In vitro alternative assays to cell-based potency assays: regulatory considerations for biologics

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Disclaimer

Please refer to any cited guidance, as this talk only refers to them at a high level. Specific regulatory issues need to be addressed with the relevant assessment team.


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Outline

- ❑ Regulations of potency for biologics and expectations on potency assays at different development stages
- ❑ Alternative assays to demonstrate potency: case studies
- ❑ Regulatory considerations when alternative assays are used

Potency definition and regulations

- PHS Act section 351 (42 USC 262):

  "…approve a biologics license application…on the basis of a demonstration that:
  (I) the biological product that is the subject of the application is safe, pure, and **potent**; and
  (II) the facility in which the biological product is manufactured, processed, packed, or held meets standards designed to assure that the biological product continues to be safe, pure, and **potent**;"

- 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 definition in § 600.3(s) of this chapter."

Additional guidance

- ICH Q6B: Specifications for Biotechnology Products:
  - Potency: 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.
  - Drug substance specifications: appearance and description, identity, purity and impurities, **potency**, quantity.
  - Drug product specifications: appearance and description, identity, purity and impurities, **potency**, quantity, general tests, additional testing for unique dosage forms.
  - "Often, 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**."

Additional guidance (cont.)

- ICH Q6B: Specifications for Biotechnology Products:
  - Importantly, a biological assay to measure the biological activity of the product may be replaced by physicochemical tests only in those instances where:
    - *sufficient physicochemical information about the drug, including higher-order structure, can be thoroughly established by such physicochemical methods, and relevant correlation to biologic activity demonstrated; and*
    - *there exists a well-established manufacturing history.*

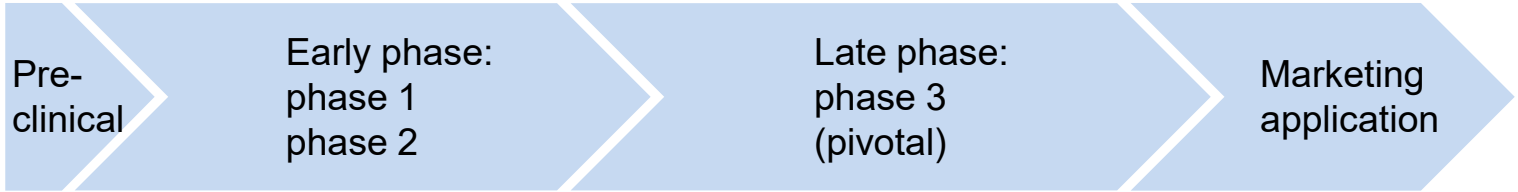
Cell-based potency assays

- Provide a more relevant and representative way to assess biological activity based on the mechanism(s) of action (MoA)
  - Physiologically relevant
- Allow evaluation of complex cellular processes or interactions
  - Cell proliferation, cell death, cell differentiation, cytokine production, cellular uptake, cell signaling by reporter gene assays, etc.
- Crucial for ensuring safety and efficacy during drug development
  - Ensure batch-to-batch consistency

Non-cell-based potency assays

- Non-cell-based potency assays usually rely on biochemical interactions
  - Ligand binding assays
  - Protein-protein interaction assays
  - Enzyme activity assays
- Typically used in early drug development
  - However, they may not reflect the physiological conditions
- Used based on the proposed MoA or when cell-based assays are not a component of the MOA
  - Products targeting soluble factors or processes that take place outside of cells (e.g., enzymatic breakdown of blood soluble substrates)
- Used when it provides more consistent and robust control/monitoring of potency than cell-based assays

Phase-appropriate potency assay development



- Initial potency assay(s) based on proposed MoA
- Binding assay is generally acceptable for monoclonal antibodies (mAbs)
- Broad acceptance criteria

- Knowledge build-up on MoA
- Cell-based functional bioassay should be developed
- Bridging of potency assays at different stages

- Validated cell-based functional bioassay
- Defined acceptance criteria

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Examples of non-cell-based potency assays

- ❑ Cell-based assays do not align with the MoA
  - Enzymes that do not have cellular uptake as part of the MoA
    - *Do not have cell/tissue targeting* → Specific activity assay and enzyme kinetics assay
    - *Have cell/tissue targeting* → Specific activity assay, enzyme kinetics assay, and cell/tissue targeting assay (e.g., target binding assay)
  - Antibodies targeting soluble factors
    - *Targeting coagulation factors (e.g., FXIIa, FIXa, X)* → ▪ Chromogenic assay ▪ Clotting assay
    - *Targeting complement system (e.g., C1, C5)* → Hemolytic assay (non-traditional red blood cell-based potency assay) and antigen binding assay
  - Products with mechanical properties
    - *e.g., lung surfactants (reduce surface tension at the air-liquid interface)* → Animal-based assay and surface tension assay
  - Radiolabeled antibodies → Antigen binding assay and radioactivity

Examples of non-cell-based potency assays (cont.)

- Alternative assays provide more consistent and robust control/ monitoring of potency

  - Products with well characterized structure-function or content-function relationships
    - *Some insulin products* → Insulin content by RP-HPLC
  - A clear correlation has been established between alternative assays and cell-based assays
    - *Effector function (ADCC, ADCP, CDC)* → Glycan profile, antigen binding assay, and FcγR and/or c1q binding assays
    - *Cell-based assays are highly variable* → Antigen binding assay and physicochemical assays

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Points to consider for justifying the use of alternative assays to replace cell-based potency assays

- Good understanding of the MoA
  - Are the proposed potency assays aligned with the MoA?
- In-depth characterization and extensive experience
  - Knowledge of how individual CQAs (and relevant process parameters) impact potency
- Well-established correlation between the alternative assays and the cell-based assays
  - Comparison under both physiological and stress conditions
  - Alternative assays have equivalent or better performance than cell-based assays

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