, and administration of the product used in the Phase 3 trials should be the same as those intended for the package insert of the commercial product.

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Once the Phase 3 studies have been completed, the data analyzed, and assuming a successful conclusion to the endpoint, submitted to the regulatory agencies, the product will be considered for regulatory approval.

3.2.2.4 Phase 4

Phase 4 studies are designed to confirm the safety and efficacy of the drug after marketing approval has been granted. These studies may be required by regulatory agencies, as in the case of accelerated approval products, or market pressures. Many biopharmaceutical agents fall into this category since they are used to treat special patient populations. These Phase 4 studies also may be used to identify new indications for the new drug. In some cases, Phase 4 studies may be Investigator Sponsored Trials (IST). ISTs are physician based studies exploring indications that are "off-label," but may be of interest to the manufacturer. Since the investigator is the sponsor, the manufacturer is only required to supply product and/or funding. ISTs are a very useful tool in minimizing the costs associated with expanding a product's label since the costs are considerably less than those associated with traditional registration studies (Phase 1, 2, and 3).

3.2.3 Comparability and Bridging

Previous sections describe the complexity of manufacturing a TBP. This complexity is amplified during clinical development because of the changes in manufacturing scale and venue that are required to support eventual commercialization. To ensure control and reproducibility over the evolution of the manufacturing process, several tools are available to evaluate and demonstrate product comparability. These are further discussed in this section of the Guide.

There are many sources of complexity in biopharmaceutical process development. In addition to numerous variables of the production bioreactor (e.g., temperature, pH, dissolved oxygen, medium components, and feed components), myriad purification steps also can affect TBP quality:

- Selection and Operating Conditions of Chromatographic Steps
- Filtration Steps
- Viral Inactivation Steps

Therefore, a robust process is necessary to ensure a consistent TBP. Changes in the manufacturing process may affect process and/or product related impurities or product related substances (e.g., altered levels of deamidation, oxidation, glycosylation, and sialylation).

As a product moves through stages of development, changes in manufacturing processes usually are required. For example, the manufacturer may change a cell line, a cell growth medium format, a purification step, and perhaps other variables to increase product yield or to lower impurity levels. Introduction of a new formulation may be needed to improve product stability. A change in scale and/or site may be required to generate sufficient product for larger clinical trials and/or commercial launch. Manufacturers also have to comply with new regulatory requirements.

Any of these changes may affect the quality, safety, or efficacy of the TBP. However, to have to repeat potentially multiple clinical trials with each process change would not be feasible (from either a time or cost perspective). The regulatory agencies recognize this, and to facilitate expeditious drug development, it has issued guidance on comparability.

To demonstrate that the TBP made by the current manufacturing process, and that of a new manufacturing process, have matching quality, safety, and efficacy, it is necessary to demonstrate that the materials are comparable and appropriate for their intended use. The two TBP need not be identical. Indeed, it is highly probable that there will be changes in specific physical characteristics (e.g., glycosylation). What is critical is that these changes do not negatively affect the quality, safety, or efficacy. If the manufacturer can obtain data that is reasonably predictive of the new process being comparable with the old, the regulatory agencies may allow the manufacturing changes without additional clinical trial(s) or animal studies.

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The regulatory agencies recommend that manufacturers consider using one or more of the following options to demonstrate comparability:

1. Analytical Testing

2. Functional Assessment (e.g., Biological Assays)

3. Animal Studies (e.g., PK Study, PD Study, Toxicity)

4. Clinical Studies (e.g., PK Study)

While each subsequent step involves greater complexity, this hierarchy of options does not mean that success or failure in one step automatically triggers the next entry. Instead, the manufacturer should decide what combination of options will best underwrite comparability. For one compound, a combination of option 1 and 2 with a PK study from option 4 may be required; a second compound may only need option 1 and 2.

Two key factors in choosing what options to employ are:

1. Stage of Development

2. Degree of Change

For the stage of development, products in later stages of development (Phase 3 or post-approval) will require greater comparability confidence than compounds in early development (e.g., Phase 1 or Phase 2).

For the degree of change, greater amounts of change require concomitant amounts of confidence.

The FDA notes that if a manufacturer is making simple changes to drug product, such as storage container or filling site, the manufacturer may need to provide only analytical release data that meets specifications and product stability data. Manufacturers often prefer making multiple changes at once, rather than undertaking a series of individual changes requiring multiple comparability efforts. Analytical and functional testing alone may be sufficient as proof of comparability, but must be agreed by open discussion with the regulatory agencies.

Manufacturers perform extensive testing of materials to support comparability beyond standard release testing methods. The FDA recommends that assays include the typical release assays for drug substance/product, as well as additional assays for characterization. To show comparability for Synagis® manufactured by two different processes, a battery of assays was employed, including: MALDI-TOF mass spectrometry, SDS-PAGE (reducing and non-reducing) capillary gel electrophoresis, capillary isoelectric focusing, Western blotting, size exclusion chromatography, microneutralization, and others.

When available, a functional assessment (ELISA-based, cellular bioassay, etc.) can provide value in two ways.

1. It can clarify whether the bioproducts from the current and new processes have similar activity (potency).

2. Bioassays can help reveal whether a change in structure (especially secondary and tertiary) may affect quality. With some compounds, a bioassay is not available, because either the mechanism of action is poorly understood or the variability of the assay is unmanageable.

Results of the analytical and functional assessment can lead to several outcomes:

- No substantial changes in the bioproduct are detected.

- Small changes in the quality of TBP are detected; based on current knowledge and manufacturer's experience, these changes won't affect the safety or efficacy.

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- No substantial changes in the quality of TBP are detected, but assays may not be sufficient to justify no impact of safety and/or efficacy; additional non-clinical and/or clinical work is needed.
- Small changes in the quality of TBP are detected and may affect safety and/or efficacy; additional non-clinical and/or clinical work is needed.
- Major differences are detected, suggesting the products of the old and new processes are not comparable.

To support comparability assessments, the agencies encourage companies to approach them with their strategy.

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4 Process Development and Manufacturing Considerations for Recombinant Therapeutic Bioproducts

4.1 Introduction

Processes to manufacture TBPs are designed by scientists with expertise from a wide variety of disciplines, including cell physiology, protein chemistry, and engineering. Initially, the molecule is identified by discovery scientists expert in molecular targets, disease states, and production/characterization of research quantities of materials. Once a candidate is nominated for development, cell lines are constructed with protein expression systems that are adequately robust to allow production of commercial quantities of materials with manageable levels of product-related impurities. After the cell line is available, representative materials produced in the early cell growth and recovery systems are used to begin experiments to identify optimal excipients for the design of a formulation to maintain the integrity of the protein during storage and use.

In parallel with formulation activities, drug substance manufacturing process development scientists create cell growth and protein recovery processes to produce materials appropriate for use in regulatory toxicology and early (Phase 1) clinical studies. During this stage of development, it is important to consider that the process partially defines the final molecule, due to limitations of analytical techniques to characterize these large, complex macromolecules. The manufacturing processes evolve as the product moves through stages of clinical testing; hence, comparability exercises of increasing sophistication are required to demonstrate that the materials used for early clinical and safety studies are representative of the protein that is taken to market. As processes near registration, each step of the manufacturing process is validated to demonstrate that it is robust under normal operating conditions, and that the limits of the process are well characterized and understood, especially with respect to the impact on the quality of the molecule.

4.1.1 Expression System Development/Cell Line Development

4.1.1.1 Introduction

Protein expression is used to provide research reagents, vaccines, industrial enzymes, and therapeutic proteins. The selection of an expression system for commercial therapeutic proteins is based upon an amalgam of technological, economic, and regulatory factors. The TBP should meet precise specifications that include the primary amino acid sequence, post-translation modifications, and biological activity. Economic factors include:

- Fermentation Expression Levels
- Downstream Processing Yields
- Processing Costs
- Product Quality
- Product Safety
- Amounts of Material Required to Supply the Anticipated Market Demand

The best system for producing a specific protein is a system that meets both the material requirements and protein quality standards with the lowest production costs; however, this may prove challenging.

The molecular biology of protein expression technology has evolved dramatically from its inception in the 1970s. Currently, there is a diverse number of expression systems available, including:

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- Bacteria
- Yeast
- Plants
- Transgenic Animals
- Mammalian Cells
- Cell Free Protein Synthesis

Manipulation of the host, expression system, and gene encoding the protein can result in higher expression levels and improved product quality, but the inherent biochemical nature of the protein product itself may significantly impact the development of a successful expression process. The three most commonly used general classes of expression systems are:

1. Bacterial Cells
2. Yeast Cells
3. Mammalian Cells

4.1.1.2 Bacterial Expression

Commercial bacterial-based therapeutic protein production has relied primarily on expression systems developed using *Escherichia coli*. There are several advantages to a bacterial approach, such as the shortened development and process times owing to the rapid growth rate of the bacterial cells, the existence of a wide variety of expression technology, and the inherent low fermentation costs. *E. coli* has historically been one of the best-studied bacteria and as a result, there is a vast amount of scientific information available. Genomic and proteomic technologies for bacterial systems are very advanced, and host engineering technologies are widely used. The availability of sophisticated technological tools often makes *E. coli* a first choice for protein expression. Several other bacterial hosts that are used for protein expression include:

- *Pseudomonas fluorescens*
- *Bacillus subtilis*
- *Bacillus megaterium*
- *Staphylococcus carnosus*

The genome has been determined and annotated for *E. coli*, *P. fluorescens*, and *B. subtilis* and genomic-based technologies such as microarrays can be utilized with these hosts.

Bacterial expression uses small DNA vectors (3 to 5 kb), that replicate independently of the bacterial genome, into which the gene encoding the protein product can be cloned. These vectors are derived from naturally occurring plasmids with different DNA replication mechanisms. The per cell copy number of vectors can vary over two orders of magnitude. Large vectors tend to have a lower cellular copy number and are not as readily maintained by the host. The vectors encode a selectable marker used to transform the vector into bacterial host and the selectable marker can be used for vector retention. Typically, a gene encoding antibiotic resistance is used, but genes that complement specific host auxotrophic deficiencies also are used. The gene encoding the protein product is transcriptionally

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controlled by the use of a regulatable promoter. Highly efficient promoters are used for bacterial protein expression, and unregulated strong promoters themselves can be deleterious to the cell and inhibit cell growth. A distinct feature of bacterial systems is their ability to regulate transcription effectively. Regulated promoters can be activated by the addition of a gratuitous inducer, by the depletion of a medium component, or by temperature changes. A regulatable expression system helps to avoid cellular toxicity caused by constitutive expression of a protein product. The gene for the protein product is followed by a transcriptional terminator that prevents transcriptional read-through into other regions of the vector. Uncontrolled transcriptional read-through can result in vector instability, and the additional of transcriptional terminators can enhance mRNA stability. Translation efficiency can be influenced by the sequence of the ribosomal binding region, by RNA secondary structure, and by the gene's codon usage.[1]

There are two bacterial expression modalities:

1. Expression into the Cytoplasm

2. Expression into the Periplasmic Space using a Secretion Signal Sequence[2]

There are examples of protein expression into the medium, but secretion into the medium cannot be performed in a predictable fashion. Proteins from the cytoplasm often retain the N-terminal methionine originating from the translational AUG initiation codon. This can be an issue, particularly for mammalian proteins that often undergo processing to remove N-terminal sequences during protein maturation. By encoding an N-terminal secretion signal sequence into the gene, proteins can be directed to the bacterial periplasm. The signal sequence is cleaved from the protein as it enters the periplasm. This provides a means of producing proteins that do not contain the N-terminal methionine, and the oxidizing periplasmic environment allows the formation of disulfide bonds. Expression levels of proteins vary widely, but often expression levels of >20% of the total cell protein and fermentation titers of 1 to 2 g/L are routinely achieved. In some cases, expression levels of >5 g/L have been achieved.

There are several potential limitations to bacterial protein expression. Proteins expressed in the cytoplasm show an increased tendency to aggregate and often form dense aggregates that are refractive under a light microscope. These aggregates have been termed inclusion bodies. Refolding of the protein after isolation from inclusion bodies is required for biologic potency. Soluble expression of protein in the *E. coli* cytoplasm can occur, but the reducing environment of the cytoplasm will limit disulfide bond formation. If disulfide bond formation is required then secretion into the oxidative periplasmic environment would be preferred. The protein folding pathways in bacterial cells are more rudimentary in comparison to mammalian cells. Although manipulation of chaperones in bacteria has been successfully employed to fold non-native proteins,[3] bacteria may not be suitable for expression of heterodimeric or multimeric proteins, and successful commercial proteins are typically small monomeric proteins such as insulin, somatotropins, antibody fragments, and cytokines. Proteolysis and proteolytic clipping of improperly folded proteins both in the cytoplasm and in the periplasm can reduce protein yields and generate product related impurities. Gram-negative bacteria such as *E. coli* produce lipopolysaccharides (LPSs) that are a component of the bacterial outer membrane. Unfortunately, the LPSs are endotoxins and can cause a wide range of non-specific pathological reactions, and must be eliminated during protein purification. Finally, although there are examples of some bacterial species glycosylating proteins, the glycoforms are distinctly different from mammalian glycoforms structures. *E. coli* and *P. fluorescens* do not post-translationally glycosylate proteins.[4] Glycosylation requires the more complex protein synthesis machinery of a eukaryotic cell.

The bacterial expression strain (host and expression vector) should be genetically stable and the cell banks should be absent of bacterial, fungal, or bacteriophage contamination. Bacterial systems are naturally free of mammalian virus. Media should be animal component free and chemically defined, and the medium components should not be produced using animal derived products.

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