

# **BIOASSAYS FOR POTENCY: An FDA Perspective**

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# POTENCY:

## Regulatory Definitions

- **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.

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# WHAT MAKES A GOOD POTENCY ASSAY?

- Reflects mode of action (MOA)
- Reproducible
- Accurate
- Robust
- Stability indicating
- Practical

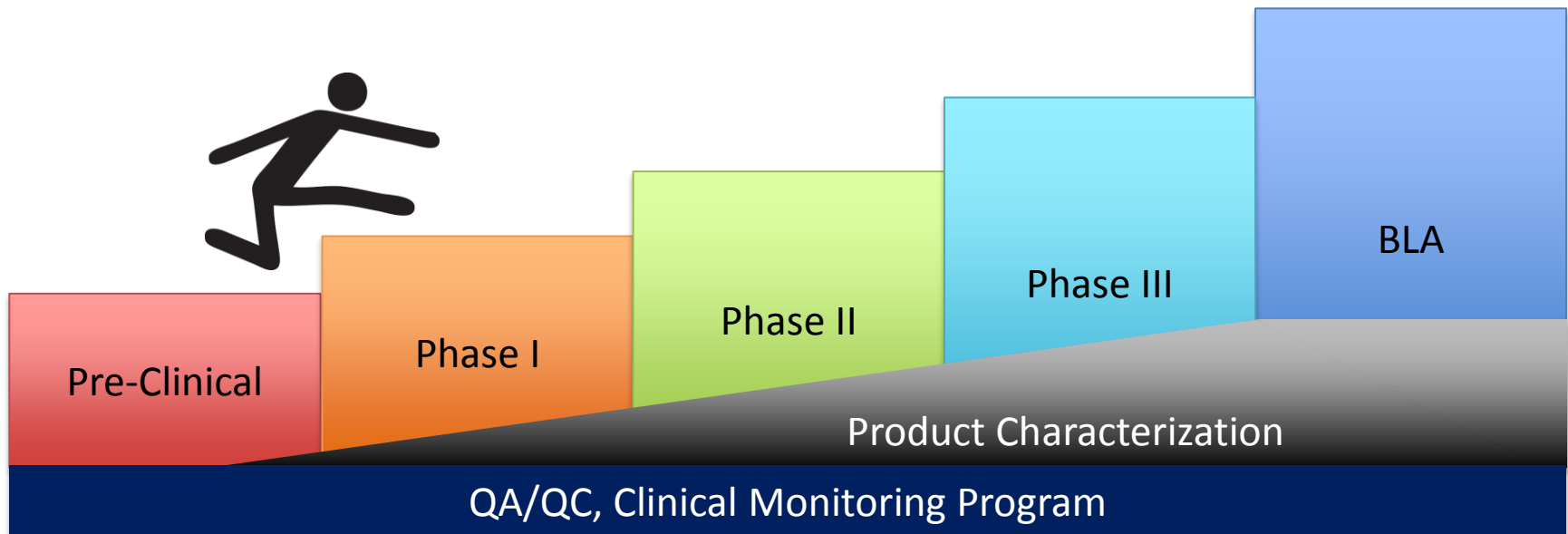


# CELL AND GENE THERAPY PRODUCTS: Challenges for Potency Assays

- Complex, variable products
- Mode of action may not be fully known
- Time constraints for release testing
- Limited material available to test
- Limited availability of reference standards and controls

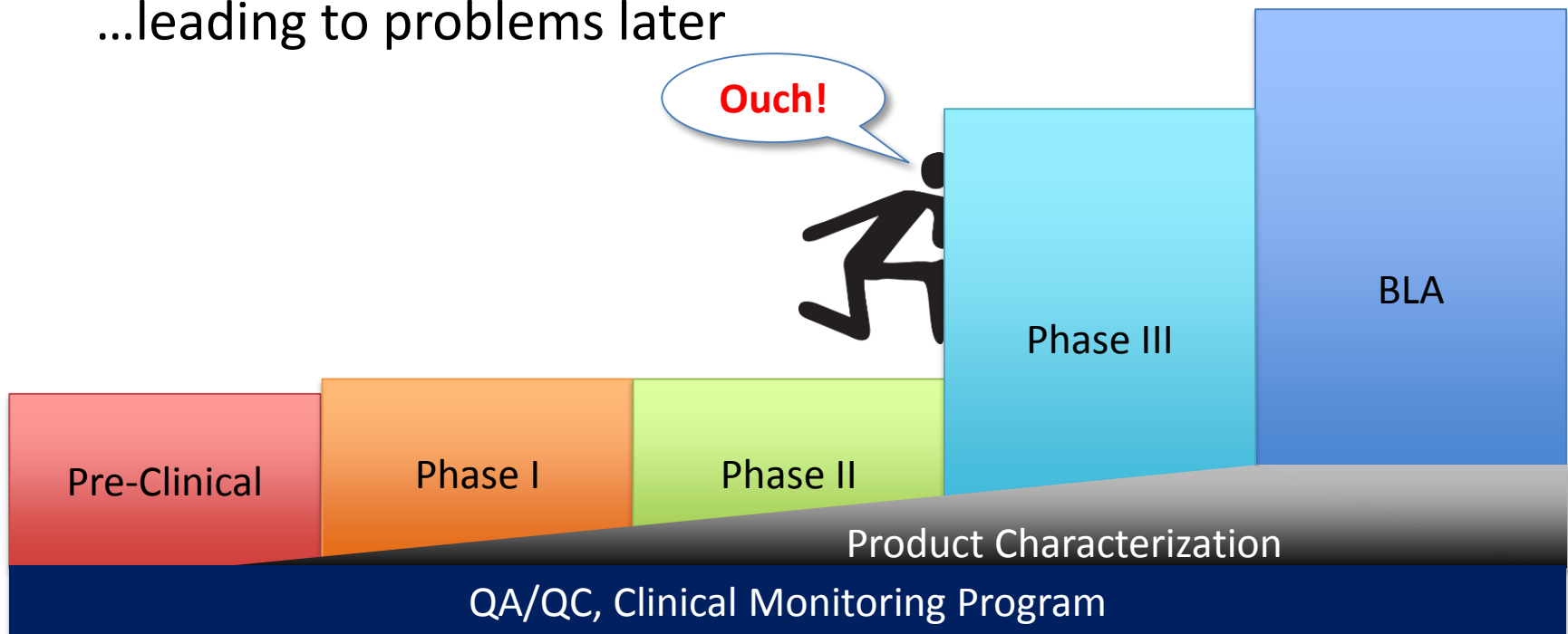
# PRODUCT LIFECYCLE APPROACH TO POTENCY MEASUREMENT: Ideal

- Stepwise assay development
  - Investigation of biological activity
  - Development of relevant potency assay



# PRODUCT LIFECYCLE APPROACH TO POTENCY MEASUREMENT: Typical

- Assay development and product characterization is often postponed once product enters clinical studies...  
...leading to problems later



# POTENCY ASSAY DEVELOPMENT

- **Preclinical and Early Clinical Development**
  - Methods guided by preclinical data and proposed MOA
  - Ensure activity/strength of product for dosing
  - Assays to determine dose should be suitable for their intended purpose (accurate, reproducible, specific)
- **Throughout Clinical Development**
  - Continue to characterize product and develop assays
  - Explore multiple measures of potency
- **By Phase III (Pivotal Studies)**
  - Well defined and qualified potency assay
  - Part of lot release: May refine specification(s) if needed
  - Part of stability test protocol
- **For BLA**
  - Validated potency assay
  - Used for lot release, product stability, product comparability



# POTENCY ASSAY VALIDATION

- **During assay development**
  - Evaluate assay performance and suitability
- **Analyze and validate all relevant assay parameters**
  - Accuracy
  - Detection limit
  - Precision (repeatability, intermediate precision)
  - Specificity
  - Linearity and range
  - Robustness
  - System suitability
- **Not all assays are created equal**
  - Some will be harder to validate than others

# REFERENCE MATERIALS AND REAGENTS

- **Qualification:** demonstrate suitability
  - Antibodies, critical reagents, assay kits, cell lines
  - Lot-to-lot variability of reagents
  - Don't be over-reliant on manufacturer
- **Availability**
  - Ensure sufficient supplies of critical materials
  - Cell banks (including for control cells, if applicable)
- **Retention samples and Reference standards**
  - Important for qualifying new reagents/reagent lots

# CASE STUDY:

## Bioassay for a Cancer Vaccine I

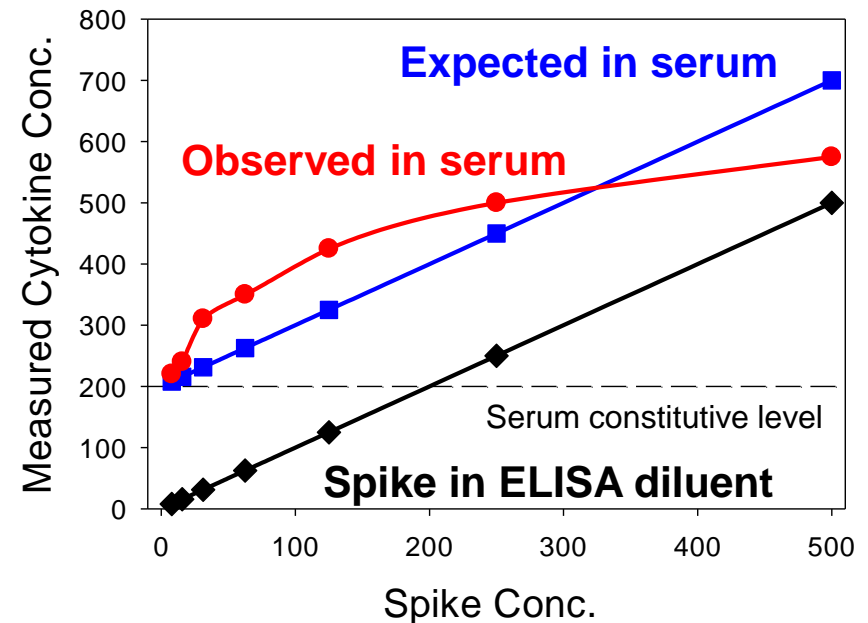
- Inject dilutions of product into mice
- Measure serum cytokine concentration by commercial ELISA kit
- Independently validate mouse assay and ELISA
  
- Good: Direct measure of product activity relevant to MOA
- But... ELISA kit measures constitutively expressed subunit shared between multiple cytokines, not bioactive cytokine
- **Know what your assay is measuring and understand the underlying biology**

# CASE STUDY: Bioassay for a Cancer Vaccine II



## Cytokine ELISA

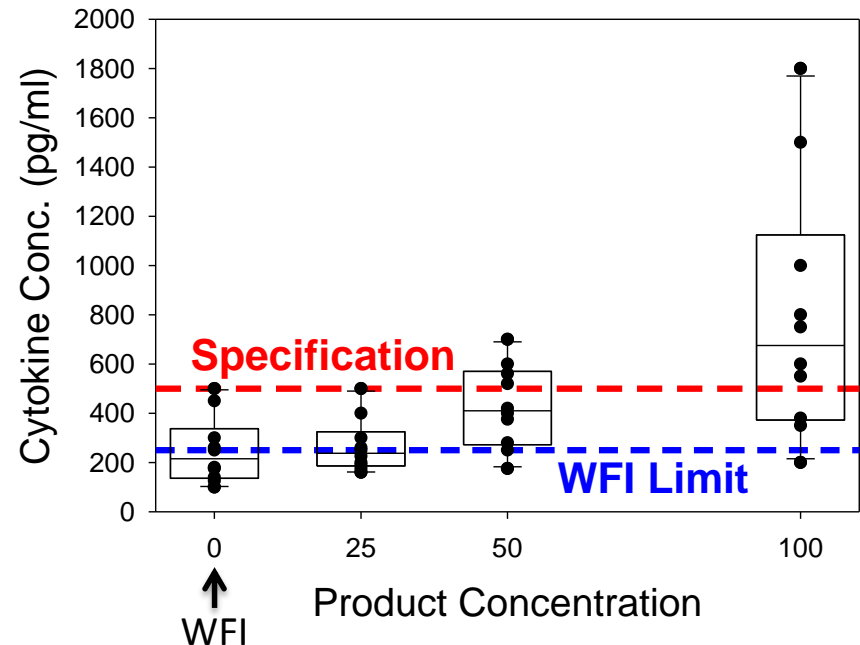
- Spike study revealed interference from the sample matrix (serum)
- Serum behaved differently to ELISA diluent buffer
- Expected spike conc.  $\neq$  observed conc.
- Assay failed on poor accuracy (but precision good)
- Only tested 1 lot of ELISA kits from manufacturer



# CASE STUDY: Bioassay for a Cancer Vaccine III

## In vivo assay

- Test 3 dilutions of product + WFI control: 10 mice/group
- Specification: Group mean  $\geq 500$  pg/ml *based on highest concentration of product*
- No upper limit set
- Assay valid if WFI group mean  $\leq 250$  pg/ml (narrow pass/fail window of test)
- Wide variability between animals in same group (15-75% CV)



- Product lot used for potency test validation was an outlier for other attributes

# CASE STUDY:

## Bioassay for a Cancer Vaccine IV: How to address the issues

- **Cytokine ELISA**
  - Revalidate assay using serum
  - Test multiple lots of kit
- **In vivo assay**
  - Reconsider dilutions used
  - Add upper limit to specifications
  - Revalidate using non-outlier products
  - High variability between animals harder to address
- **Consider alternative assays – cell based?**

# SUMMARY

- **Start working on potency assays early**
  - Test different assays, discard ones that don't work
- **Continue throughout product development**
- **Product characterization**
  - Understand mode of action
  - Understand what product attributes contribute to biological effect
  - Use to guide assay development
- **Validated assays for BLA submission**
- **Talk to regulators about challenging issues**



# OTAT contact information

- **Regulatory Questions:**

Contact the Regulatory Management Staff in OTAT at

[CBEROCTGTRMS@fda.hhs.gov](mailto:CBEROCTGTRMS@fda.hhs.gov)

or [Lori.Tull@fda.hhs.gov](mailto:Lori.Tull@fda.hhs.gov)

- **References for the regulatory process for OTAT**

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/ucm094338.htm>

- **OTAT Learn Webinar Series:**

<http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm>





# Public Access to CBER

- CBER website:  
<http://www.fda.gov/BiologicsBloodVaccines/default.htm>  
Phone: 1-800-835-4709 or 240-402-8010
- Consumer Affairs Branch (CAB)  
Email: [ocod@fda.hhs.gov](mailto:ocod@fda.hhs.gov)  
Phone: 240-402-8010
- Manufacturers Assistance and Technical Training Branch (MATTB)  
Email: [industry.biologics@fda.gov](mailto:industry.biologics@fda.gov)
- Follow us on Twitter:  
<https://www.twitter.com/fdacber>



**Jennifer Swisher, Ph.D.,  
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# Changing Bioassays



- When a new assay has a justifiable advantage over the current assay
  - More reflective of the mechanism of action
  - More stability indicating or better at detecting potency changes due to product variants/ degradation products
  - Improved accuracy, precision, sensitivity, etc.; more “QC friendly”
- Side-by-side testing should demonstrate equivalence/superiority with a variety of types of samples
  - Multiple lots of product (retains, release and stability)
  - Accelerated and stressed stability samples
    - Photostability, pH, UV, chemical stress, etc.
  - Charge variants (deamidated, oxidized, etc.), glycan variants, proteolytic fragments, aggregates
- Post-pivotal trials, acceptance criteria of new assay need to be linked to clinical experience through the acceptance criteria of the current assay

## CASE STUDY 2

# From Binding Assay to ADCC Surrogate

- Proposed mechanism of action: ADCC
- Potency: Target binding assay (phase 1-2)
- Additional assays for characterization:
  - FcγRIIIa (V158) binding
  - Bridging assay (simultaneous binding of target, FcγRIIIa)
  - Surrogate ADCC: engineered effector and target cells; measures FcγRIIIa-driven reporter expression

**Agency and Sponsor agreed that an assay to evaluate ADCC activity should be implemented as a release assay prior to pivotal trials**

# CASE STUDY 2: Substitution of surrogate ADCC for binding assay

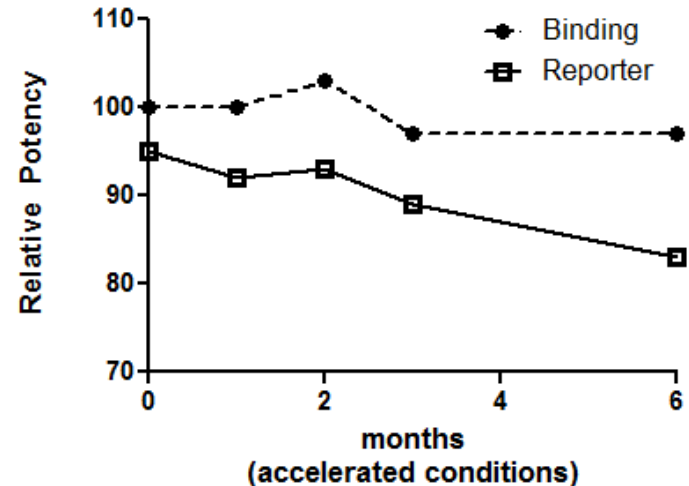
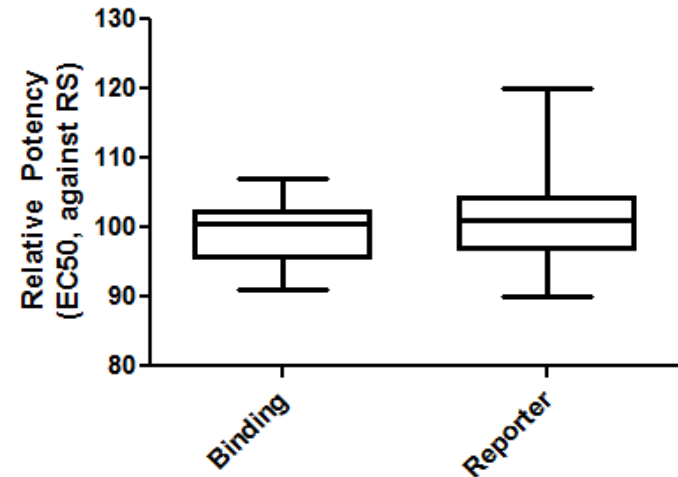
## Prior to Phase 3:

- Tested a limited number of lots of DP, side by side (current process, retains)
- Limited stability sample testing

## Initially failed to test:

- Substantially stressed stability samples
- Samples enriched in modifications known to affect potency

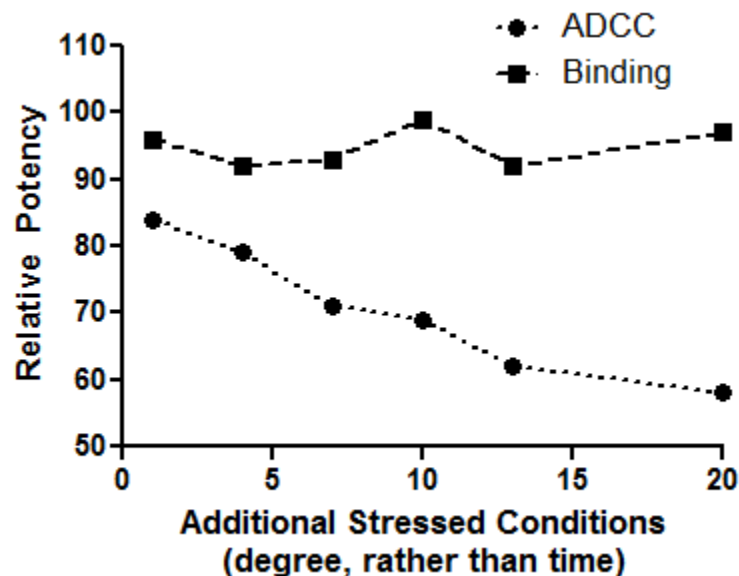
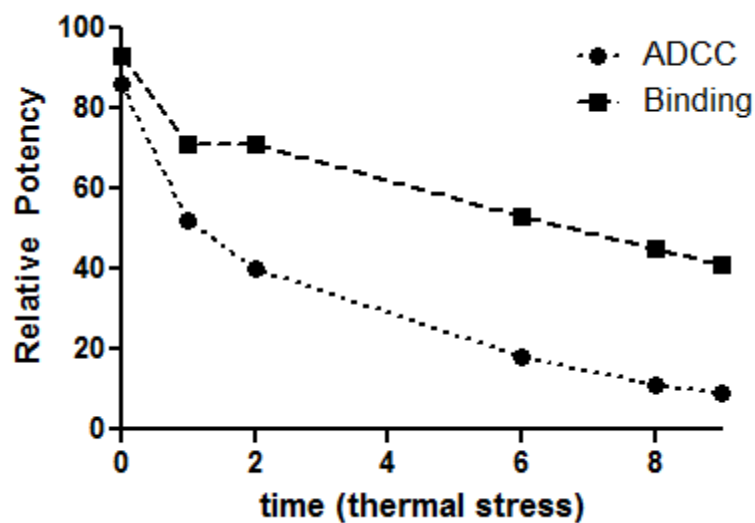
**Additional information was required...**



# CASE STUDY 3

## Substitution of ADCC assay for binding

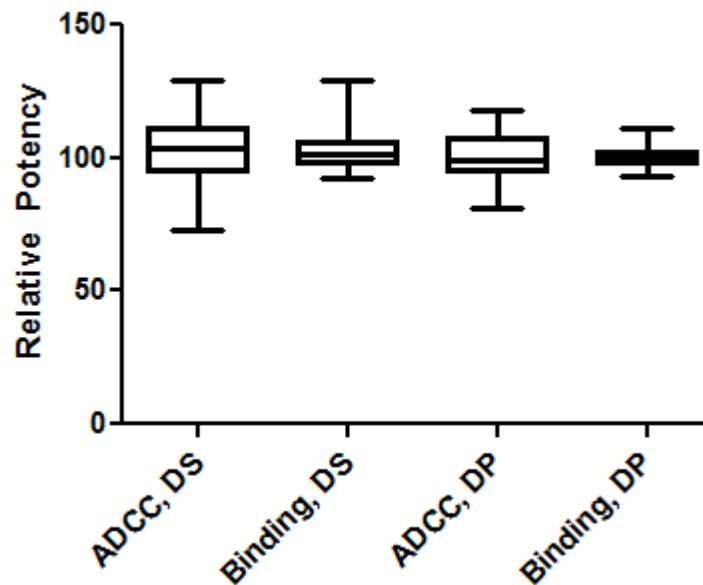
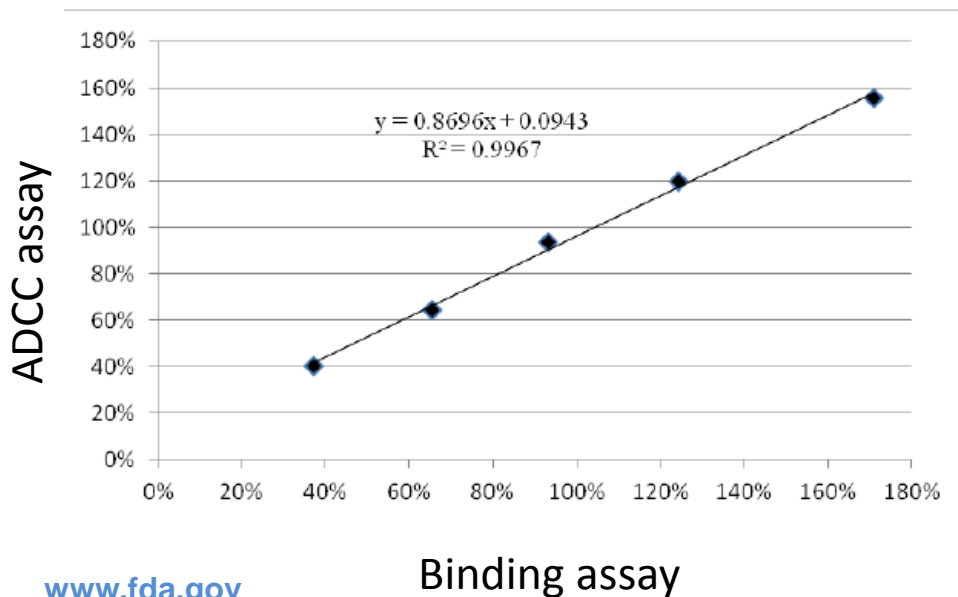
- Similar situation, substitution to Case Study 2, although a direct ADCC (killing) assay is replacing a binding assay
- First, showed a side-by-side comparison of the assays using samples treated with two distinct, suitably harsh sets of stressed conditions



# CASE STUDY 3

## Substitution of ADCC assay for binding

- Made a dilution series of the reference standard to demonstrate that the ADCC assay was just as accurate across the same range as the binding assay
- Tested several dozen DS and DP retain samples (release and stability) in parallel with the two assays



# CASE STUDY 4

## Substitution of binding assay for cellular

- Original, cellular assay was more representative of the mechanism of action; contained downstream steps
- Mechanism of action believed to be simple (binding only)
- Side-by-side assay comparison included:
  - Enriched charge variants
  - Enriched deamidated variants (for each amino acid separately)
  - N-terminal clipped variants
  - Aggregates
  - Retains from different processes (~15 lots DP, 60 DS, 40 stability)
  - Forced degradation samples from all processes
- Binding assay was found to be equivalent to the cellular assay in all cases, but was likely superior in lower risks to critical raw materials/ reagents.



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