Regulatory Considerations of Bioassay Lifecycle Management For Biologics

Bioassays, April 16, 2024

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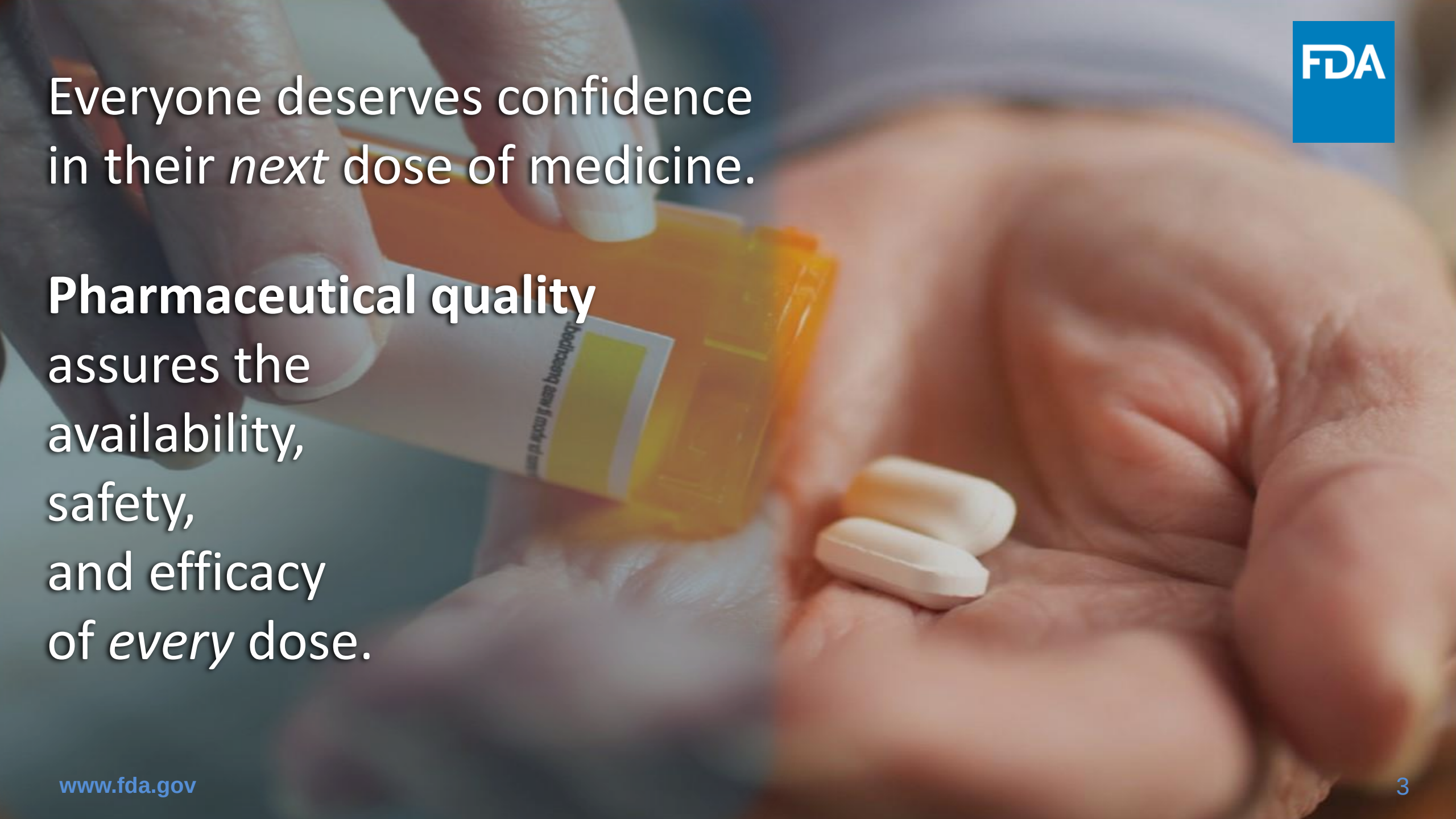
Cases and examples may be hypothetical.



Everyone deserves confidence in their *next* dose of medicine.

Pharmaceutical quality assures the availability, safety, and efficacy of *every* dose.

Outline

- Potency regulation and definition
- Expectations and considerations for potency assays
- Phase-appropriate bioassay development
- ICH Q14 approach on analytical lifecycle management

Potency Regulation and Definition

- **PHS Act section 351 (42 USC 262):** Regulation of biological products

  "…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...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.

ICH Q6B on Potency

- 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.
- 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**.

Potency Assays

Potency assay format

- Animal-based (organ/tissue; inhibition of tumor cell growth)
- Biochemical assays (enzymatic reaction rates, ligand/receptor binding assays)
- Cell-based
  - Early response (signaling pathway; tyrosine phosphorylation)
  - Late response (cell proliferation/apoptosis, cytokines)
  - Multiple cell types (mixed lymphocyte reaction /MLR, cell-cell adhesion)

Bioassay

- Is an analytical procedure measuring the effective constituent in a biological product that *utilizes a biological reporter system**.*
- Is used for:
  - Product development
  - Product release
  - Stability testing
  - Manufacturing changes: comparability assessment
  - Clinical outcome correlation

* A. R. Mire-Sluis, Pharmaceutical Sciences, 1997 (3: 15-18).

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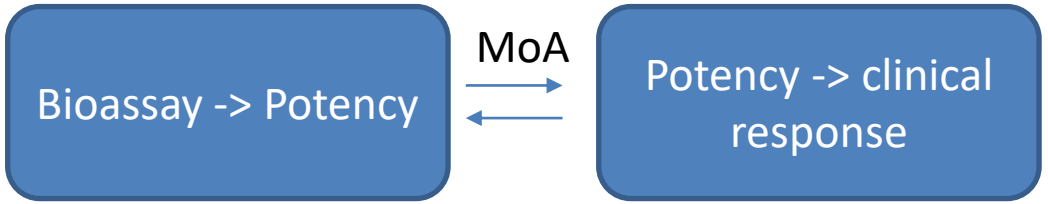
Expectations for Potency Assays

Potency assays should:

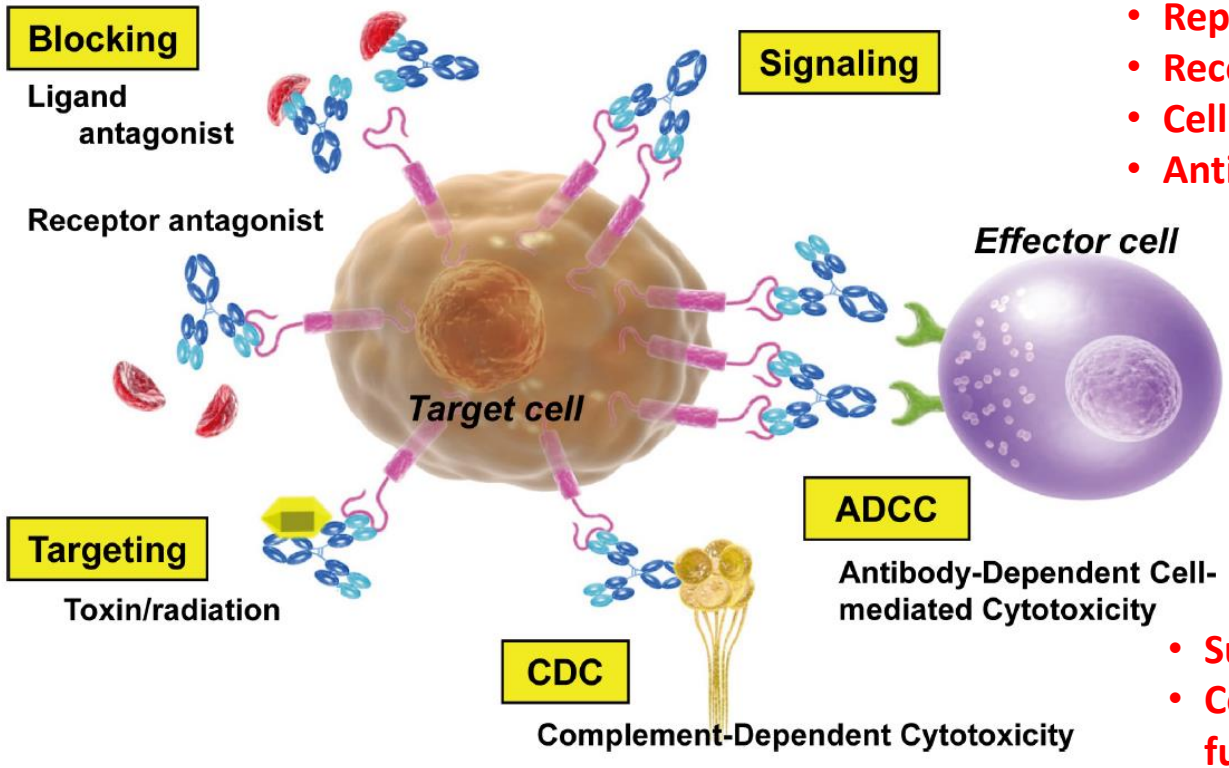
- Reflect the (primary) proposed mechanism of actions (MoAs)
- Quantitatively measure biological activities that are relevant to clinical efficacy
- Be suitable for quality control environment
- Be stability-indicating
- Account for all biologically active constituents of the product (masked and unmasked)

  E.g., bispecific antibodies, antibody-drug conjugates, other antibody-fusion proteins (cytokines, enzyme, etc.)



Common MoAs and Potency Assays for Therapeutic Antibodies

- Virus neutralization assay
- Binding assay (cell-targeting)
- Non-cell targeting (e.g., specific reversal agents)



- Reporter gene assay
- Receptor phosphorylation (Ser/Tyr)
- Cell proliferation/apoptosis
- Anti-differentiation

- Cytotoxicity assay

- Surrogate FcγIIIa or C1q binding
- Control of critical glycans (e.g., fucose, high mannose)
- Cell-based ADCC/ADCP or CDC assays

Modified from *Suzuki M, et al., J Toxicol Pathol 2015 (28: 133–139)*

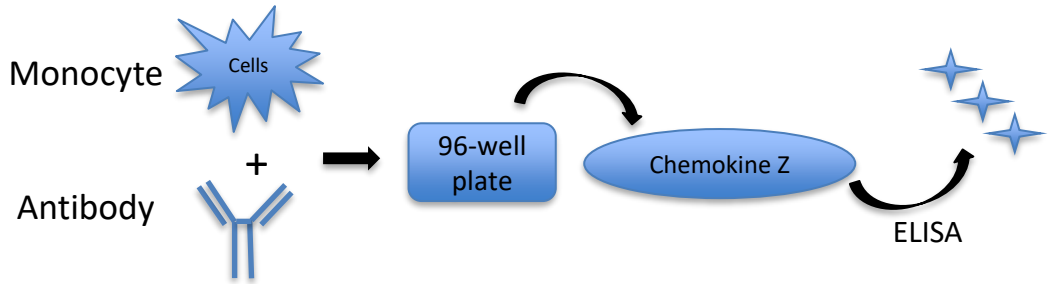
Case Study #1

Product and MoA

- Antibody X is a humanized antagonistic IgG4 mAb
- Fc is engineered with mutation to avoid Fab-arm exchange and formation of half antibodies
- Binds to membrane target Y
- Induces reprograming of M2- to M1-like macrophages for oncology indication

Bioassay

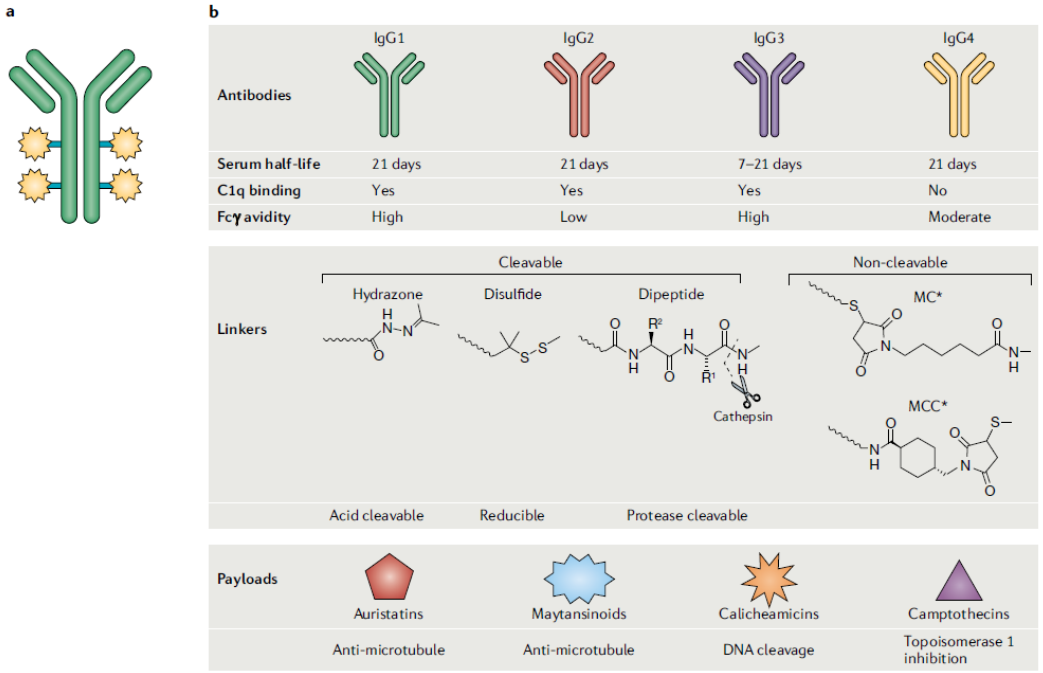
- Uses a monocyte stable cell line
- Measures the ability of antibody in activating the cells upon binding to target Y
- Activation is measured by secretion of chemokine Z, positively correlates to the antibody concentration
- Chemokine Z indicates monocyte activation to macrophage, but does not distinguish M2 vs M1 → the assay does not fully reflect the intended MoA for macrophage reprograming



Antibody-Drug Conjugate (ADC)

ADC mechanisms of action

- Binding to target (could be multi-specific)
- Internalize
- Release payload
- Disrupt internal target (tubulin or DNA)



Drago JZ et al., Nature Reviews, 2021

Typical Potency Assays Include:

- ELISA for target binding
- Cytotoxicity assay for the payload
  - Indicates binding, internalization, release of payload, and cell killing

Additional considerations

- Effector functions
- Bystander effect (target free cells?)
- May not need all assays across mAb intermediate, DS, and DP (justification).
- Drug to antibody (DAR) ratio informs potency control and ensures consistent dosing and safety (off-target effects)

Potency assays should be suitable for the intended use and reflect the (primary) MoAs for all biologically active constituents.

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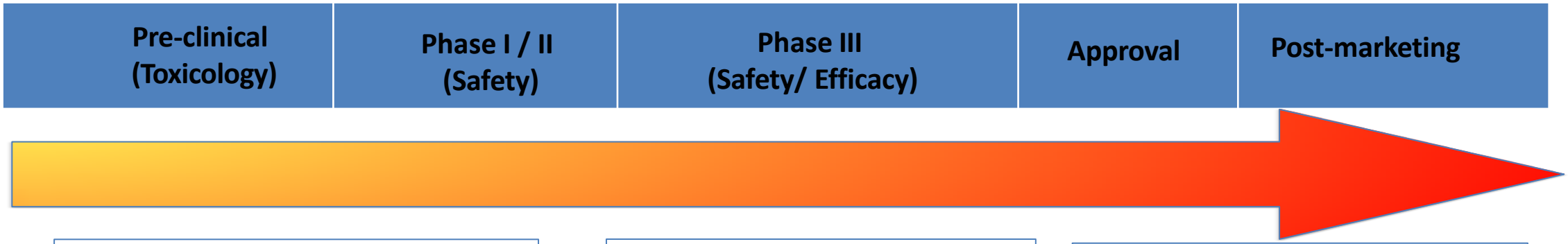
Phase-Appropriate Bioassay Development



Early phase
- Appropriate bioassay may be used for candidate selection
- No or limited information on bioassay submitted to INDs
- Large assay variability
- Wide acceptance criteria

Late phase
- Further developed and well-characterized
- Generally fully qualified with less variability
- Approaching GMP standards and controls
- Better defined acceptance criteria

BLA (and post):
- Fully validated
- Well defined SST criteria and GMP standards
- Tightened and justified acceptance criteria: release and stability testing (IPC)

Early Phase Clinical Development: Phase 1

- Many bioassays may be developed during the early product development
  - Some may be used for candidate selection and characterization only
  - Some as potency assays for release and stability testing

- For cases where binding ELISA is the only potency assay for release and stability testing, the following comment may be issued.

  Example Comment:
  While the current potency assay (i.e., antigen binding ELISA) is acceptable for initiating the proposed phase 1 clinical study, **cell-based potency assay(s)** that reflects the mechanism(s) of action of XXX should be developed and incorporated into the drug substance and drug product lot release and stability testing **prior to entry into a major efficacy trial**. Sufficient retain samples should be appropriately stored for bridging studies to support the development of a new potency assay and ensure lot-to-lot consistency with regard to potency.

Late Phase Bioassay Development: Phase 2/3

- **21 CFR 312.22 (a)**

  *FDA's primary objectives … in Phase 2 and 3, to help assure that the **quality of the scientific evaluation of drugs is adequate** to permit an evaluation of the drug's effectiveness and safety.*

- Therefore, bioassays, reflective of the (primary) presumed MoA, should be developed by phase 3, and fully qualified.
  - New bioassay may be developed and incorporated into specification
  - More than one potency assay may be required as the MoA is further defined
  - Potency assay is expected to be stability-indicating

- Specifications should be tightened based on product manufacturing experience.

Case Study #2

Product and MoA

- Product is a humanized antagonistic IgG1 mAb
- Has Fc mutations that improve half life and enhance the cellular uptake of the mAb-target complex.
- Results in antigen neutralization and prevents it from being cleaved by protease into active form; for a genetic disorder.

Potency Assay Development

- Binding SPR was used as potency assay for IND opening study; not stability-indicating.
  - A cell-based potency assay was recommended
  - A cell-based reporter gene assay had been developed for nonclinical studies (described in Module 4)
- During clinical phase 1/2, the sponsor developed a cell-free assay that reflects the key MoA (preventing the conversion of target antigen into active form in competition with protease) and is stability indicating.
- The cell-free assay appeared to be superior to the cell-based assay, and eventually was deemed adequate for DS/DP release and stability testing.

Bioassay Development at BLA Stage

- The final potency assay(s) is chosen for lot release and stability testing (need to balance assay complexity vs. QC feasibility)

- Sufficient data are provided to define and justify the acceptance criteria

- Assay variability should be reduced by understanding the fickle variations in a cell
  - Cell culture conditions (pH, media composition, growth factors)
  - Density of cells (e.g., may impact cell surface receptor expression)
  - Handling of cells and the assay should be done in a very reproducible manner

- Full assay validation data are available for all the selected assays

- For accelerated development timeline with limited number of lots, the acceptance criteria may need to be further tightened using additional commercial lots.

Validation of Bioassays

21 CFR 211.165 Testing and release for distribution:
The **accuracy, sensitivity, specificity, and reproducibility** of test methods employed by the firm should be established and documented.

Validation parameters per ICH Q2(R2)

- Assay Specificity (product discrimination; stability-indicating)
- Assay Range (linearity, sensitivity: LOD/LOQ)
- Assay Accuracy (% recovery, degradation product)
- Assay Precision (repeatability, intermediate precision, reproducibility)
- Assay Robustness (method parameter variations, SST)

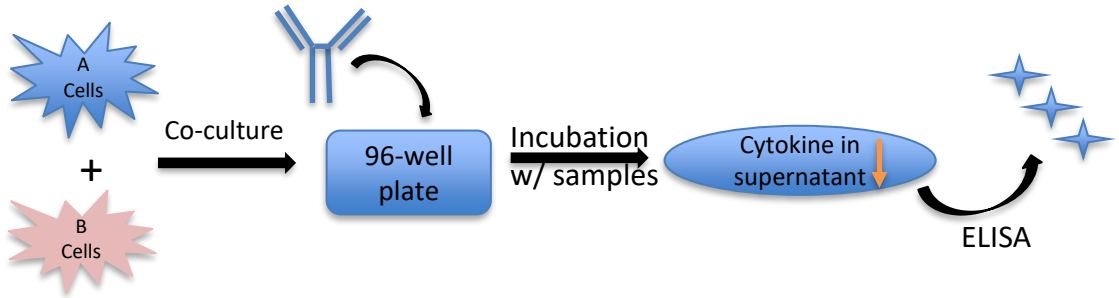
Case Study #3

Product and MoA

- Product is a humanized antagonistic IgG1 mAb
- Fc is engineered to silence potential effector functions
- Binds to membrane target
- Induces immune activation for oncology indication

Bioassay

- Uses co-culture of two types of cells:
  - Cell A is engineered to express the target
  - Cell B is engineered to express target receptor
  - Co-culture leads to cytokine expression
- Assay measures the antibody's ability in blocking the cytokine expression, measured by ELISA
- Samples are tested in duplicate; SST requires that the duplicate determinations CV ≤ 30%.

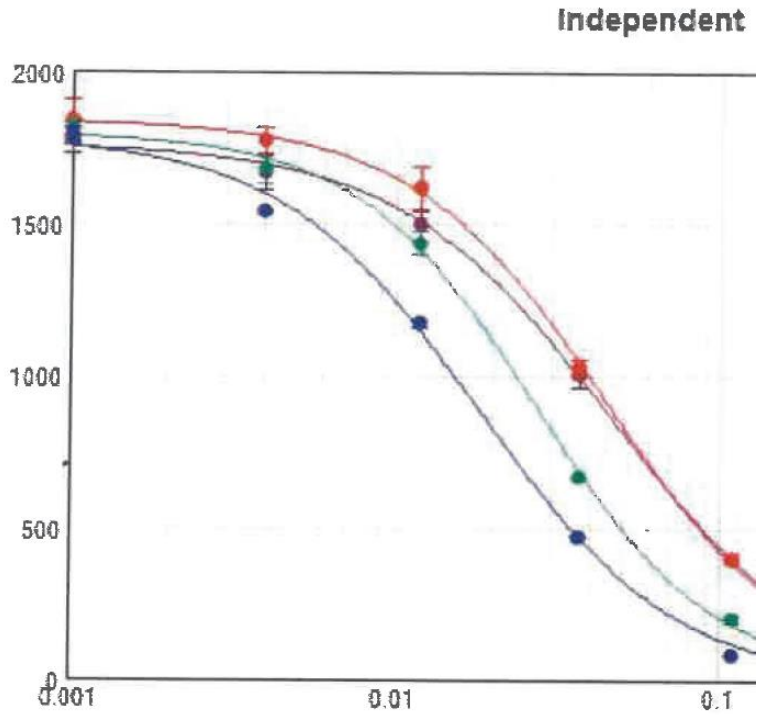


Case Study #3 (Continued)

During inspection, it was found that:

- The cell culture supernatant from duplicate samples were mixed, prior to ELISA assay; the purpose was to reduce the assay variability (CV%)
- Inadequate SST and curve similarity controls (control for hillslope but not upper or lower asymptote)
- High variability at upper asymptote
- Invalided assay show high CV%

Assay being very complex and NOT QC friendly! (and inadequate SST control)



Bioassay Changes

Changes may occur throughout the lifecycle. The degree of data needed to support a change depends on:

- The development stage
- Types of changes e.g.,
  - New testing method
  - New testing site
  - Changes to the reagents or instruments

Overlapping data using both assays are required to support assay change and bridging between assays, non-clinical, and clinical data. Samples for method bridging may include:

- QC release samples (multiple lots)
- Stability samples
- Forced degradation (high temperature, pH, photostability, F/T)
- In-process samples
- Aggregates/degradants

A new assay should have a justifiable advantage over the existing assay

- Better accuracy, precision, sensitivity
- Product variants/degradants
- Stability-indicating

Case Study #3

change in analytical procedures assessed using stressed conditions

	Assay#1		Assay #2	
Samples and Conditions	Mean	%CV	Mean	%CV
Control, Normal Storage Temperature	96	8	99	5
Elevated Temperature– short term	86	8	71	4
Light Protection	99	6	97	2
High Light Exposure	70	5	47	3
0% Oxidizing Agent	103	8	100	4
5% Oxidizing Agent	94	7	80	4
Acidic pH Conditions	97	4	86	1
Basic pH Conditions	90	5	77	3

The new assay #2 shows advantages over stability-indicating and precision.

Testing Site Changes

Inspection Finding:

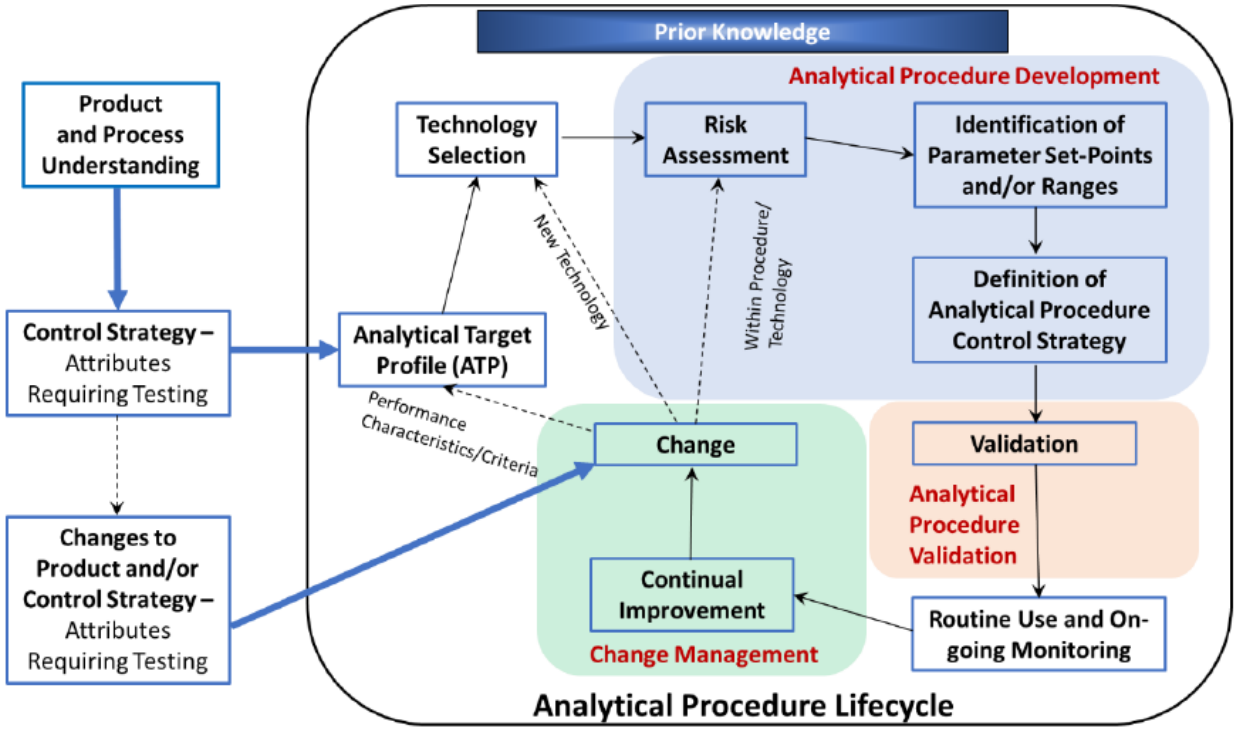
- Bioassay was transferred from BLA applicant to CMO
- CMO has 4 testing sites: A, B, C, and D
- Only site A did assay transfer and validation
- However, internal GMP documents list all 4 sites as the testing sites for the assay.

21 CFR 211.194 Laboratory records

(a)(2) A statement of each method used in the testing of the sample. The statement shall indicate the **location of data** that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested…. The ***suitability of all testing methods*** used shall be verified under ***actual conditions of use***.

ICH Q14: Analytical Procedure Development (November 1, 2023)

Figure 1: The analytical procedure lifecycle



Analytical target profile (ATP) as the basis for analytical procedure development, ongoing monitoring, and continual improvement.

Tools from Q12 are applicable for analytical procedures

- Established conditions (ECs)
- Product lifecycle change management (PLCM)
- Post-approval change management protocols (PACMPs)

ECs are primarily focused on method specific performance criteria

- Requires strong understanding of the relationship between method parameters and method performance

Summary

- **Potency assays should reflect the (primary) proposed MoAs, stability-indicating, and suitable for QC testing.**
- **Bioassay should be suitable for the intended purpose and follow a phase-appropriate development timeline.**
- **Sufficient data should be provided to support the assay changes under actual conditions of use.**
- **Analytical target profile should be the basis for bioassay development and lifecycle management.**

Acknowledgements

OPQA III Colleagues:

- Rapti Madurawe
- Leslie Rivera Rosado
- Chana Fuchs
- Marjorie Shapiro
- Nailing Zhang
- Gerald Feldman
- Susan Kirshner

Thank You!