Selection of Potency Assays for Lot Release During Early Development of Therapeutic Monoclonal Antibodies

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The changing landscape of therapeutic mAbs: why not use a binding assay for every mAb?

Role that Mechanism of Action (MoA) plays in choosing lot release format

Strategies for use of cell-based assays (bioassays) and binding assays at various stages of product development

Case studies of mAbs with different MoAs
Conjugated mAbs
- Radioisotope labeled
- Toxin conjugated

Enhanced effector functions
- ADCC
- CDC
- ADCP

Ablated effector functions
- IgG4
- Fc mutants
Increasingly Complex MoAs May Limit Usefulness of Binding Assays

- As mechanisms of action become more complex, it is increasingly difficult to assess potency using ligand binding assays alone.

- Consider a toxin-conjugated mAb:
  - Does the mAb still bind its target?
  - Does the complex still get internalized?
  - Is the chemical linkage to the toxin intact?
  - Does the toxin get released?
  - Does the toxin bind its target?
  - Does the toxin have consistent on-target potency?

- Are individual binding assays even useful for such molecules?
Key Decision Points in Choosing a Lot Release Assay Format

- Can the expected MoA of the product be modeled using a binding assay?
  - Does mAb simply bind and neutralize soluble ligand?
  - Does mAb have altered effector functions?
  - Does mAb engage multiple cell types?

- Are bioassay and binding assays comparable in detecting changes that affect potency (i.e. deamidation in CDR, etc.)?

- Is there historical precedence for the use of bioassays for your product (i.e. interferon-related therapeutics)

- Can a bioassay be made suitable for its intended purpose?
Multiple Strategies Exist for Implementation of Bioassays and Binding Assays

- Can a binding assay be used from IND through BLA?
  - If not, when to change to bioassay and why?

- Can a binding assay be used first, followed by bioassay later?

- When should a bioassay be used for lot release from the beginning?

- Should a bioassay always be present in some capacity (i.e. as a characterization assay)?
**Strategy 1: Binding Assay as Lot Release From IND Through BLA**

**Pros**
- Short development process
- Short assays
- High precision and accuracy

**Cons**
- Greater regulatory risk
- Must ensure MoA is binding only
- Bioassay still required to demonstrate comparability
- Binding assay and bioassay may prove to be non-comparable
- If bioassay becomes required for BLA, little time to validate
Strategy 2: Bioassay as Lot Release From IND Through BLA

**Pros**
- Low regulatory risk
- Low risk of missing changes that affect potency of molecule
- Ample time to collect data and prepare bioassay for validation

**Cons**
- Resource intensive
- If bioassay is variable early on, data may be less useful for decision making
- May need multiple assays if MoA is unclear or if multiple indications
**Strategy 3: Bioassay First, Binding Assay at BLA**

**IND/Ph 1** | **Ph 2** | **Ph 3** | **BLA**
---|---|---|---
Bioassay (Lot Release) |  | Binding Assay (LR) |  
Characterization

**Pros**
- Low risk that relevant degradation pathways missed by bioassay
- Accumulated data to ensure comparability of methods
- Long-term experience with bioassay reduces validation risk if binding assay not acceptable

**Cons**
- Significant resources spent on early bioassay development
- High regulatory hurdles to demonstrate comparability of methods
- Binding assay may not be accepted
Strategy 4: Binding Assay First, Bioassay at BLA

Pros
• Little resources required to IND/Ph1
• If program terminates at Ph1, minimal loss of time
• Typically easier to switch from binding assay to bioassay

Cons
• Bioassay less able to assist in decision making early in lifecycle
• Complex bioassays may be difficult to validate in time for BLA
• Sample load may increase during later-stage development at the same time bioassay must prepare for validation
Case Study #1: Binding Assay as Final Selection for Lot Release

- mAb binds to virus and prevents virus-receptor interaction
- MoA expected to be binding only
- Both bioassay and binding assays developed
  - Cell-based viral neutralization
  - ELISA-based viral protein binding
- Correlation of binding and CBA demonstrated previously

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Product-Specific Testing Required to Ensure Binding Assay “Fit for Use”

Case Study #1: Conclusions

- Extensive studies were performed to verify binding was the MoA
- Previous data demonstrated strong correlation between binding and bioassays
- Binding assay deemed suitable as the lot release method
Case Study #2: Started with Binding Assay and Moved to Bioassay

- mAb X binds to a soluble ligand
- mAb X does not neutralize the binding of Ab-ligand complexes to receptor
- Ab-ligand complexes inhibit functional receptor signaling
Initial Lot Release Strategy

- Binding assay utilized initially as lot release potency assay

- Reporter gene bioassay used as characterization method
A Deamidation Site was Identified Within the CDR of the mAb

Does this deamidation site cause a change in potency?

Material with varying levels of deamidation was purified from heat stressed samples
- Deamidated samples had mixture of 0, 1 and 2 CDR deamidations

Samples tested in both binding assay and bioassay
Impact of CDR deamidation on potency was detected by bioassay, but not binding assay.

Bioassay had been run in parallel as a characterization assay.
- When change was made, reduced effort to make bioassay lot release ready.

Bioassay replaced ELISA as the lot release assay.
- ELISA dropped from product specifications.
Case Study #3: Bioassay From the Beginning

- Antibody-drug conjugate
  - Targeting cellular receptor overexpressed on solid tumors
  - Range of drug molecules per antibody molecule
  - Complex mechanism of action leading to apoptosis

Apoptosis
Complex MoA not capable of being modeled by binding assay(s):

- MoA consists of:
  1. Binding
  2. Internalization
  3. Toxin release
  4. Toxin binding
  5. Toxin activity

Cell death
A Homogenous Bioassay Developed for Lot Release

- Simple, homogenous format allows for accurate data generation

- Plate tumor Cells
- O/N
- Add mAb serial dilutions
- 72 hr Incubation
- Add viability reagent
- 0.5 hr
- Read on luminometer

![Graph with data points for mAb+toxin, Naked mAb, and Irrelevant mAb.](chart.png)
Studies performed to determine if both assays can detect potency differences due to drug:Ab ratio

No difference was detected using an ELISA-based binding assay
Case Study #3: Conclusions

- Complex MoA suggested use of bioassay for lot release from onset
- Bioassay capable of detecting changes in product potency due to drug:Ab ratio
  - Changes could not be detected by binding assay
- Binding assay no longer used for potency assessments
Multiple strategies exist for implementation of cell-based potency assays during a product’s lifespan

Lot release assay must be “fit for use” for a given product

Parallel tracking of binding and bioassay data provides long term risk mitigation and ensures that most appropriate format is chosen for lot release assay
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