Lessons Learned for Potency Assays for Bispecific mAbs for Accelerated Programs

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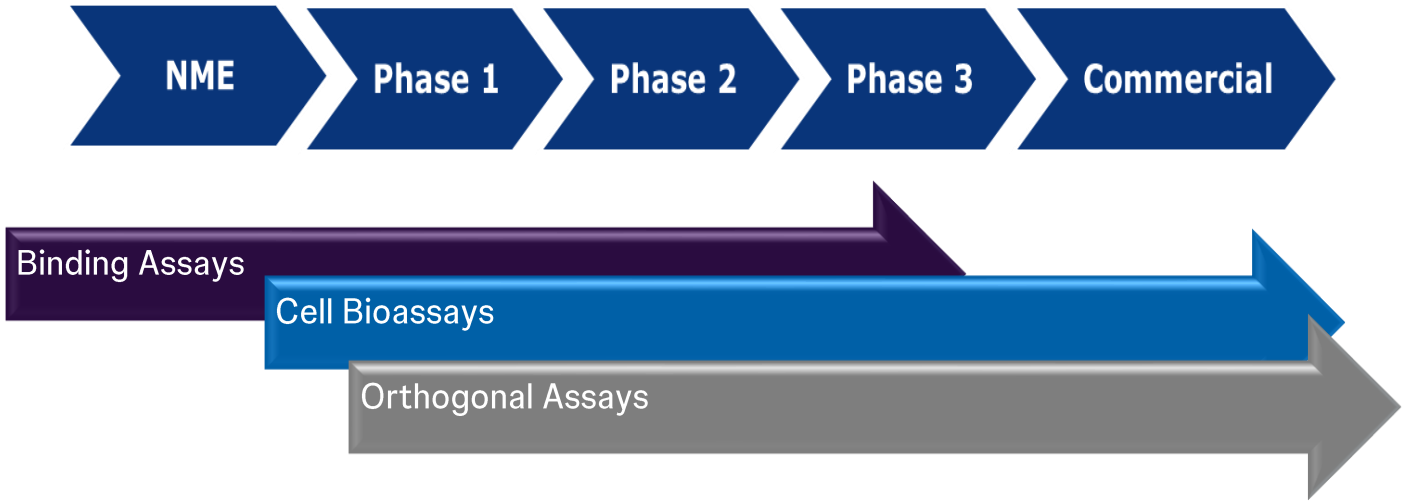
Phase based potency assay approach for Mabs

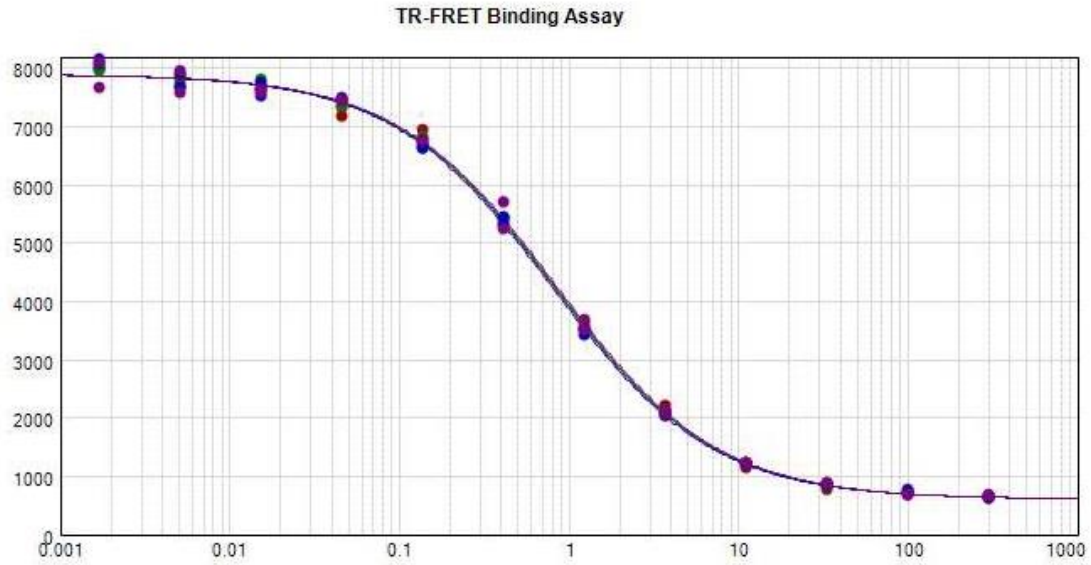
Binding assays: implemented in early development (Preclinical to FIH)

Cell bioassay development deferred until after FIH



Orthogonal functional and binding assays: for Characterization and to establish Control Strategy

Binding Assay Platform approach globally implemented



Competitive Assay format

- Mix and Read assay
- Development time- 2 weeks
- Easily Automated

Control of critical reagents required

Implemented in > 6 testing labs over >50 NMEs

Validation for clinical phase

- Precision <15 % -typically 5-10%
- Control trending shows variability ~5% in routine use

Platform Technology also used for Characterization Assays such as FcRs, across programs

- Methods easily modified to accommodate engineered Fc regions.

Phase appropriate Potency assays

Cell based bioassay(s) representative of MOA are required to fully characterize biological activity during clinical development.

For Mabs the goal should be a single potency assay on release with appropriate biological characterization assays

- This can be a Binding assay.

- **What happens with a Bi-specific** ???

Potency Approaches for Bi-Specific Antibodies

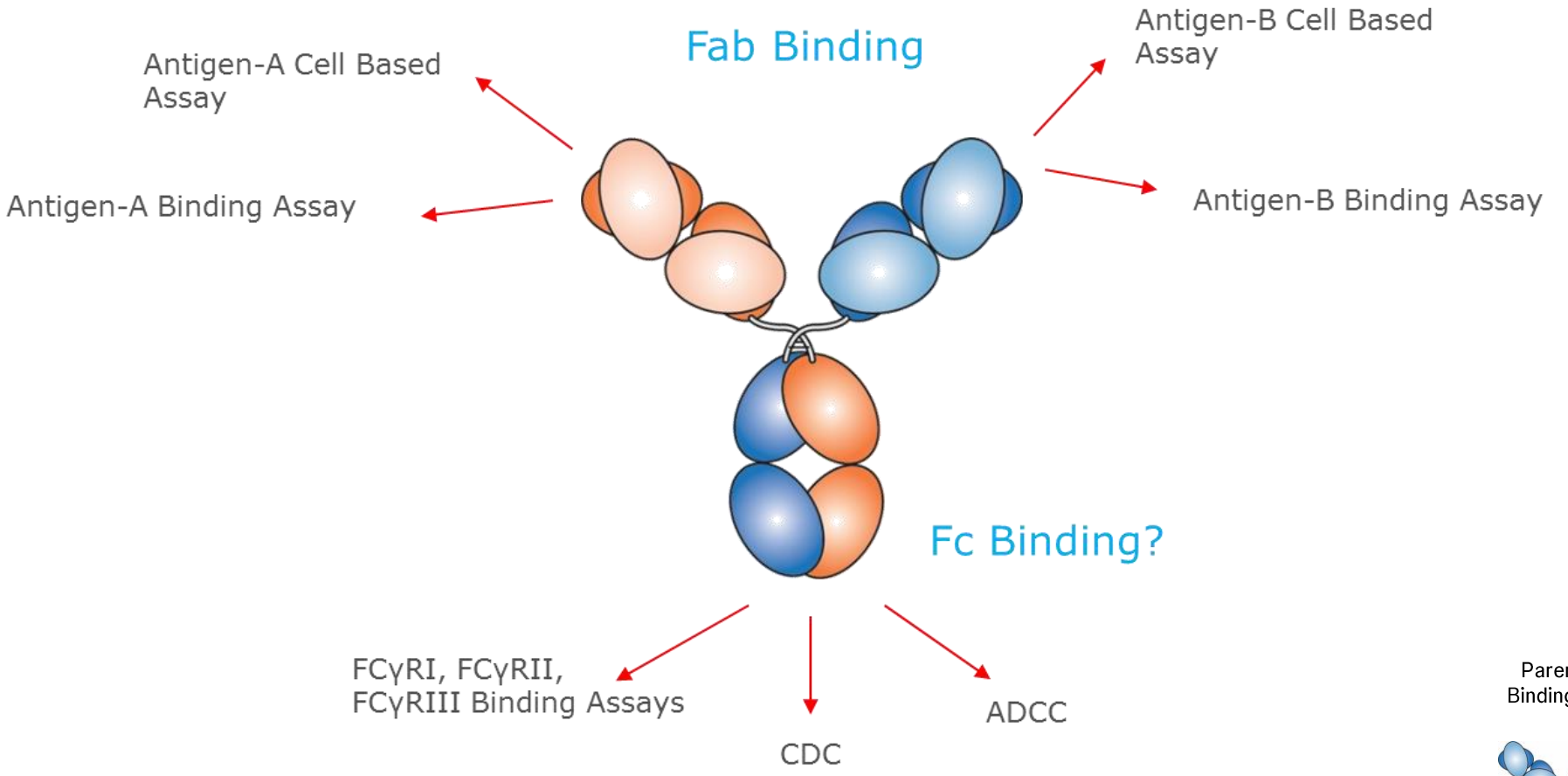
- Phased approach Bioassay method development:
  - Develop platform binding assays rather then cell based assays.
  - TR-FRET: ‘simple’ single plate assays.
  - minimize time and cost at early phases.

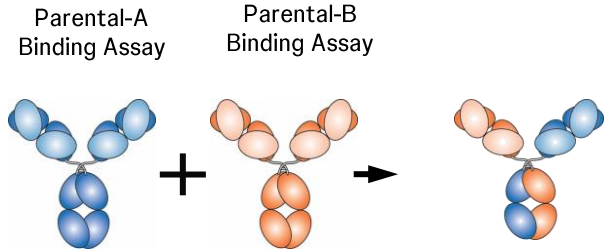
  - **separate assays to monitor binding of each Fab arm**.

- At **late phase** development cell based bioassays:
  - must reflect mode of action.
  - must define reference material (and control) strategy.
  - assay bridging studies
  - Robust retain strategy

  - Due to complexity of MOAs don’t delay development !!

How Many Assays?- It depends !





Oncology Bi-Specific-BsAb #X

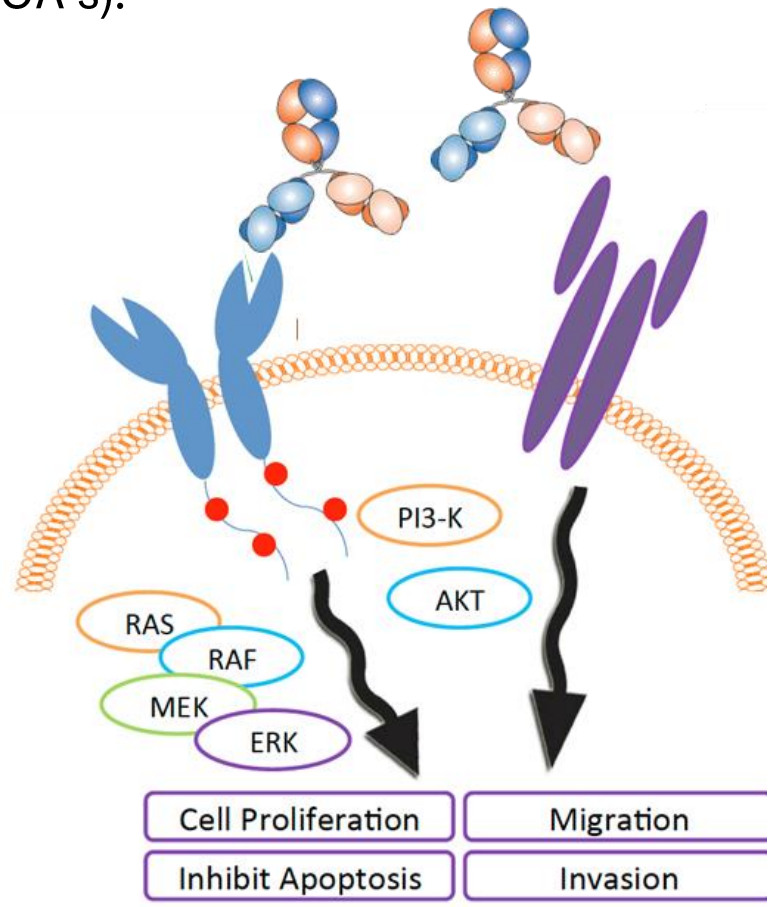
- Bi-Specific Targeting 2 cell surface receptors (5+ potential MOA's).

  - 2 x TR-Fret Binding Assays (2x Fab)
  - 3x Binding assays (Fc)

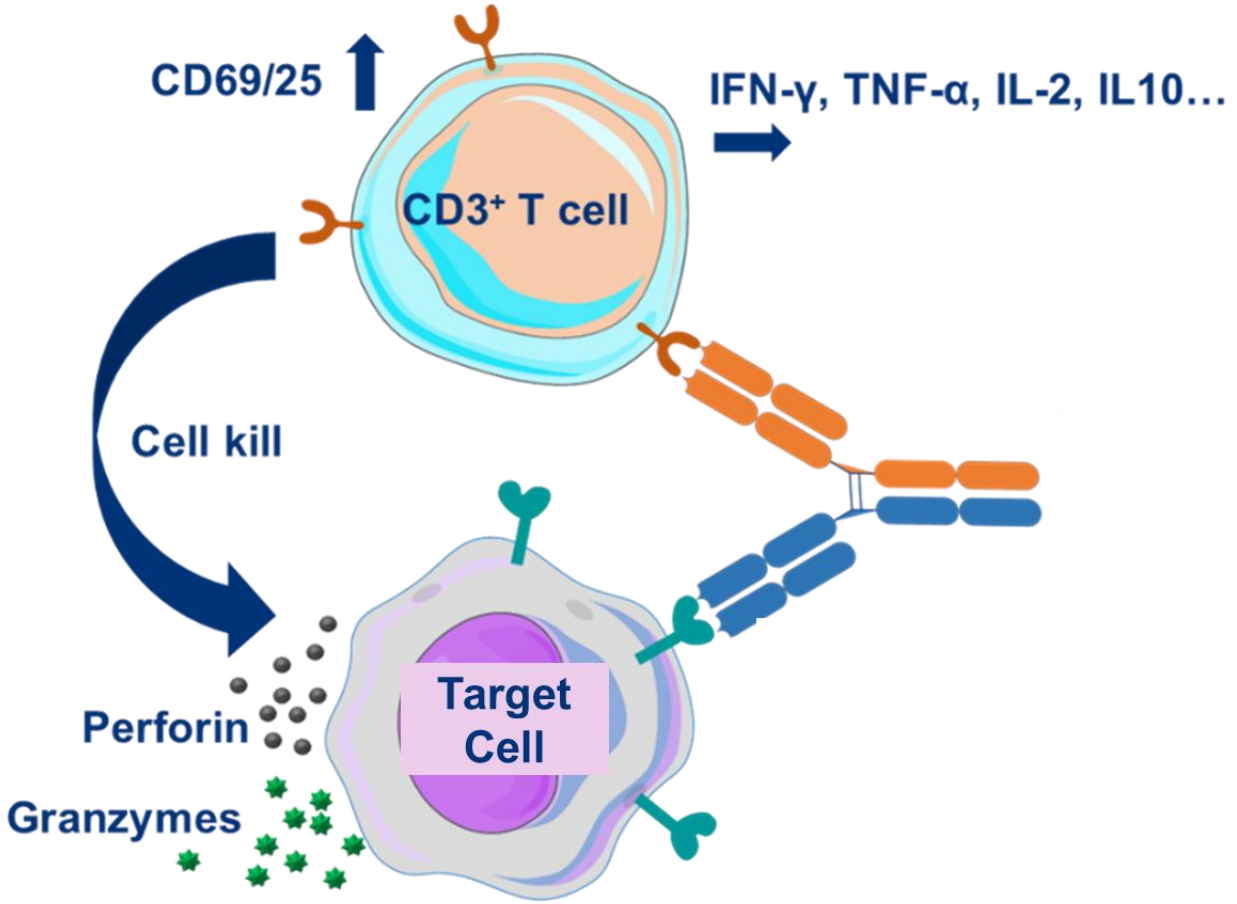
  - Cell Death Cell Based Bioassay
  - Cell based Reporter assay (eg ADCC)
  - Inhibition of cell signalling assay (s)

  - 2 x Parental Binding Assays (IP)

  – Ref Std, CT's, Qualified Reagents & Cells



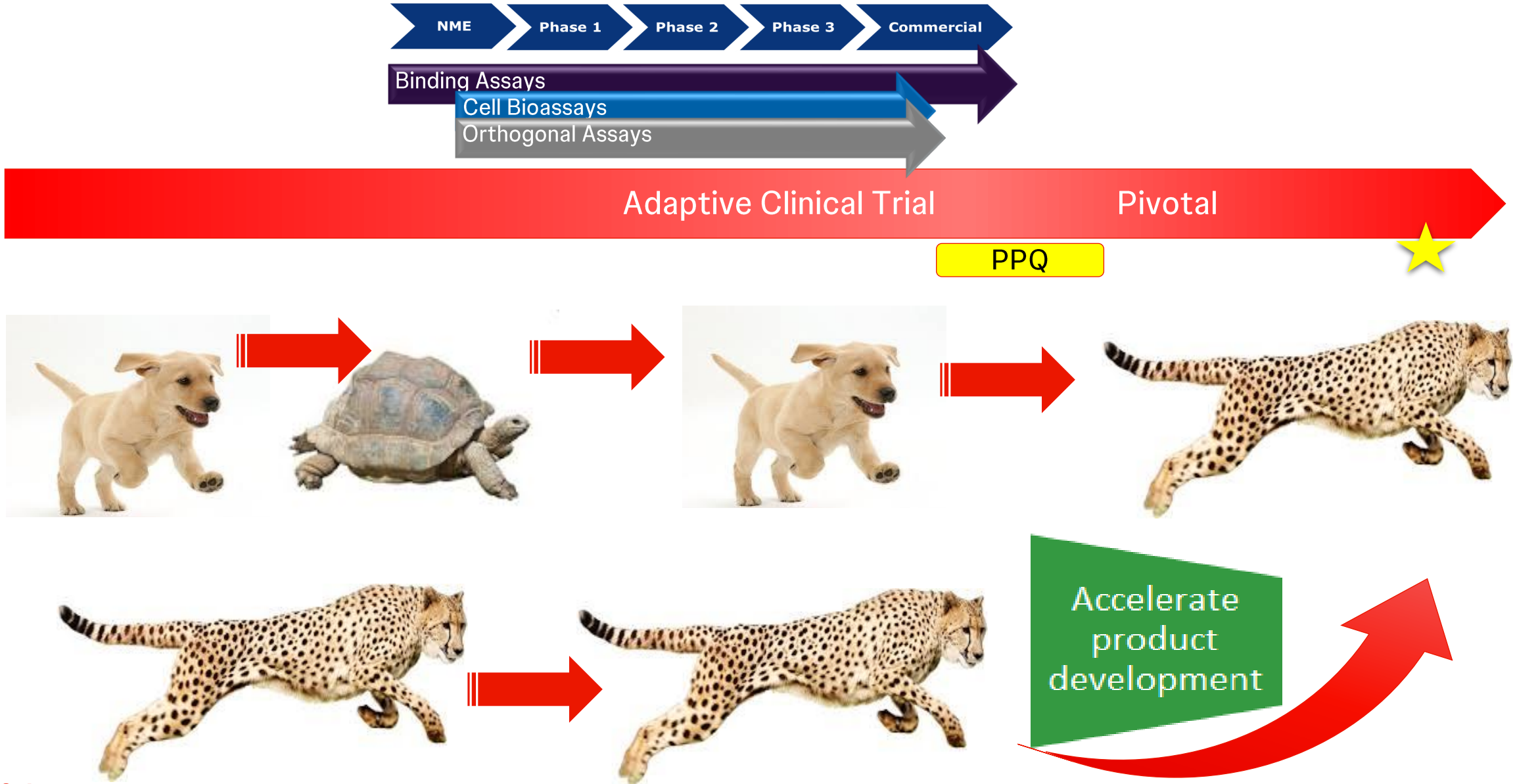
BsAb #Y Simple or Complex ?



Co-engagement is Key !

MOA dependent strategy needs to be defined.

Development Timelines- Fast to FIH , fast to BLA or both



Challenge #1 Assay Development

- Binding assays used for FIH
- Cell-based assay development delayed pending clinical data
  - Prior experience with a similar type assay
  - Initial feasibility completed, MCB and WCB available and assay development was paused.

- Numerous unexpected issues in development when restarted !
  - Prior experience with Platform technology doesn't guarantee success
  - **Everything changes with new Mab and cell line**

- Implementation of assay required for Biological activity !

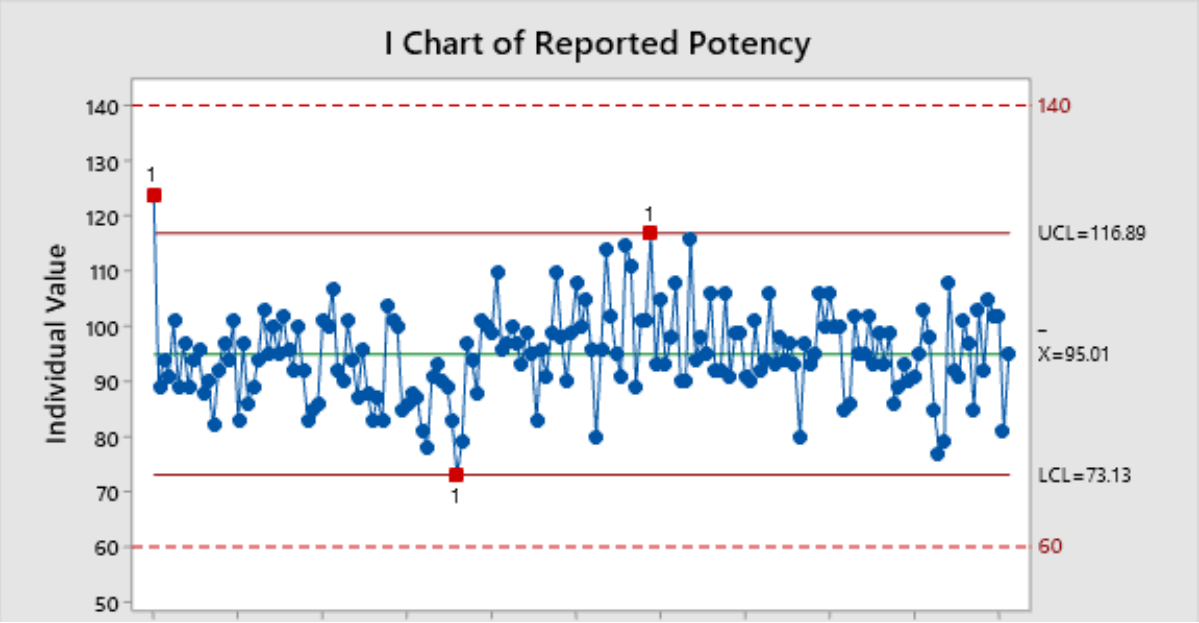
Challenge #2 Assays Move all over the Globe

- Prior experience doesn't guarantee success- similar assay format changes with every different Mab
  - New cell lines
  - Updated assay format
- Analysts may change from implementation to routine testing
  - Training and knowledge transfer is key .
- Usual suspects-
- Reagents and plastic ware, availability in different regions
- Instruments

Challenge #3 Retain Testing

- Introduction of potency assay when program in “cheetah” phase
  - Retains of clinical batches release and stability
  - Large volume of testing in a short period of time

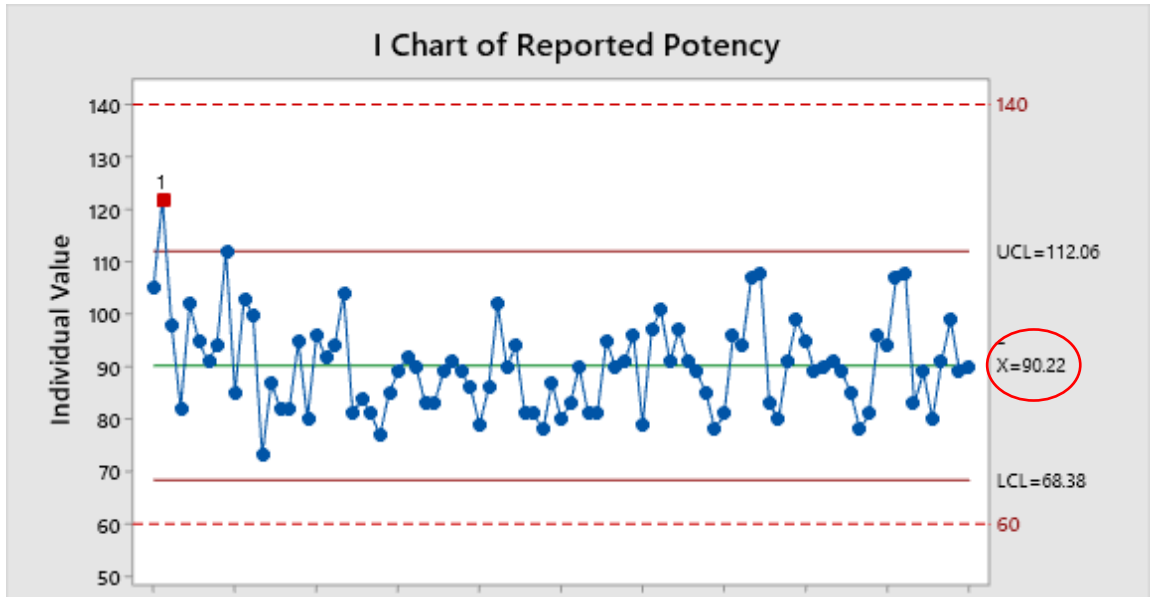
  - Assay transfer by co qualification testing and development lab
    - Post transfer assay trending showed site differences



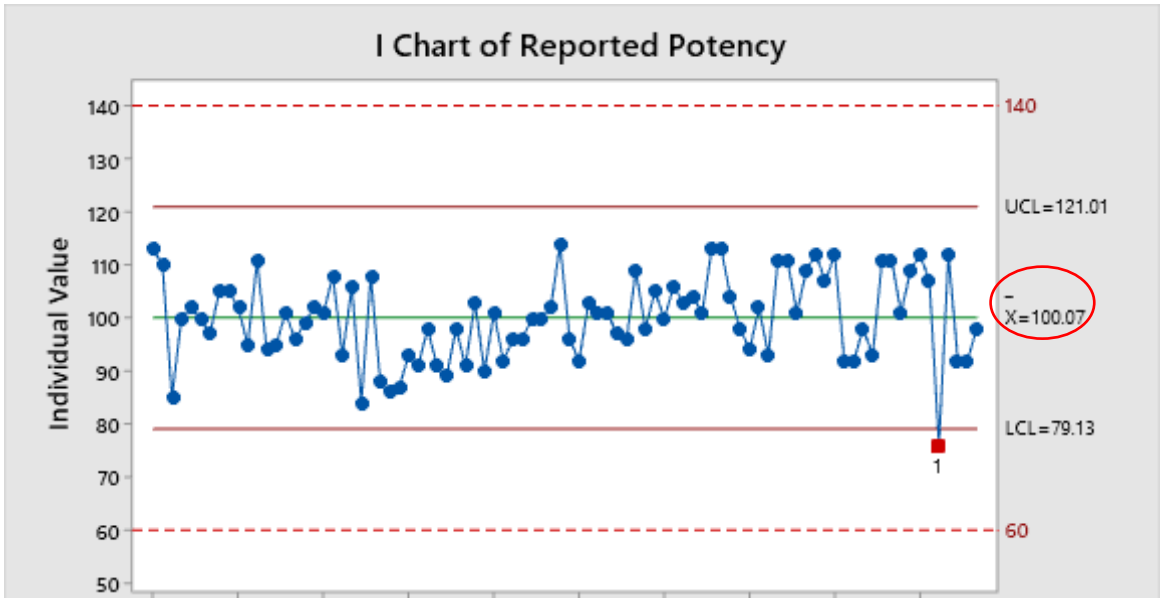
Control Trending indicating bias between sites ~5%

Trending by position on plate

S1 trending <10%lower than S2

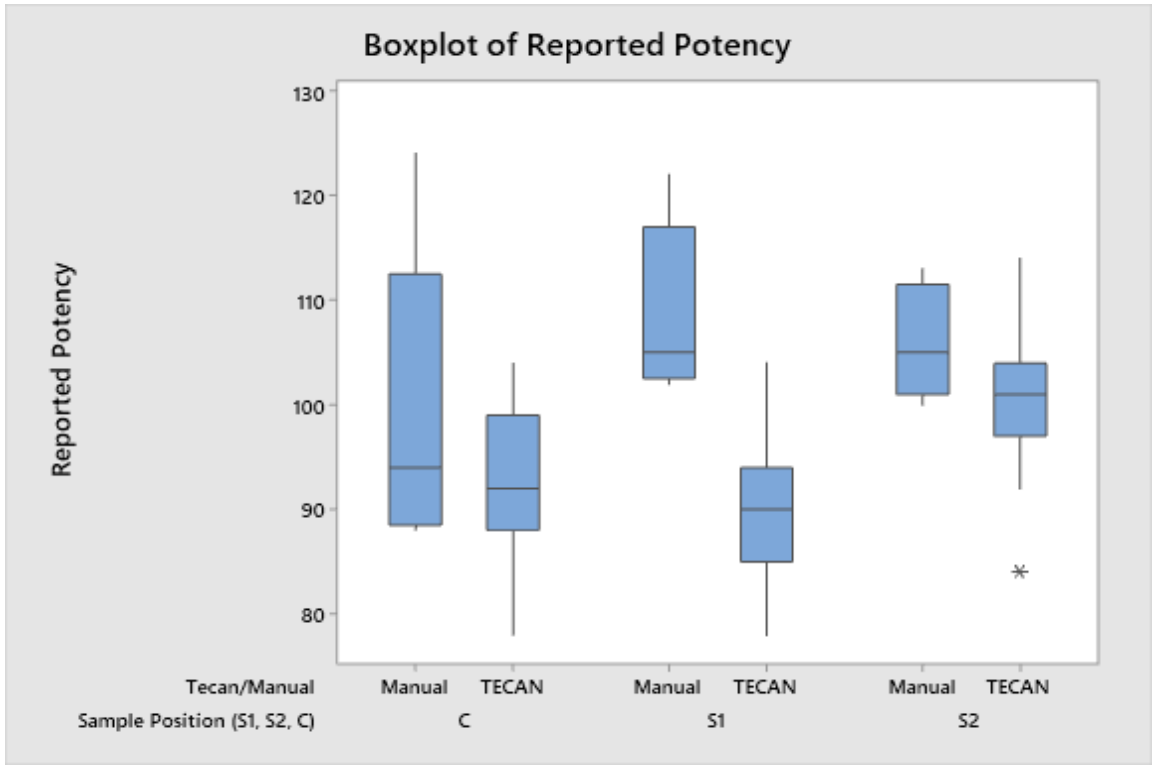


Sample Position 1

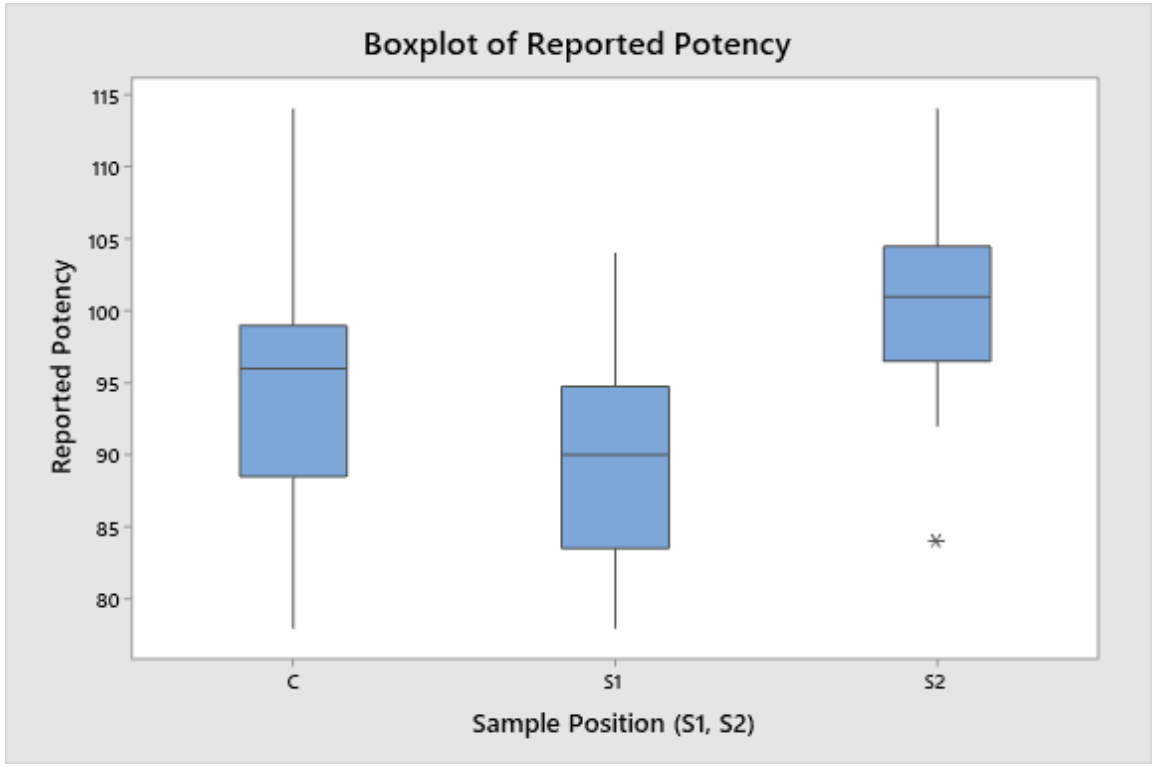


Sample Position 2

Automation vs Manual Execution- **It's the liquid Handler**



S1 positional Bias with automation



- **New liquid handler became available – same script**
  - **No Issues**

More Challenges in Assay Development

- **#1** Binding assays used for FIH

- Cell-based assay implemented soon after FIH
  - Binding assays replaced and used Characterization

- extensive experience and continuity of data collection during developmer..
  - No retain testing required
  - Implementation in QC

- **#2** Cell based assay for FIH

- New MOA assay format
  - Binding assays not technically possible
  - Early phase assay
  - Updated for Late development

  - Extensive experience and continuity of data collection during development
  - No retain testing required
  - Implementation in QC

After the submission

Some general observations on global submissions

Wide variation in questions and IR across submissions to different HAs for the same or even similar programs

Considerations

- What was submitted ? What have they seen ?
- Many responses are restating what is in the submission and pointing to the sections

Technical questions

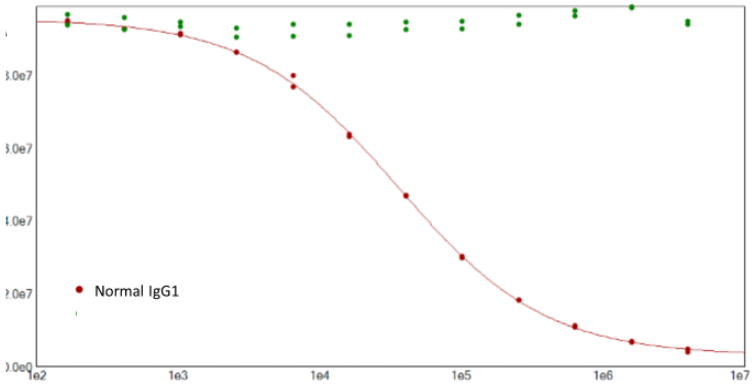
- Requests for potency assays to be added to specification for **all** potential MOAs mentioned in non-clinical Pharmacology section
- Requests for separate binding assays to be added to specification when cell-based assay requires co engagement

More general observations

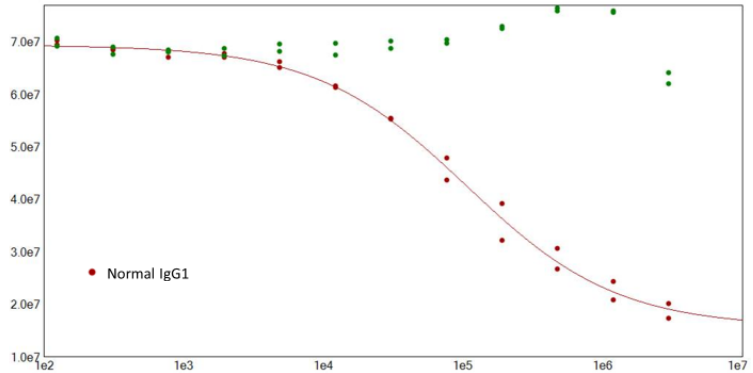
Increased focus on Fc effector functions

- Functional assays requested when not part of MOA
  - Including for non cell surface targets
- Functional assays requested for engineered Mabs with known mutations

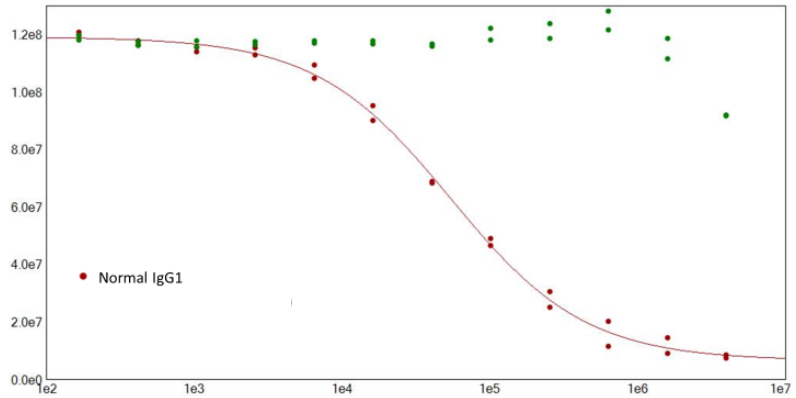
FcγRI



FcγRIIa



FcγRIIIa



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Increased focus on Fc effector functions

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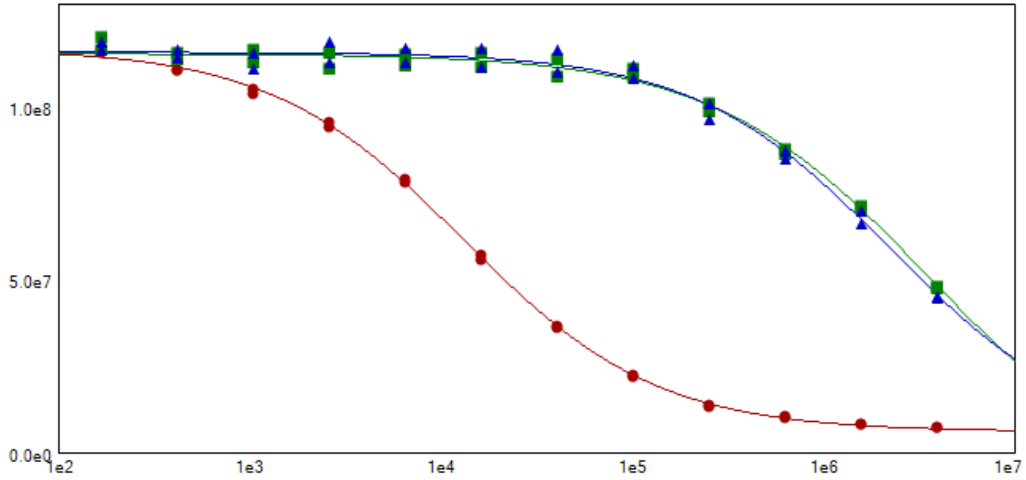
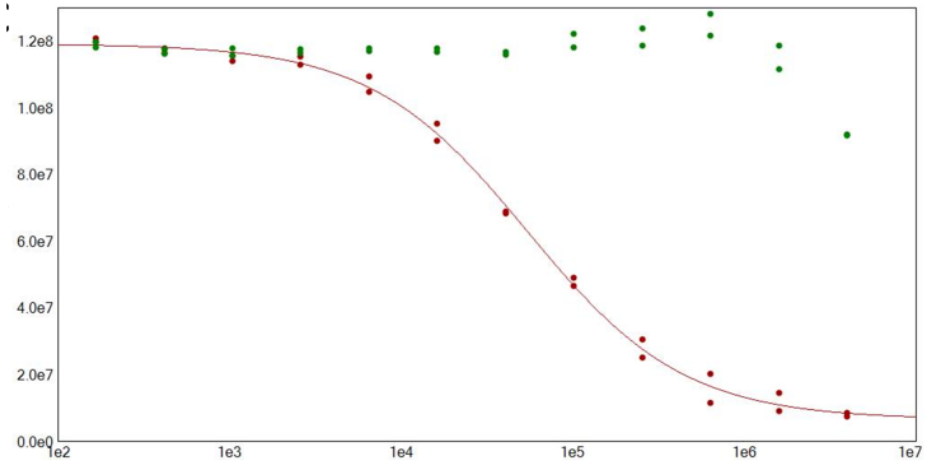
FcγRIIIa IgG4



FcγRIIIa



Summary

- Bi specifics - more complexity in biological activity assessment
- Accelerated timelines changes assumptions for “ phase based” strategy
- Global filings produce many challenges
- Expect the unexpected.....

Acknowledgements

- Global AD Bioassay Team
  - Special Mention : Christian Schnarr, Meghan Banh, James Psathas, Marion Kennefick , Thomas Maguire, Chi So, Peter Gray, Lata Chitikila
- AD Automation Team
- QC Bioassay Labs

Thank you

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