Potency Assays Throughout Product Development: Perspectives of an FDA Reviewer

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Disclaimer: some of the views expressed in this presentation are my own and may not necessarily represent FDA policy.

Potency – The Regulations

“The word potency is interpreted to mean the specific ability or capacity of the product (…) to effect a given result.” 21 CFR 600.3(p)

“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 of this chapter.”

21 CFR 610.10

Potency - ICH Q6B

• “…for 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”

• (Glossary)
The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties.

• Other procedures such as ligand and receptor binding assays may be acceptable.

A well-designed bioassay will reflect the mechanism of action of the product.
Application of a Bioassay

- Indicates biological activities specific/relevant to the product (mechanism of action) (…to effect a given result)
- Clinical correlate (if possible)
- Product release
  - Confirm higher order structure/activity
- Stability program
- Manufacturing changes - Comparability
- Immunogenicity: neutralizing activity

Types of potency assays:

- Binding assays
- Bioassays
  - Animal based
  - Organ or tissue based
  - Cell culture based
    - Early response (signaling pathway)
    - Late response (proliferation, cytokines)
    - Multiple cell types (MLR, cell-cell adhesion)
  - Biochemical
    - Enzyme activity assays

Biological relevance of potency assays:

Clinical efficacy reflects mechanism of action

TNF antagonists for treatment of rheumatoid arthritis:
- **Mechanism of efficacy** - reducing inflammation and destruction of joint integrity
- **Mechanism of action** - inhibition of TNF binding to its receptor
- Bioassays:
  - Prevention of joint inflammation in animal model
  - Blocking TNF mediated inhibition of cell line proliferation
  - Impact on downstream signaling components

G-CSF treatment for neutropenia:
- **Mechanism of efficacy** - increasing production of leukocytes by bone marrow
- **Mechanism of action** - stimulating cell proliferation through receptor binding
- Bioassays:
  - Reconstitution of leukocytes in animal model
  - Stimulation of leukocyte production in bone marrow assays
  - Stimulation of cell line proliferation
  - Tyrosine phosphorylation of downstream signaling components
Mechanism of efficacy – reducing tumor burden
Mechanism of action – blocking signaling through growth factor receptors

Potential bioassays:
- blocking growth of tumor cells in animal model
- blocking in vitro proliferation of cell line
- induction of apoptosis
- effects on downstream signaling components of the receptor

Enzyme Assays

- Optimal enzymatic assays utilize physiologically relevant substrates (by phase 3)
  - e.g., small molecule vs large, complex substrate
- Important structural information derived from determination of the kinetic parameters:
  - $K_M$ (binding affinity for substrate)
  - $k_{cat}$ (turnover of substrate)
- Replacement enzymes may need more than one potency assay (cell uptake, regulatory domains).

Timeline for Bioassay development:

**Phase I**

- A potency assay is needed for phase 1
- Ideally based on intended mechanism of action of drug product
  - Monoclonal antibodies do not always require a functional bioassay for phase 1 (can have a binding assay), but other therapeutic proteins do.
  - Can use multiple assays for protein activity(s) characterization
- Bioassay requirements depend on protein product, indication, therapeutic window, etc. Potency assay for products with a narrow therapeutic window may require a good validated bioassay for phase 1.
- There should be provisional preliminary specification established for the potency assay on release and stability. Depends on therapeutic index and dosing.
Timeline for Bioassay Development

- 21 CFR 312.22: For Phase 2 and 3, FDA’s primary objectives include:
  - “to help assure the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug’s effectiveness and safety”.
  - Therefore product characterization assays (e.g. potency) should be “adequately qualified”.

Timeline for Bioassay Development

**Phase 2:**
- Potency assay(s) further developed
- Bioassay is added if binding assay was used for phase 1.
- Refined specifications

**Phase 3:**
- Potency assay well-characterized and near full GMP/GLP
- Specifications tightened and justified
- (Nearly) validated
- Employed on the proposed clinical lot(s)

Timeline for Bioassay development: Phase 2/3

- Often, a new assay may be developed and transitioned in during this time period
- More than one potency assay may be required as the mechanism of action is further defined
- A good potency assay has usually been found to be stability-indicating.
- When planning manufacturing changes or scale-up programs, product comparability may have to be demonstrated. A well developed potency assay is very useful at such times.
When would a binding assay be an acceptable potency assay?

• When the mechanism of action of the antibody has been clearly demonstrated to be appropriate for such a potency assay

• No effector functions have been demonstrated

• Requires extensive structure/function understanding.

table

example

Therapeutic protein: Radiolabeled mAb used as therapeutic for oncology indication

Target antigen: an extracellular matrix protein secreted by the cancer cell.

• Mechanism of action: binding in proximity to the cancer results in localized irradiation of the tumor.

• Cell based bioassay: Not required based on the mechanism of action. (for release and stability. May still be useful for characterization)

Biological Activity: Structure = Function

• Biotechnology products can have more than one activity
  – IFNa (anti-viral vs anti-proliferative)

• The structural requirements for different activities may differ

• A particular biologic activity may not reflect the structural requirements of active product
  – indication specific

• If there are multiple possible mechanisms, more than one assay may be required.
Case study

- Fusion protein with multiple active sites required for its Mechanism of Action
- Manufacturing changes resulted in some differences seen on charge based assay - different ratios of the existing species.
- No differences were detected on the bioassay
- However - clinical data identified differences between pre- and post- manufacturing change products!
- FDA required that additional bioassays be developed and added to release and stability testing to reflect the multiple activities necessary for protein function in vivo. Additional bioassays identified differences.

Changing Potency Assays

- The degree of data needed to support a change will depend upon
  - The development stage of the product
  - The type(s) of assay(s) being exchanged
- A new assay should have a justifiable advantage over the old assay
  - Stability indicating
  - Product variants
  - Degradation products
  - Accuracy, precision, sensitivity, etc.
- Overlapping data using both assays is required to support assay change and link between assays, non-clinical and clinical data.

Summary

- Bioassays are the only assays that can directly measure the biological activity of biological therapeutics.
- Many different types of bioassays possible
- Whenever possible, the bioassay should reflect the mechanism of action of the drug product and should be designed to capture the integrity of all structural components necessary for activity.
- Binding assays should measure and be correlated with all functional activities of the protein.