Development of Biotechnology Drug Products: Critical Issues in Manufacturing and Quality Controls

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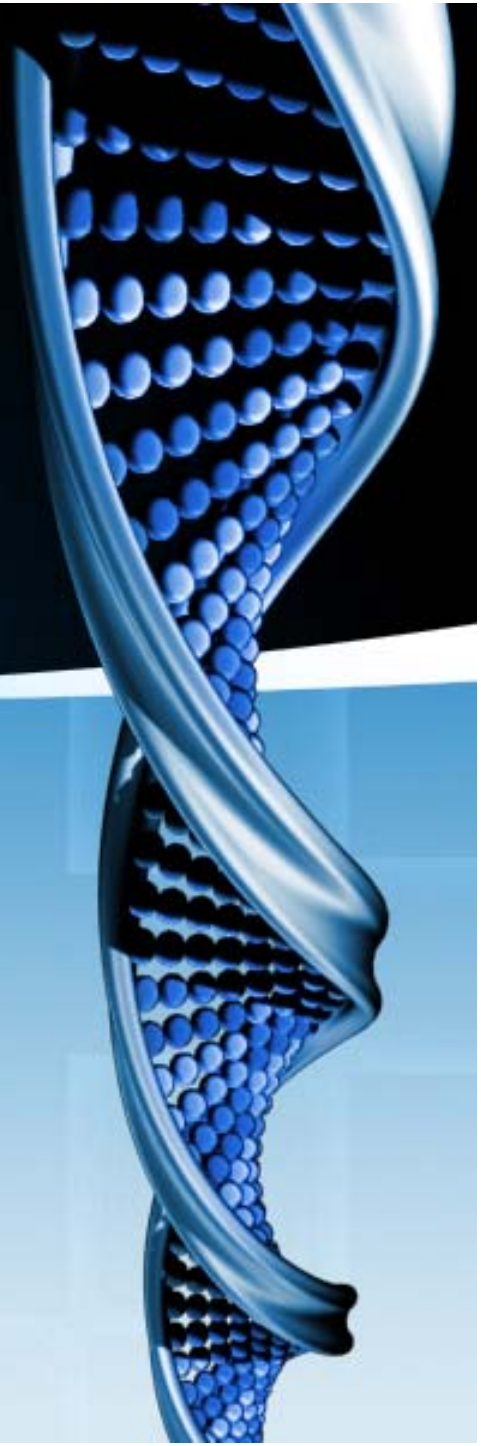
A Balance Between Science and Common Senses

生物技术药物的生产与质量控制

- 建立科学与常识之间的一种平衡

2010 International Forum of Pharmaceutical Engineering,
June 2-3, Beijing, China



Disclaimer

My remarks today do not necessarily reflect the official views of the US Food and Drug Administration (FDA).

声明：

1. 所讲内容纯属个人观点，不代表 FDA 官方立场。
2. 引用时请注明出处。

Agenda

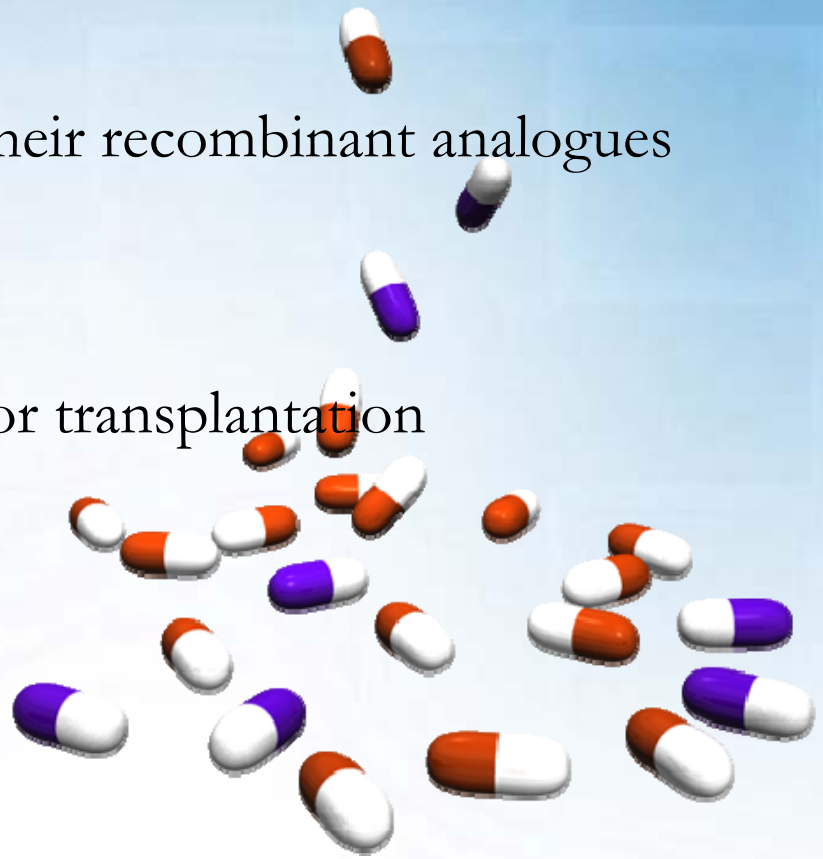
- Biological Products
  - Product types
  - Biologics vs. Drugs
  - Manufacturing process
  - Quality controls
- Major Issues
  - Heterogeneity 不均一性
  - Immunogenicity 抗原性
  - Comparability 可比性

Types of Biological Drug Products
生物制品药物的种类

- Therapeutic proteins
- Monoclonal antibodies for human use

- Blood, plasma derivatives, and their recombinant analogues
- Allergenic products
- Vaccines
- Human tissue/tissue products for transplantation
- Cells & gene therapies

- Combination therapies
  （e.g. pre-filled syringes)



Therapeutic Proteins
蛋白质药物

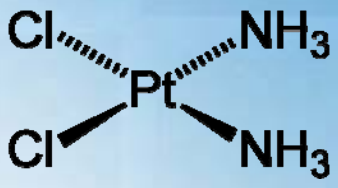
- Cytokines (interferon-α, β, γ; interleukins)
- Chemokines
- Growth factors (EPO, G-CSF, PDGF)
- Human Growth Hormones
- Immunomodulators
- Enzymes (pancrelipase, tPA, urokinase)
- Toxin conjugates (DT, Ricin)
- PEGylated proteins
- Derivatives from plants, animals, or microorganisms, and recombinant versions of these products

Unique Attributes of Protein Therapeutics

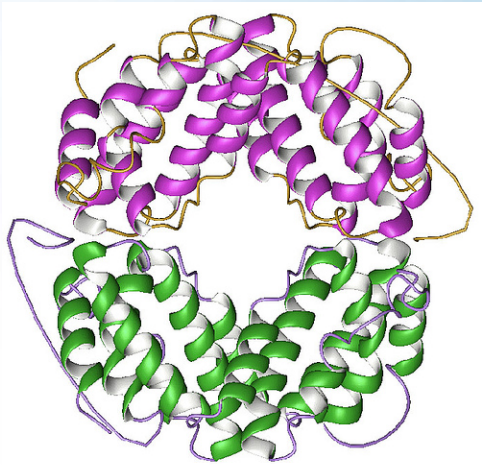
蛋白质药物的特殊属性

- Larger size ~ 5000 – 300,000 Da
- Higher order structure
- Complex manufacturing process
- Source of living organisms
- (Ability) to transmit infectious agents
- Usually must be injected or infused directly into the bloodstream to be effective – IV, SC or IM.
- Heterogeneity
- Immunogenicity

Cisplatin
(MW = 300)

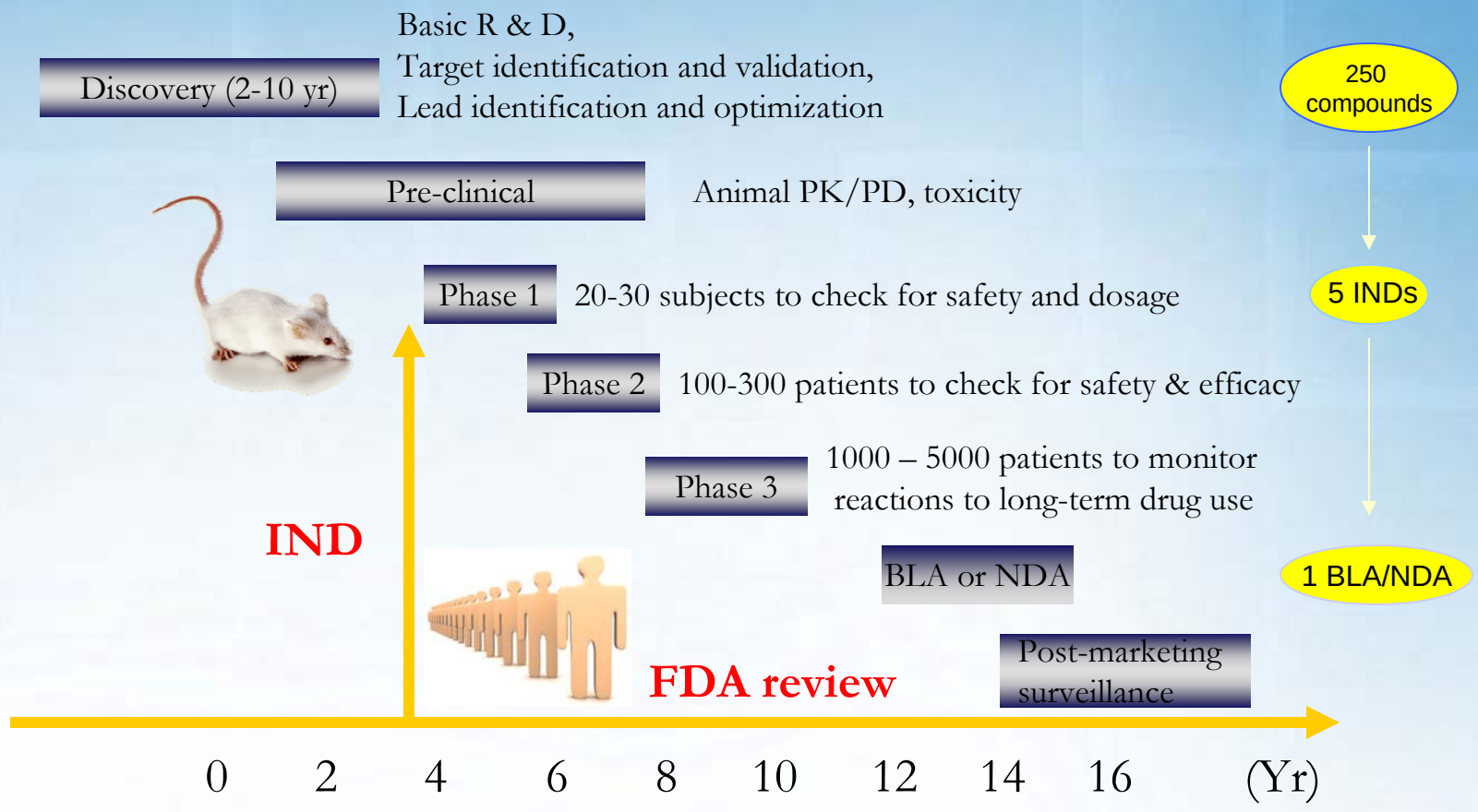


Interferon-γ
(MW =17146)



Stages of Drug Development

药物开发研制的主要阶段



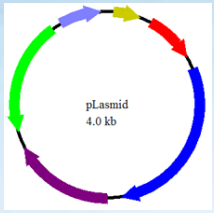
Typical cost: 10-15 years; $800 M; <20% approval (Ernst & Young).

The Evolution of Biotechnology
生物技术的演化

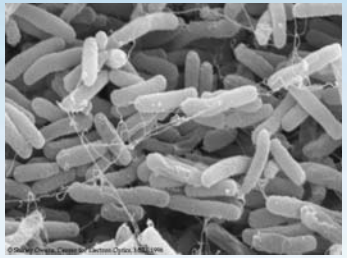
- 1970s, recombinant DNA technique (Herbert Boyer).
- 1982, recombinant human insulin (Humulin) approved by FDA as the first biotech therapy (Genentech & Eli Lilly)
- 1986, first therapeutic monoclonal antibody anti-CD3 (Janssen-Cilag).
- As of May 2010, FDA approved ~ 360 biopharmaceutical drugs (biopharma.com).
- By 2010, >50% of newly approved medicines will be biotechnology-based products. (BIO)

Manufacturing Process for Recombinant Protein Products 重组蛋白质药物的生产过程

Cell Banking



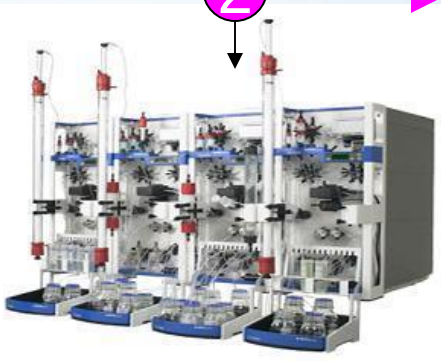
1



Fermentation

2

- Process Design
- Optimization
- Scale-up



Purification

3

Drug Substance

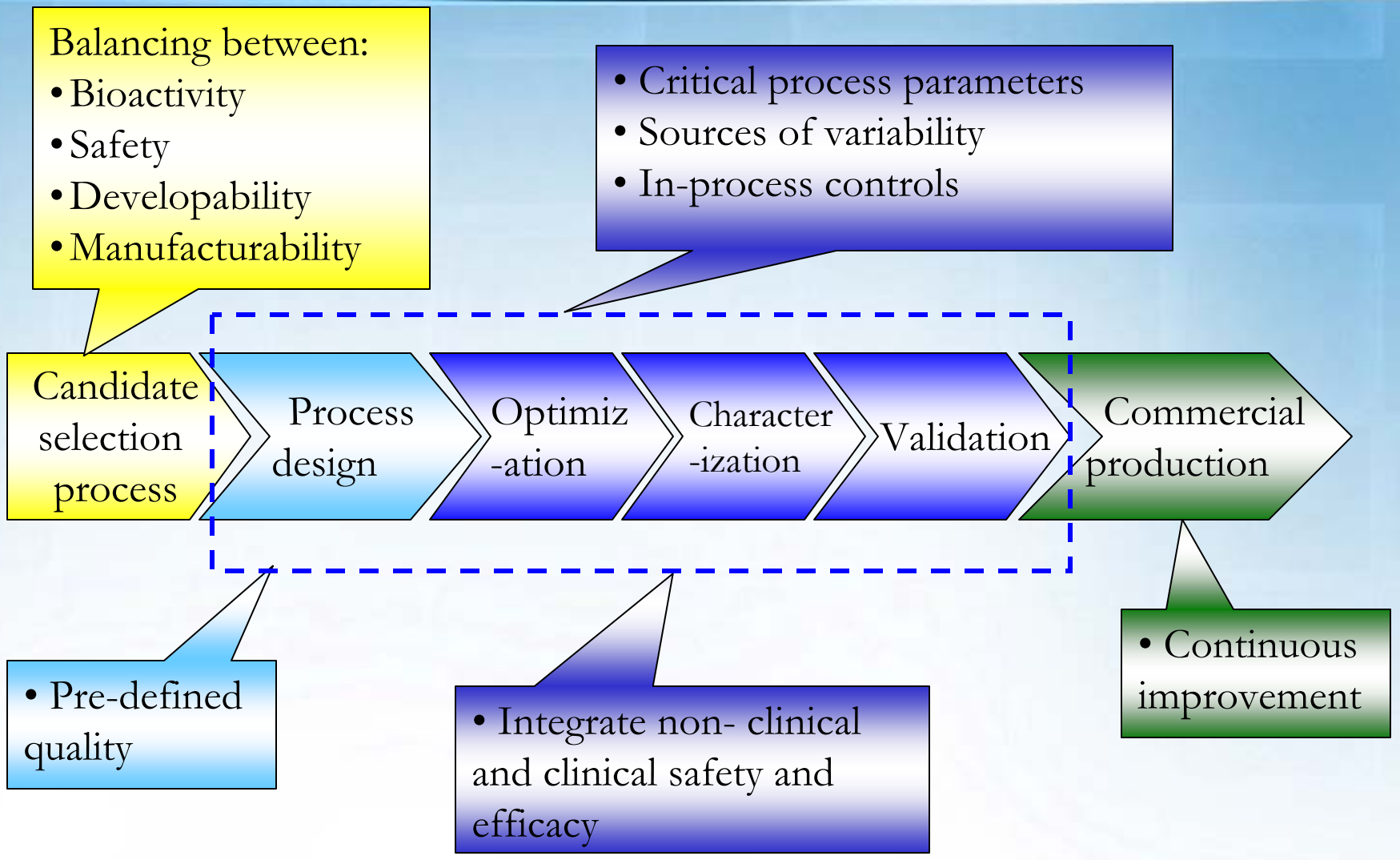
4

Drug Product

- Characterization
- Formulation

- Packaging
- Labeling
- Release testing

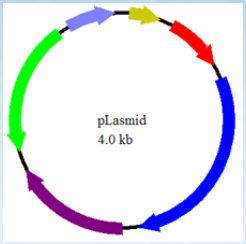
Process development
生产工艺流程的设计与改进



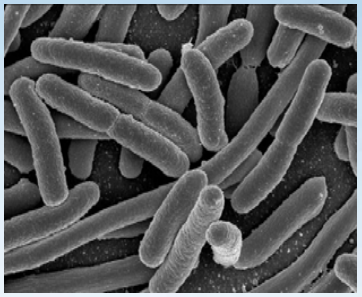
Expression System – A Critical Decision to Make
基因表达系统 – 成功的关键

Gene → Cloning → Plasmid → Transfection → Host Cells

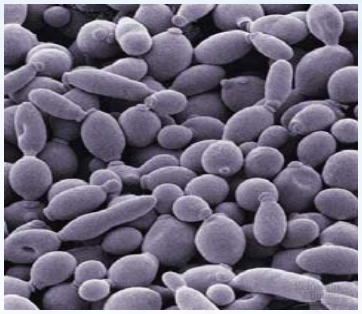
Source Sequence Modification

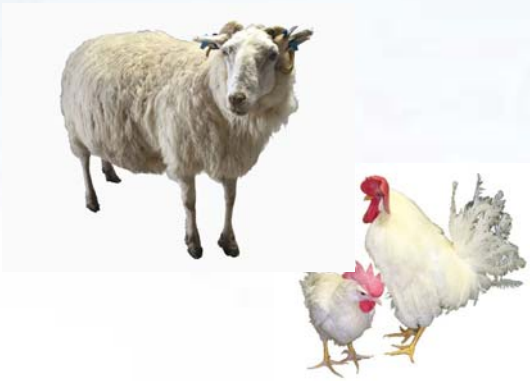


Origin Map

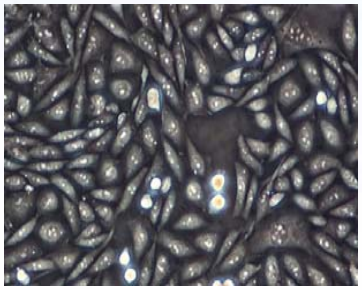


Pichia pastoris





CHO
HEK293

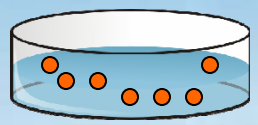


Advantages

E. Coli:
- Simple vector construction
- Rapid cell growth
- High intracellular expression levels
- Inexpensive media
- Yield: up to 7 g/L

Yeast & CHO:
- Post-translational modifications
- Natural secretor
- Yield: 80's (5-50 mg/L)
  Future (10-20 g/L)

Cell Banks 细胞库

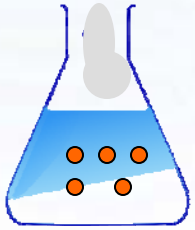


Single clone

Master Cell Bank (MCB)

主细胞库

Single vial



Working Cell Bank (WCB)

工程细胞库

Characterization:

- Identity
- Purity
- Copy #
- Viability
- Microbial contamination
  (Bacteria, fungus, mycoplasma)
- Adventitious agents
- Genetic stability (EPC)

End of Production Cells (EPC)

Protein Production
蛋白质的生产与纯化

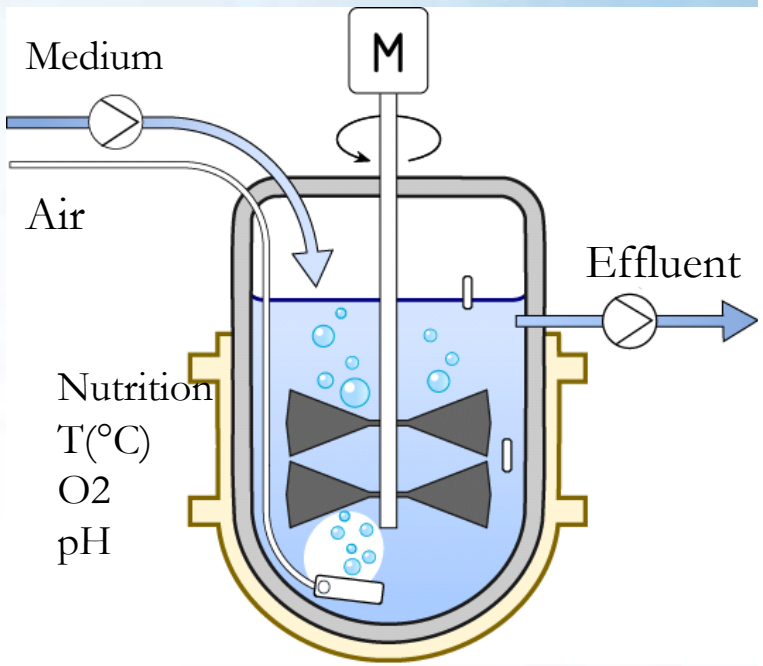
Fermentation:

- Nutrition – serum vs serum-free medium
- Environment – pH, temperature, oxygen supply
- Raw materials – any animal origin?

Purification:

- Product-related impurities
  - aggregates, degraded species
  - charge variants
  - mis-folded species
- Process-related impurities
  - host cell protein
  - host cell DNA
  - media components

1 L – 20,000 L



Formulation 配方/制剂

- Formation: Lyophilized or liquid
- Buffer composition: Surfactants, salts, polymers, pH
- Container-closure
- Storage conditions: -80, -20， 4-25°C
- Key points to consider:
  - Stability
  - Convenience of delivery
  - Economy

Typical Release Tests for Protein Therapeutics
原料药和成品药的验收质量检验指标和方法

Tests	DS	DP	Methods/Assays
Appearance	√	√	Visual
pH	√	√	USP<791>
Strength	√	√	UV
Identity	√	√	Peptide mapping, N-, C-terminal sequence, WB
Purity	√	√	RP-HPLC, SEC, SDS-PAGE
Potency (bioassay)	√	√	Product-specific
Impurities	√	√	RP-HPLC, SEC, SDS-PAGE
Host Cell Protein	√	√	ELISA for Total HCP
Host Cell DNA	√	√	< 10 ng DNA/dose (WHO limit)
Endotoxin	√		USP<85>, 5 EU/kg body weight/hr
Moisture	√	√	USP<921>
Particulate Matter		√	USP<788> NMT 6000 particles ≥ 10 μm; 600 ≥ 25 μm,
Bioburden	√		USP<61>, total microbial count, fungus
Sterility		√	USP<71>

Why a Potency Assay?
测定生物活性/效价

- ICH Q6B "Complex molecules, the physicochemical information may be extensive but unable to confirm the higher-order structure…, which, however, can be inferred from the biological activity"
- To assure consistent dosing of the product – protein mass *vs.* bioactivity
- To assure manufacturing consistency
- To assure comparability of product lots

Design of Potency Assay
生物活性测定方法的设计

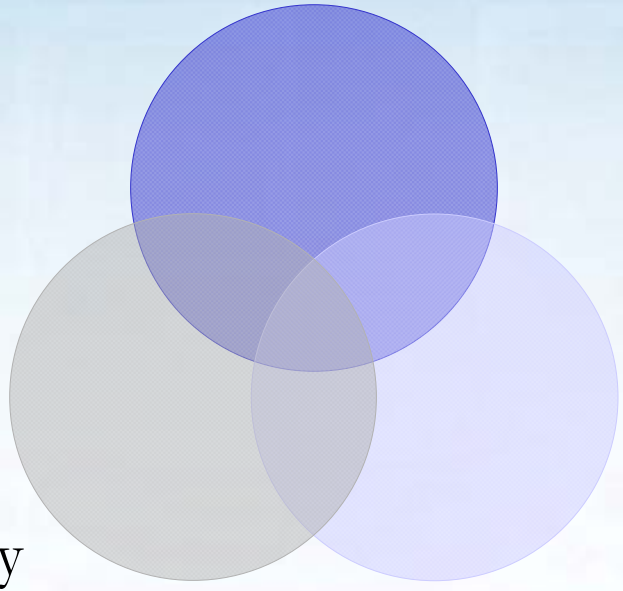
- A potency assay should reflect as much as possible the intended mechanisms of action (MOA) of the drug product.

- The assay should be designed to capture the integrity of structural components necessary for the activity.
  - Cell line-based
  - Late response (proliferation, cell viability, cytokine release)
  - Early response (phosphorylation of upstream signaling components)
  - *In vitro* enzymatic assay
  - Binding to targeted molecules
  - Animal based

Major Issues

不均一性

Heterogeneity



Immunogenicity

抗原性

Comparability

可比性

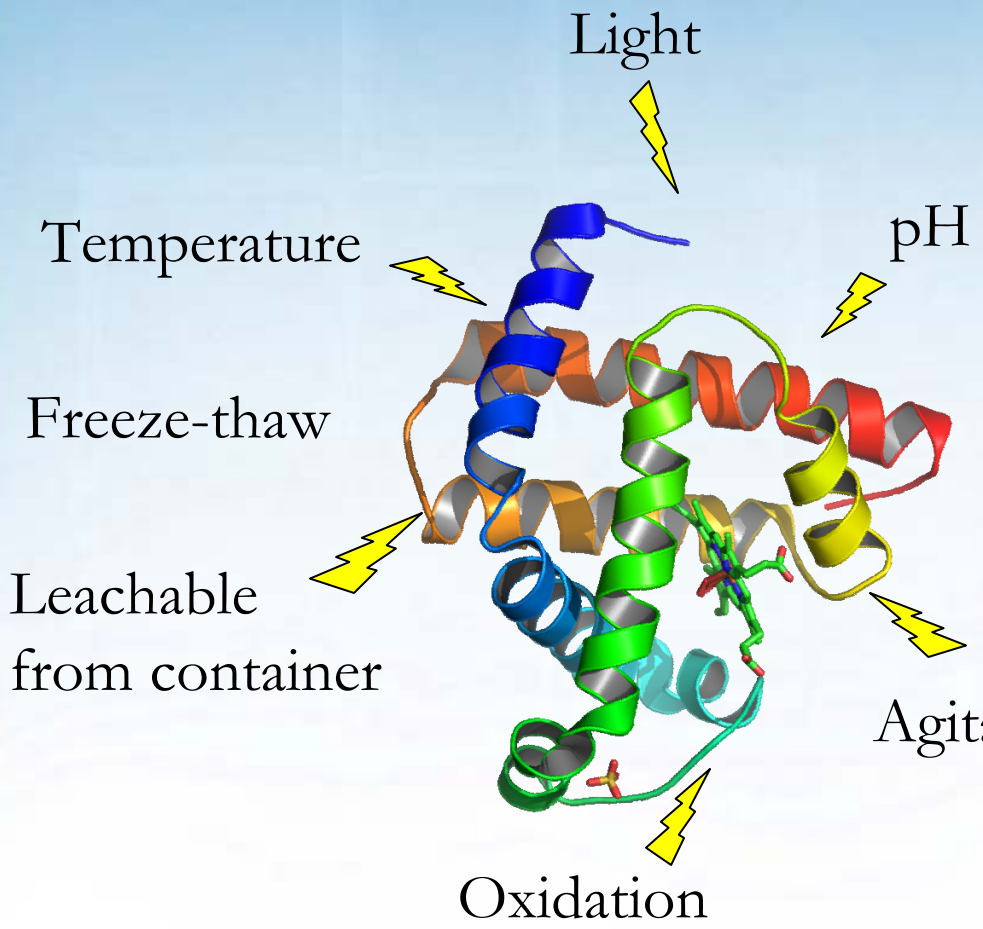
Heterogeneity of Protein Product
蛋白质药品的不均一性

- Product-related variants
  - aggregates (dimer, trimer, etc.)
  - degraded products
  - charge variants
  - mis-folded species
  - oxidized species
- Process-related impurities
  - host cell residuals (HCP & DNA)
  - contaminants (endotoxin & adventitious)
  - media components (antibiotics, growth factors)
  - leachables (heavy metals, resin)

Heterogeneity - what makes it more complicated…

- *In vivo* post-translational modifications
  - Glycosylation
  - Proteolysis
- *In vitro* modifications
  - PEGylation
  - Conjugation
- Derivatives during storage
  - Aggregates
  - Degraded products
  - Oxidized products
  - Deamidated products

Protein Degradation Pathways
蛋白质的降解途径



- Aggregation
- Degradation
- Oxidation
- Deamidation

Stability Issues 稳定性注意事项

- Real-time stability under proposed storage conditions
- Stability under stressed and accelerated conditions
- In-use stability
- Shipping validation
- Expiration date
- Amount of stability data depends on the stage of development

Immunogenicity of Protein Therapeutics
蛋白质药物的抗原性

- Immunogenicity is a primary clinical safety concern for protein therapeutics
- Clinical consequences:

  - Triggering hypersensitivity responses (allergic reaction)

  - Altering PK and PD profiles

  - Decreasing the product efficacy if the antibody has neutralizing activity to the product

  - Causing deficiency syndromes if the antibody has neutralizing activity to the endogenous counterpart

Potential Causes of Clinical Immunogenicity
引起蛋白质药物免疫反应的主要因素

- Product attributes:
  - Product inherent amino acid sequences (e.g. T-cell epitopes)
  - Product impurities - aggregation, oxidation, proteolysis, degradation, deamidation, glycosylation, misfolding
  - Process impurities - host cell proteins, container leachables and/or adjuvant effects
  - Formulation conditions – excipients and/or adjuvant effects

- Other risk factors:
  - Route of administration – SC>IM>IV>Oral
  - Dose, frequency, and duration of treatment – Chronic vs acute
  - Biological redundancy
  - Concomitant medication – Immune suppressants, chemotherapies

- Protein physicochemical properties or animal models are not necessarily predictive of immunogenicity in humans

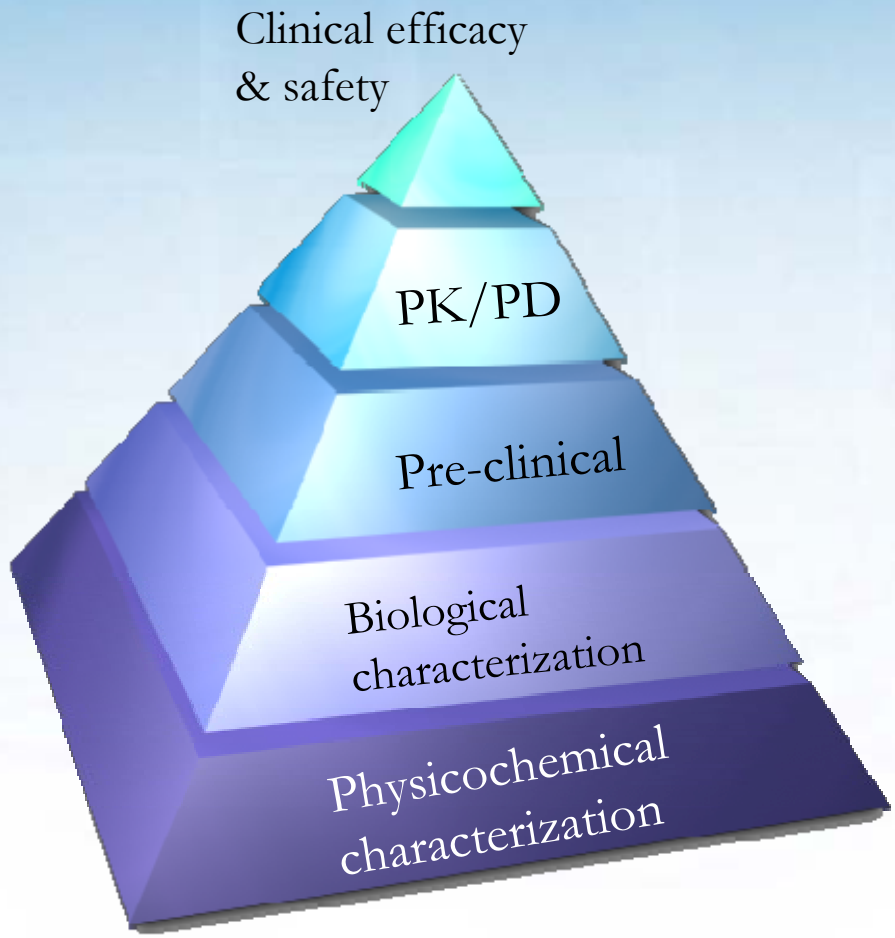
Monitoring Clinical Immunogenicity
药物免疫反应的临床检测

- Acute hypersensitivity response to therapy (allergic reaction)
- Frequency of antibody formation (% of patients)
- Neutralizing vs non-neutralizing activity to product
- Effects on clearance (PK/PD)
- Neutralizing activity to endogenous counterparts
- Time course of development and disappearance of antibody responses
- Isotype (IgG, M, E)

Comparability of Development Batches
建立蛋白质药物的可比较性

- Manufacturing changes:
  - Cell bank (expression vector, host cells)
  - Raw material (serum vs serum-free)
  - Fermentation process
  - Purification process
  - Scale-up
  - New manufacturing sites
  - Formulation
  - Dosage form
- The manufacturing process defines a protein product
- A minor manufacturing change can have significant impact on product quality

The Comparability Exercise



Comparability
Similarity

"Comparable"
≠ "Identical"

Design specifications for the product and manufacturing process

Quality management across the product life cycle 横贯产品生命周期的质量控制和改进

Comparability & Consistency.

It's time to harvest…

- The product is safe, pure and potent.
- The facility(ies) meet standards designed to assure that it continues to be safe, pure, and potent."

安全，纯净，有效。
持之以恒。


Questions?