Bioassays: The Superstars of Drug Development

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Bioassays: Scientific Approaches & Regulatory Strategies
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Disclosure of potential conflicts of interest

Helena Madden is a Sr. Director of Regulatory CMC at bluebird bio, Inc. and owns equity in the company.

This presentation reflects the views of the author.

Remember Me?

- In the beginning…….
- Scientific Approaches
- Regulatory Strategies

} **Not a static subject matter**

- The objective and the secret sauce = ***conversation***
- We are all humming the same tune
- Thank you

agenda

- Bioassay Basics
- Bioassay Challenges
- What Can Bioassays Tell Us?
- Words of ~~Wisdom~~ Experience

Bioassay Basics

Bioassays Have Been Around For A Long Time

A bioassay shows that a drug candidate "does something" and "has an activity" in a living system

Early ancestors chewed a berry, smoked a leaf, drank tea, or made a paste from plant parts and watched for a medicinal effect

1894 - Paul Ehrlich

- Animal bioassays
- Test vs Standard Preparation
- Defined Unit of Activity based on activity of a specific mass of the Standard Preparation

Today

Bioassays play a role in many industries and in everyday life

Images from shutterstock

In Biological Drug Development Bioassays Can Be Used To:

- Measure an *in vivo* or *in vitro* response in a drug discovery or research setting
- Determine product activity in animal models of disease
- Select drug candidates, drug targets, and production clones
- Show pharmacokinetics (PK) and bioavailability
- Evaluate immunogenicity
- Influence design of clinical trials
- Demonstrate understanding of a therapeutic biological pathway or mechanism of action
- Characterize and control a drug manufacturing process
- Test a drug in a QC lab for assurance of biological function and molecule stability
- Show the function of a transgene
- Evaluate biosimilarity
- Evaluate the use of a product for a new indication or new Mechanism of Action (MoA)

Why Do We Need Bioassays?

All Health Authorities have expectations and requirements for assurance of identity, purity, **POTENCY**, and stability of biotherapeutic drug substances and drug products used through all phases of clinical development

ICH Q6B - Specifications, 2.1.2 Biological Activity

- Assessment of the biological properties constitutes an equally **essential step** in establishing a complete characterization profile.

- A valid biological assay to measure the biological activity should be provided by the manufacturer. A **relevant, validated potency assay** should be part of the specifications for a biotechnological and biological drug substance and/or drug product.

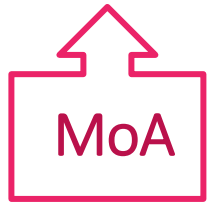
➢ **Examples include:**

  - **Animal-based biological assays**
  - **Cell culture-based biological assays**
  - **Biochemical assays** (enzymatic reaction rates or biological responses induced by immunological interactions)
  - **Other procedures**, such as ligand and receptor binding assays, may be acceptable

Definitions of Potency

- **ICH Q6B:** **Potency** is the **quantitative measure of biological activity** based on the **attribute** of the product, which is **linked to the relevant biological properties**.

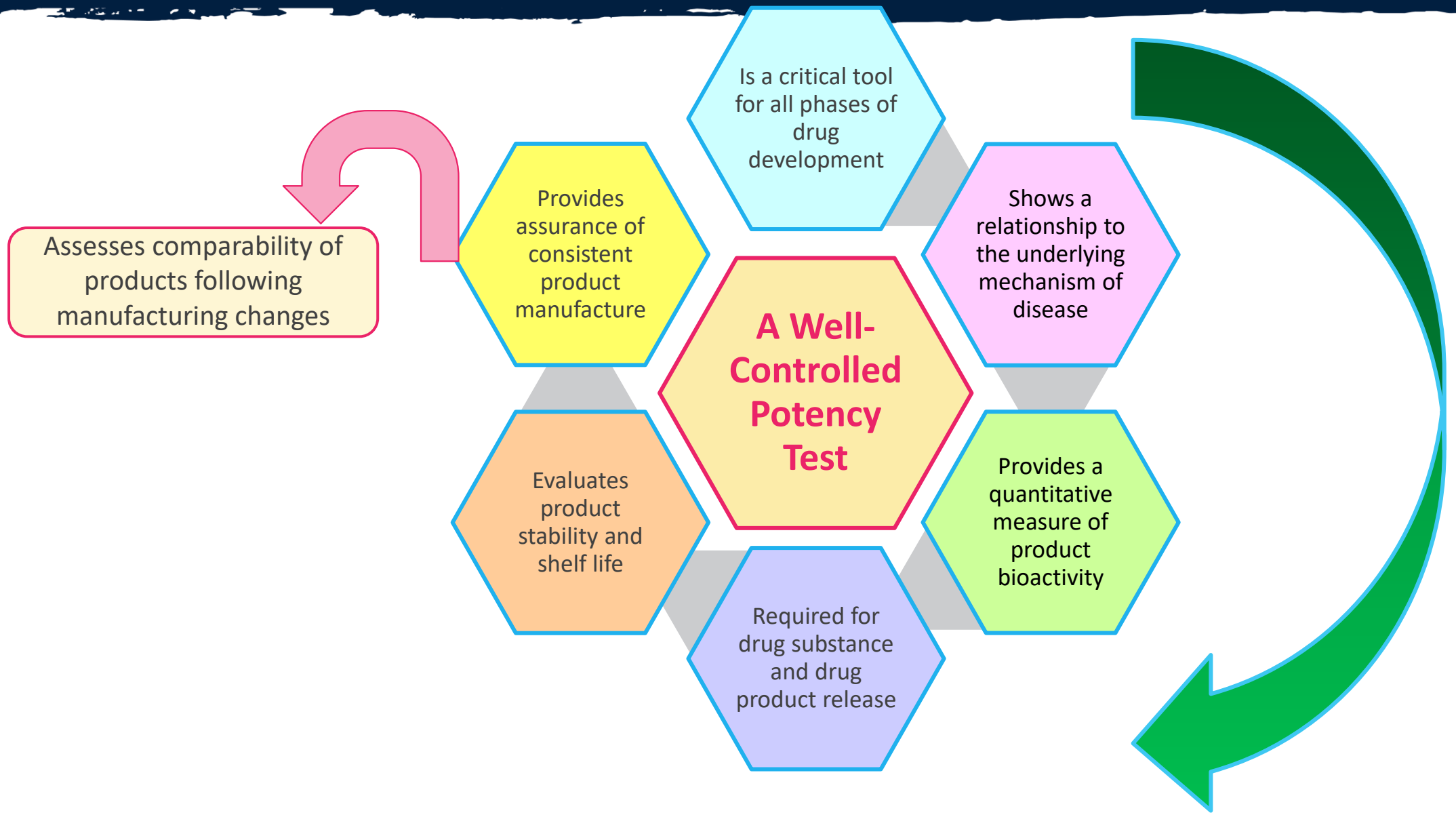
- The **assay** demonstrating the biological activity should be **based on the intended biological effect** which should ideally **be related to the clinical response**.



- **21 CFR 600.3(s):** The word ***potency*** is interpreted to mean **the specific ability or capacity of the product, as indicated by appropriate laboratory tests** or by adequately controlled clinical data obtained through the administration of the product in the manner intended, **to effect a given result**.

- **21 CFR 610.10:** **Tests for potency** shall consist of either **in vitro or in vivo** tests, or both, which have been **specifically designed for each product** so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in §600.3(s) of this chapter.

The Bioassay Bottom Line



Bioassay Challenges

Bioassays are Unique Analytical Tests

- Bioassays and potency tests are specifically designed for **each product or target**

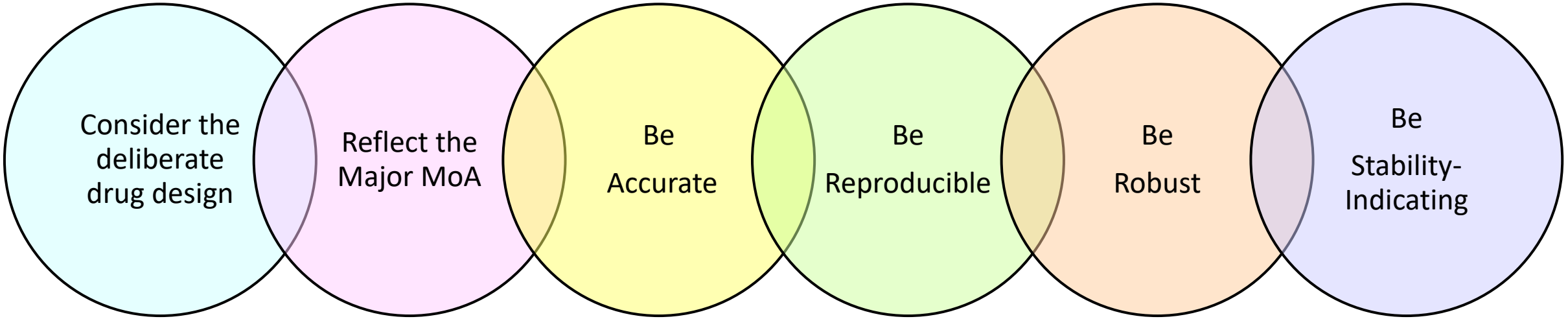
- Should represent the mechanism(s) of action for a specific product
  - Delivery or binding to cell and uptake into cell could be pivotal functions, **but is there more?**
  - **Clinical (Primary) MoA**: – **product binds and then what**? → blocks, activates, expresses, kills, etc.?
  - **Secondary MoA:** *example* - Fc Effector Function – product binds to specific Fcγ receptors on effector cells → ADCC (antibody-dependent cellular cytotoxicity, ADCP (antibody-dependent cellular phagocytosis), binds receptor C1q → CDC (complement- dependent cytotoxicity) **NOTE: Fc effector function could be a primary MoA**
  - **Work with your Research Group to verify that the *in-vitro* format of the bioassay is relevant to the MoA**

- **Multiple methods may be needed to define potency**
  - More than 1 MoA
  - When effector function cannot be eliminated as a MoA, need assays to monitor effector function
  - Clinical MoA unknown
  - Bioassay to control manufacturing process → often same as release bioassay, run in a different format
  - Bioassay for DS/DP lot release → assay performance is key, more replicates and fewer samples per run
  - Bioassay(s) for molecule characterization → provides useful information; use for specific situations

Bioassays are Unique Analytical Tests

- **Complex products** are difficult to characterize therefore **potency is a critical paramter**
- A potency test or combination of tests can model the interactions of complex pathways in an in-vitro environment
- Potency test results show that **a consistently manufactured drug product** is administered during all phases of clinical investigation
- A Phase-based or incremental approach for development of a bioassays program is acceptable and advised (except for biosimilar development)
  - **Early Phase**: Target binding → qualified
  - **Late Phase**: Cell-based functional → validated by pivotal trial and Ph3 GMP campaign
    - **Develop as early as possible**
- Analytical information generated over the course of development **using different assays must be bridged**

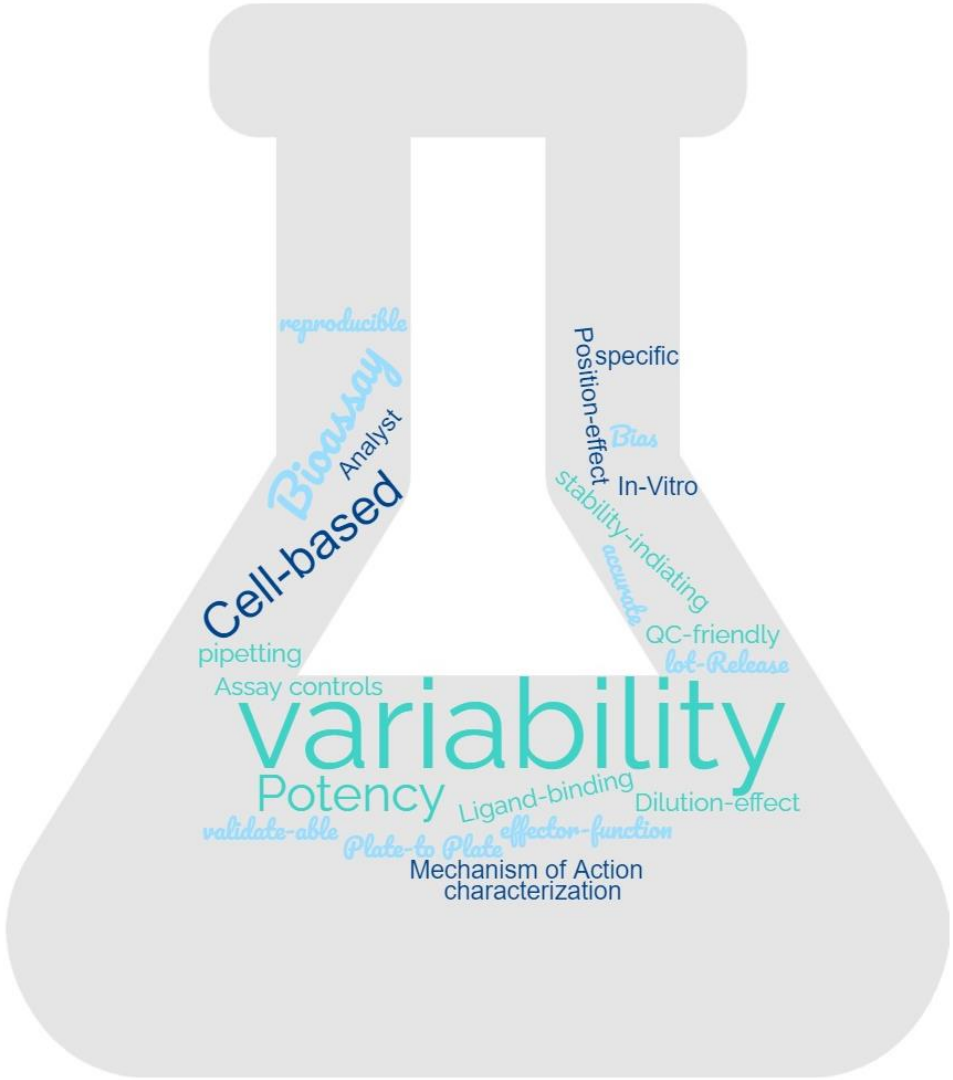
A “Gold-Standard” Potency Assay Should:

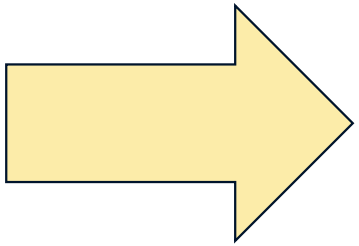


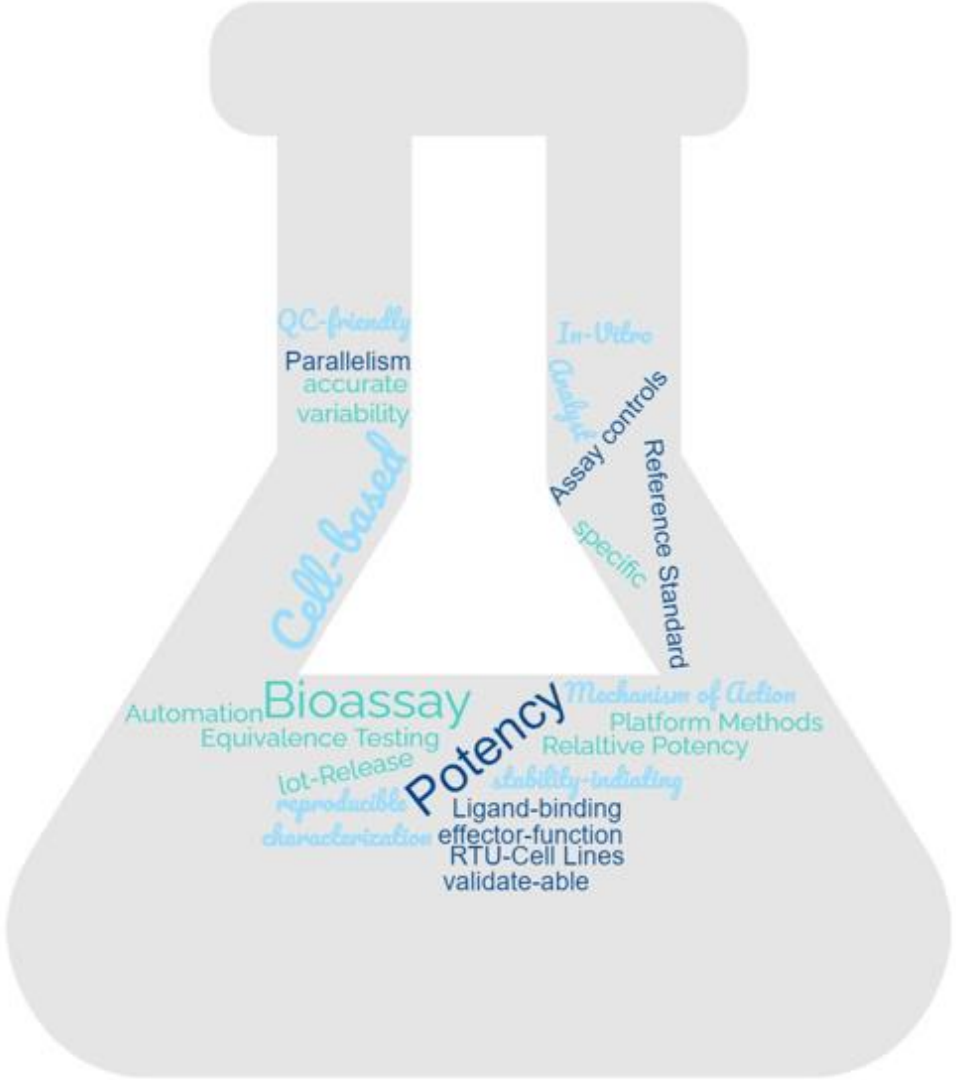
Be Practical

- ✓ Simple Format
- ✓ Cell lines available & stable
- ✓ Critical reagents (e.g., commercial assays, antibodies) available & stable
- ✓ Lot to lot variability of critical reagents evaluated
- ✓ Reference Standards appropriate and available
- ✓ Instrumentation and equipment dependable, qualified / validated
- ✓ Clear methods for analyses and results reporting
- ✓ One control chart for all testing sites
- ✓ Retain samples for evaluation of future method changes

Bioassays Then and Now







Points to Consider: Reduction of Variability

United States Pharmacopeia (USP)<1032> Design and Development of Biological Assays

*"Because of the inherent variability in biological test systems, an **absolute** measure of potency is more variable than a measure of activity **relative** to a Standard"*

*"Assuming that the **Standard** and **Test** materials are biologically similar, statistical similarity should be present, and the Test sample can be expected to behave like a concentration or dilution of the Standard."*

- **Determine Relative Potency** → Tests for parallelism, equivalence, curve similarity, etc.
- Use **Platform** technologies (e.g., Luciferase reporter, FRET binding, etc.)
- Use **Platform** instrumentation
  - Equipment should be qualified and routinely calibrated
- **Standardize** cell culture systems
  - Use easy to culture cell lines
  - Create or buy Ready to Use (**RTU**) cells
  - Consistent cell number and cell age
  - Consistent culture conditions

Points to Consider: Reduction of Variability, cont'd

- Sufficient **assay development and performance optimization**
  - Evaluate and optimize **every method step** including **data analysis**
  - **DoE approach** – repeat evaluations to confirm conclusions
  - Study and optimize plate-layout and plate-uniformity
  - Shorten incubation times and amplify signal

- Verify **sample concentrations** at time of assay

- **Automation** of method unit operations (liquid handling systems, pipetting, wash, detection, analysis)

- Develop meaningful
  - **System Suitability** Criteria: Is the assay valid?
  - **Sample Suitability** Criteria: Is the potency estimate for the test article valid?
- Analyst Training
- Well-designed **method transfers**
  - Co-validate methods (Transferring and Receiving groups)

Points to Consider: Control Critical Reagents

What reagents are critical?

- Cell lines, Abs, Ab conjugates, recombinant proteins, FBS, assay controls, Reference Standards, commercial assays, etc.

Points to consider:

- In-house production vs commercially sourced
- Characterization
- Qualification
- Handling (aliquoting, freeze thaw, etc.)
- Storage and stability (expiry dating, re-testing)
- Documentation (SOPs, work instructions, CONTROL CHARTS, etc.)
- Changing critical reagents
  - Use pre-defined procedure and acceptance criteria for new reagent lots

A Word about Reference Standards (RS)

ICH Q6B, 2.2.1 Reference Standards and Reference Materials

- At the time of submission, the manufacturer should have established appropriately characterized in-house primary reference material, prepared from lot(s) **representative of production and clinical materials**. In-house working reference material(s) used in the testing of production lots should be calibrated against this **primary reference material**.

- The potency of bioassays is typically expressed as percentage of potency in comparison to a RS (% RP)

- A link needs to be maintained between the **potency** of the RS used during clinical trials, in characterization studies, and in commercial release and stability tests
  - Use the appropriate number of replicates for qualification of RS potency

- **Acceptance criteria** (AC) for **RS potency qualification** should not include manufacturing variability. Don't use potency data from multiple lots to set AC; DO use multiple replicates (e.g., 9 independent runs) of a single lot

Resource: CASSS Bioassays 2019, Postcards from the Edge: Regulatory Reflections, Susan Kirshner, CDER, FDA

What Can Bioassays Tell Us?

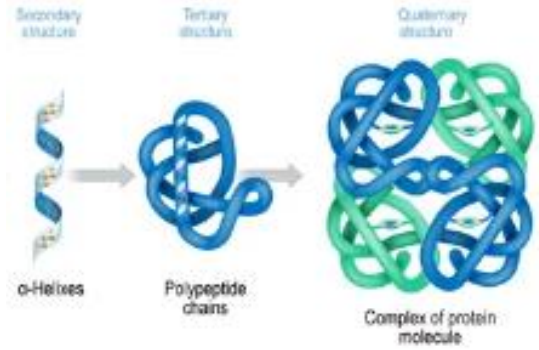
So How Does Your Product Work?

- **Continuously** learn from clinical trial results and **continuous** analytical characterization of your product

- Conducting clinical trials for evaluation of every product variant or manufacturing change is impractical
  - Time, Expense, Sensitivity

- **Bioassays can be used to correlate biological activity to structure and MoA → link to the clinical response**

- Relationships can be derived between bioactivity and protein structure
  - Higher Order Structure (HOS)
  - Posttranslational modifications (PTMs)
    - Phosphorylation → can influence cell cycle, growth, apoptosis, signal transduction
    - Glycosylation → absence, presence, type of carbohydrate can influence bioactivity
    - Product Variants – e.g., oxidized variants or clipped variants can result in the loss or gain of activity



Characterize your product to understand what product attributes contribute to biological effects

  - Measurement of a PTM may be more sensitive than a method that measures bioactivity

Is a Potency Assay Always a Biological Assay?

ICH Q6B - Specifications, 4.1.4 Potency

- A relevant, validated potency assay should be part of the specifications for a biotechnological and biological drug substance and/or drug product. When an **appropriate potency assay is used for the drug substance**, an alternative method (physicochemical and/or biological) may suffice for quantitative assessment of the **drug product**. However, the rationale for such a choice should be provided.

Justify your rationale using supportive scientific data

- A cell-free assay that reflects the MoA could be used to assess potency
- Examples
  - Receptor binding - microtiter plate-based
  - Receptor phosphorylation using a cell membrane preparation
  - Direct binding to a specific ligand

Is a Potency Assay Always a Biological Assay?

- **Correlation to a cell-based bioassay is an expectation**
  - The cell-based assay is the link to the clinic effect
  - Is the cell-free assay superior? Show this using data!
  - Propose to use cell-based assay for comparability studies, qualification of a new Reference Standard, etc.

- **Surrogate analytical assays may be used to demonstrate potency if the following are shown**:
  - A solid understanding of product bioactivity and other critical product quality attributes
  - Correlation to relevant, product-specific bioassay results
  - Qualified or validated assays are used
  - Results are from multiple lots of appropriate (active, inactive, degraded, etc.) test articles and/or patient samples

The amount and types of correlation data needed to justify your approach depends on product complexity

Correlations Are Powerful Friends

The Role of Bioassays in Determining Relationships Between Structure and Function of a Biotherapeutic

- Technology advancements in the analytical evaluation of protein structure have been significant
  - Structure is necessary for bioactivity, but data from structural assays, **alone**, are not enough to assure molecule potency
  - Perturb the structure and investigate → does this structural change impact bioactivity?

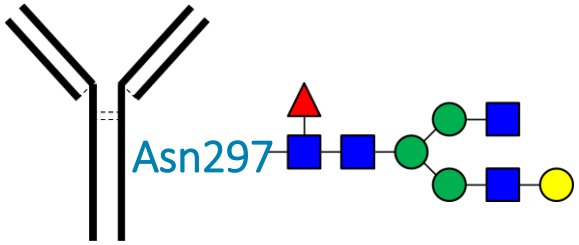
- Bioassays can inform about a change in product chemistry or product structure
  - But **only if** that chemical or structural component plays a role in activity
  - Consider the overall impact of intentional structural change – did any unintended changes occur?

- Demonstrating linkages between the structure of a biotherapeutic and its function increases product knowledge; use product knowledge to your advantage

- Orthogonal methods that measure physiochemical and/or structural components of a product can serve as surrogate measures of potency, but don't necessarily replace potency assays in the overall assessment of pharmaceutical quality
  - MORE work is needed!! A LOT of work is needed to build scientific rationale for using surrogate tests

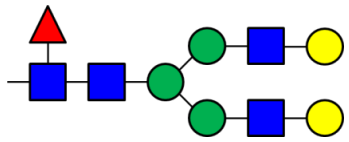
Can Physicochemical Methods Replace Traditional Bioassays?

- MANY literature references show the impact of N-glycans on IgG1 Mab bioactivity, safety, and efficacy

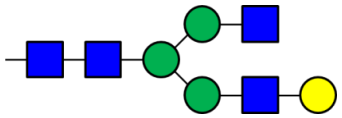


- For example:

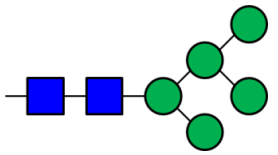
Increase Gal = ↑ CDC activity



Decrease Fuc = ↑ FcgRIIIa binding + ADCC activity



High Man, No Fuc = ↑ FcgRIIIa binding + ADCC activity



Fuc: Fucose, GlcNac: N-Acetylglucosamine, Man: Mannose, Gal: Galactose, ADCC: Antibody-Dependent Cellular Cytotoxicity, CDC: Complement- Dependent Cytotoxicity

- Production of Mabs and Fc fusion proteins in mammalian cell culture systems results in heterogeneous N-glycan patterns
- N-glycans affect the conformation of antibodies
- N-glycans modulate interactions with Fc receptors on immune cells
- N-glycans can impact antibody effector function
- N-glycan composition may be a critical quality attribute; monitor to assure process and product consistency

Factors Needed for Successful Structure Activity Relationship Studies

- **Strong partnerships** between bioassay specialists, analytical chemists, and biophysicists

- **Resource planning** – *it takes more time and more people than you think*

- **Sample generation**: express Mab in a fucosyltransferase-deficient cell line, use enzymes, enzyme inhibitors, chromatography enrichment, etc. to create a library of variants on a Mab
  - De-glycosylated (mannose and/or galactose)
  - Hyper-glycosylated (mannose or galactose)
  - Hypo-glycosylated (mannose or galactose)
  - + / - sialic acid
  - + / - fucose
  - Mixtures

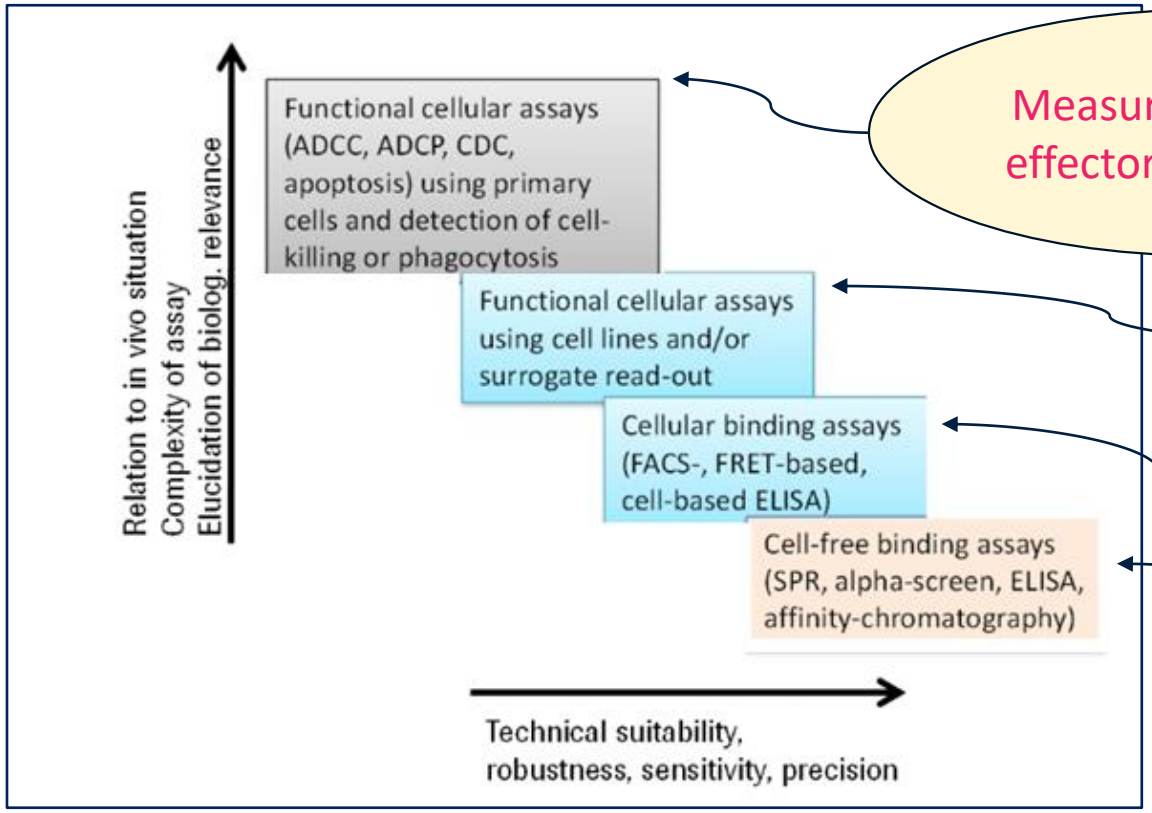
  - Chromatographic separation and enrichment of each N-glycan variant type

  - Characterize each variant:
    - Size exclusion chromatography (SEC) – aggregation? Capillary Electrophoresis methods – fragmented forms?
    - Mass Spectrometry – glycan structure, any modifications (oxidation, deamidation, etc.)?
    - Evaluate and consider variant stability

  - Create mixtures containing various amounts of N-glycan variants

Evaluate Bioactivity of Each Variant Using Well-understood Bioassays

Analysis of N-glycan variants vs Reference Standard Using Potency Assay Matrix



Measures binding & effector cell function

Use of 1° cells leads to ↑ variability

Measures binding & signaling (e.g., cytokine release

Measures binding, assesses conformation, readout = sensitive

Measures binding, reduces complexity

Bioassays 2022, A Better Understanding of Bioassays, Florian Cymer, Hoffmann La Roche, Ltd.

- Bioassays are a critical tools to show how Mab structure influences bioactivity & potency
- **Principles and learnings can be applied to other modalities**

Potency of Complex Products

- Antibody Cocktails, BiSpecific Abs, Antibody Drug Conjugates, Gene Therapies, Cell Therapies, etc.

- **Potency** is a **key parameter** for complex products which have unknowns & are difficult to characterize

- A **combination of multiple bioassay methods** may be needed to evaluate potency of complex product
  - During drug candidate development
  - For product characterization
  - To control DS and DP manufacture
  - For DS and DP release and stability testing
  - For comparability studies
- **Provide rationale** for bioassay application strategy: what bioassay to use, for what purpose, and why
- What analytical tests are performed? How do they compliment or strengthen bioassays results?

Combinations of all product quality attributes matter, but **Bioassays are the superstars**

Drug Candidate Failures

- All fingers point to the bioassays

- Bioassay won't work?
  - The mechanism of action may not be fully known
- Do you understand your drug candidate?
  - Is effector function part of MOA? Other?
- Bioassay transfer to QC is difficult
- Bioassay validation fails
- Drug design or indication evolves → did you change your bioassay if needed?

Listen to your Bioassay

Words of ~~Wisdom~~ Experience

Words of ~~Wisdom~~ Experience

- Start potency assay development as **early** as possible
- **Understand** the product mode of action
  - Reflect the physiological situation as closely & as practical and possible in your potency assay
- Develop **multiple measures** of potency and **document** assay performance & suitability
- Potency assay development **should be continuous** throughout product development
- Use a product life cycle approach (e.g., simple binding → cell-based, functional)
  - Validation of your potency assay is needed for pivotal trial DP and for BLA submission
  - **Plan ahead for future comparability**
  - Measure potency in stability studies

Words of ~~Wisdom~~ Experience

- **Test multiple** bioassay reagent lots, instruments, analysts; **evaluate method robustness**

- Understand what **product attributes contribute to the biological function**
  - Evaluate contribution of post translational modifications
  - Evaluate bioactivity when protein structure changes

- Talk to regulators about potency strategies, reference standards, and challenging issues
  - **Listen to regulators!!**

Remember that: Combinations of product attributes matter, but

Bioassays are the superstar methods

Keep the conversations going between industry and regulators

Acknowledgements

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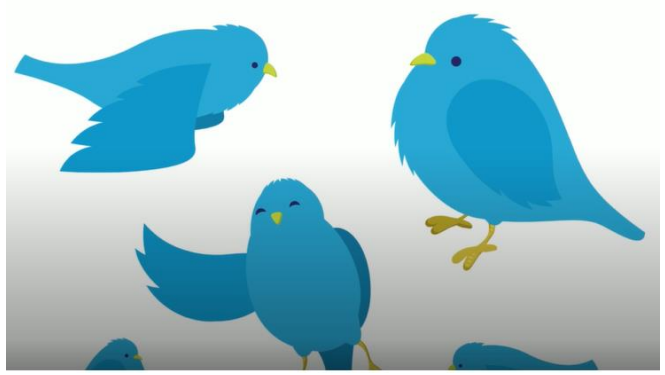

THANK YOU!!!

and a *"SHOUT OUT"* to

***EVERYONE* who thinks about Bioassays!!**

References

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