Neutralizing Antibody Assays

CLB vs. Bioassays

Deborah Finco

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Outline

- Assay formats
- History
- Regulatory guidances
- Case Studies
- Nab Data
- Strategy
- Regulatory experience
- Conclusion

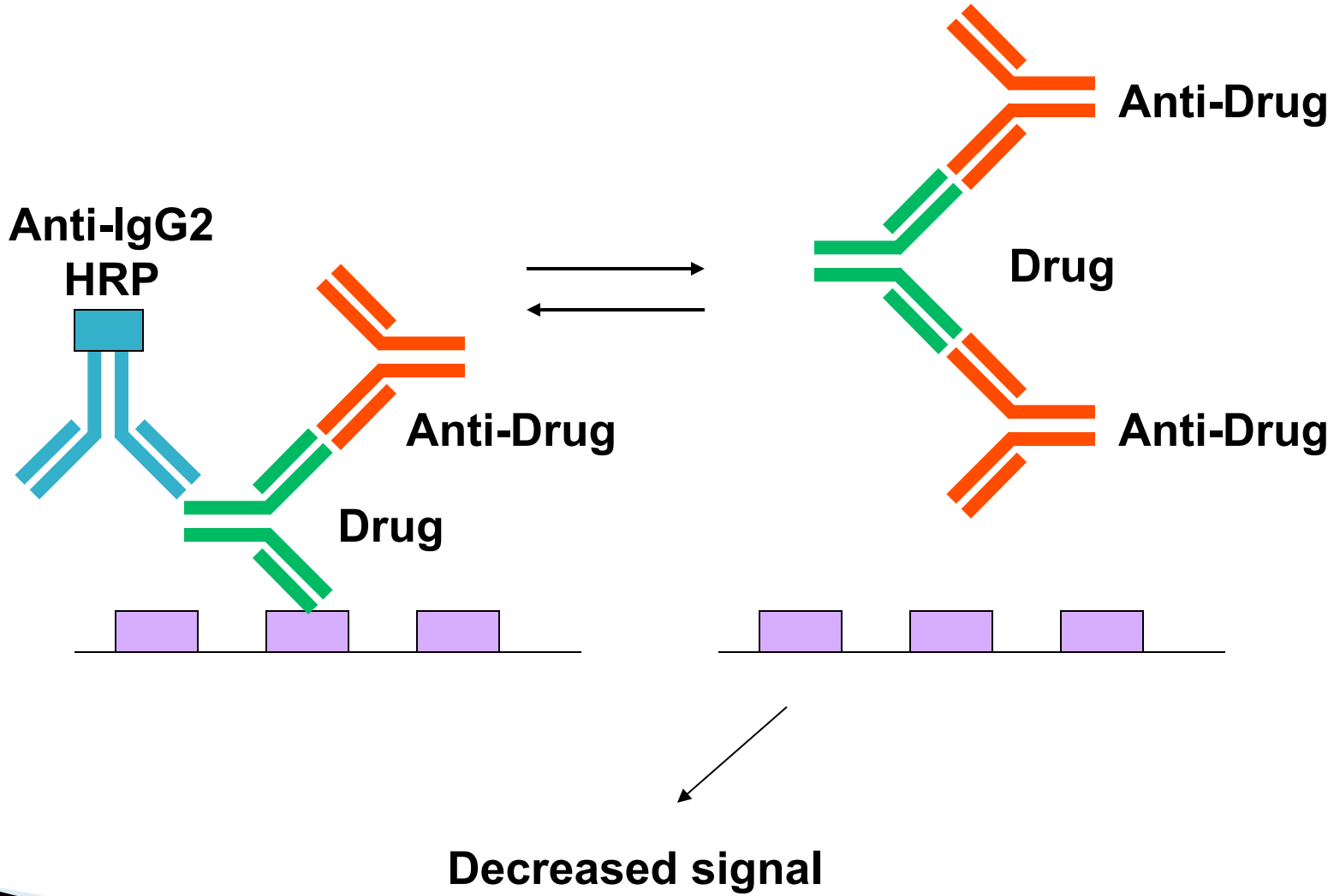
Neutralizing Assay Formats

Two Types

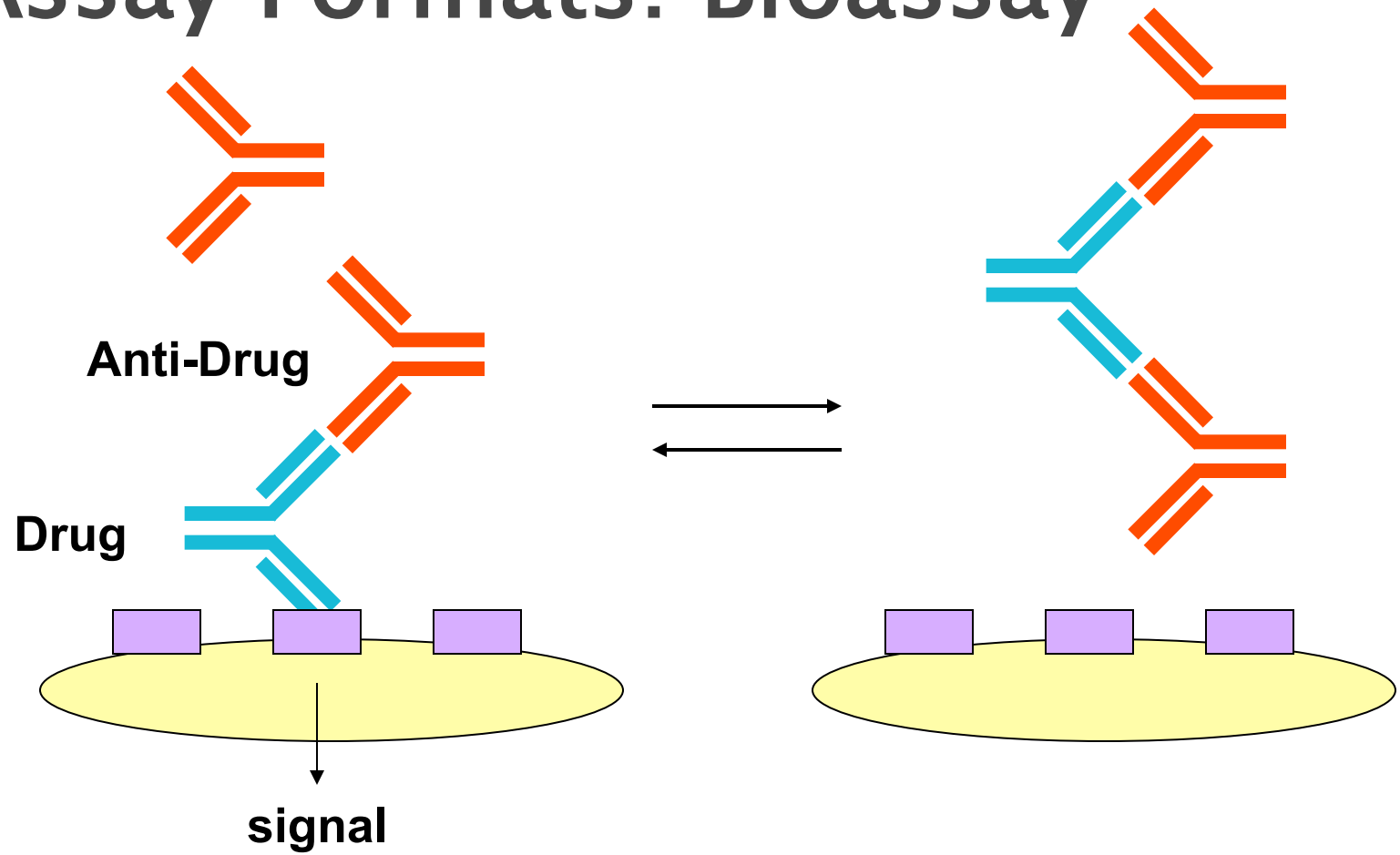
1. Bioassays
   - *Direct*: Drug binding to target results in biological response. NAbs block biological response.
   - *Indirect*: Ligand binding to target results in biological response. Drug blocks ligand-target binding. NAbs restore biological response.

2. Competitive Ligand Binding (CLB/LBA) Assay
   - Drug binds to target (ligand, receptor) coated on microplate. NAbs block drug binding to target.
     - Downstream signaling events critical for drug action may be missed using a CLB
     - Molecules that have MOA that requires ADCC may not be able to suitably be measured by CLB even if one does assay to target and assay for Fc

Assay formats: CLB



Assay Formats: Bioassay



- Proliferation
- Receptor tyrosine phosphorylation
- Cytokine secretion
- mRNA or target protein expression
- Change in expression of a cell surface marker
- Re-distribution of cellular proteins within the different compartments
- CPE
- Others

The selected response should assess some aspect of the drug's biology

CLB vs. Bioassay History- mine

- 2006- Competitive ligand Binding vs. Bioassay based neutralizing antibody Assessments- Munich
- 2007- Practical Application of Immunogenicity Assays- Immunotoxicology Summer School-Lyon France
- 2009-Neutralizing Antibody Assays: Relative Merits of Competitive Ligand Binding Assays and Cell-Based Bioassays-Berlin
- 2010- Nonclinical Case Study: Competitive ligand Binding and Cell-based Assay Formats for Assessing Neutralizing Antibodies- AAPS (D. Baltrukonis)
- 2011- Manuscript article: Comparison of competitive ligand-binding assay and bioassay formats for the measurement of neutralizing antibodies to protein therapeutics. J PBA 54(2):351-8
- 2011-Comparison of Competitive ligand Binding Assay and Bioassay Formats for the Measurement of Neutralizing Antibodies to Protein Therapeutics- Barcelona

Regulatory Guidance's

EMA Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. EMEA/CHMP/BMWP/14327/2008

- Assessing the neutralizing capacity of antibodies usually requires the use of bioassays
- If neutralizing cell-based assays are not feasible/available, competitive ligand binding assays or other alternatives may be suitable. However, when these are used it must be demonstrated that they reflect the neutralizing capacity/potential in an appropriate manner

EMA Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use(EMA/CHMP/BMWP/86289/2010.

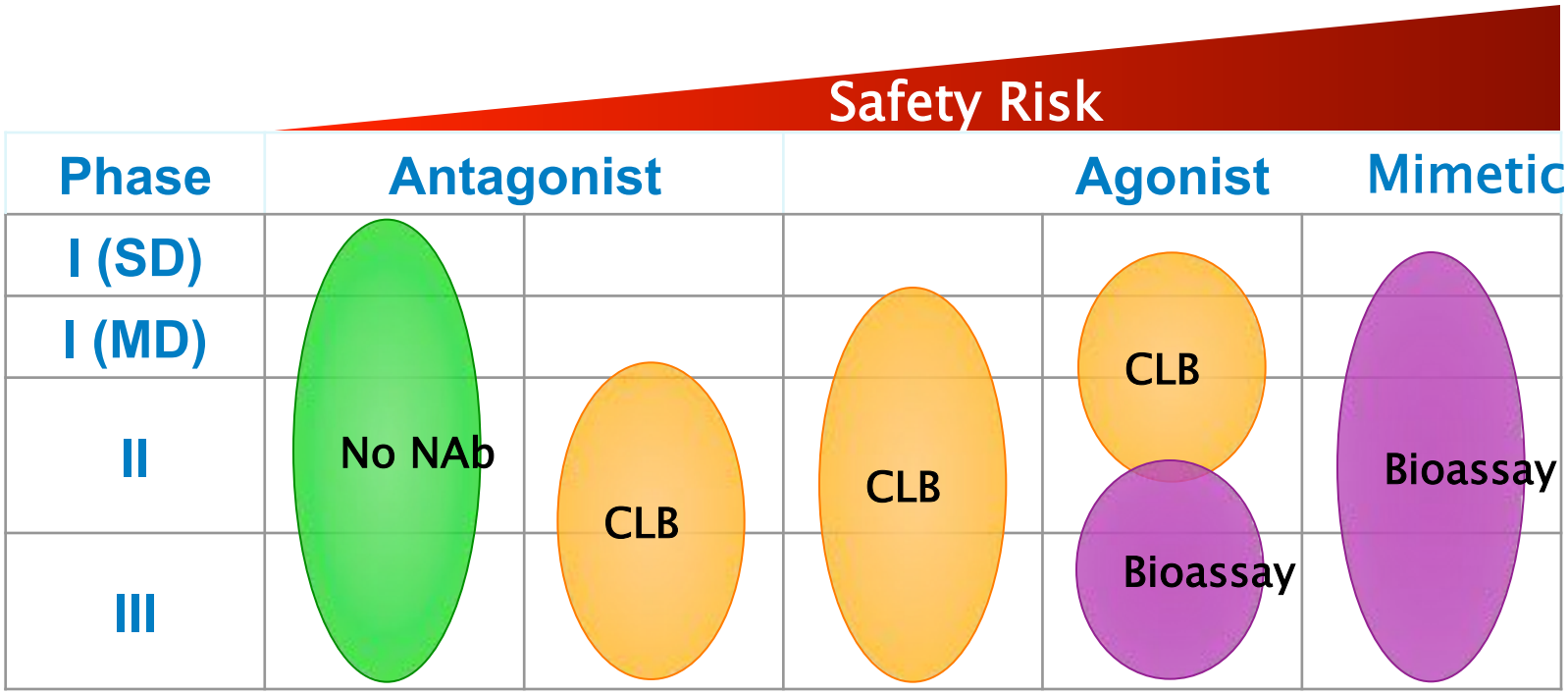
For most biological products the most appropriate assay is a bioassay…however ….competitive ligand binding assays may be the neutralizing assay of choice for MAbs rather than classic bioassays

Regulatory Guidance's

FDA: 2009 DRAFT Guidance On Assay Development for Immunogenicity Testing of Therapeutic Proteins

- Two types of assays have been used to measure neutralizing antibody activity: cell-based biologic assays and non cell-based competitive ligand-binding assays. While competitive ligand-binding assays may be the only alternative in some situations, generally **FDA considers that bioassays are more reflective of the in vivo situation and are recommended**.
- Generally, bioassays have significant variability and a limited dynamic range for their activity curves. **Such problems can make development and validation of neutralization assays difficult and FDA understands such difficulties. Nonetheless, we will recommend such assays** because they are critical to understanding the importance of patient immune responses to therapeutic proteins.

Example of Assay Selection and Implementation Strategies



Case Studies

- PDL Antagonist MabX (targets a cytokine receptor and blocks binding of cytokine- y) [1]
- Agonist Monoclonal (anti-CD40R) with oncology indication [2]
- Antagonist Mab to TNF [3]

1.Caras, I. Evaluation of Immunogenicity for pre-clinical and clinical Investigations , in Biopharm knowledge Publishing: Immunogenicity To Biologics. 2007.
2. Baltrukonis et al. Journal of Immunotoxicology. 2006.
3. Finco et al. 2011

Case Study 1

PDL MabX

- Targets a cytokine receptor
- Blocks binding of cytokine-y (antagonist)
- The binding of the cytokine results in multiple downstream events which could serve as endpoints

1.Caras, I. Evaluation of Immunogenicity for pre-clinical and clinical Investigations , in Biopharm knowledge Publishing: Immunogenicity To Biologics. 2007.

Endpoints

- Phosphorylation of a signaling molecule STAT (early signaling event)
- Cytokine production (intermediate)
- Cell proliferation (late)
- Binding event

CLB

- Direct measurement of MabX binding to target cells
- Measured by flow cytometry
- Nab would prevent MabX from binding to cells
- Rationale: since the primary mechanism of action of MabX is to bind to target receptor and prevent ligand from binding to its target, hence this approach is valid

Nab Endpoints & sensitivity

- STAT phosphorylation : ~ 5 ug/mL
- Cell Proliferation: ~ 6 ug/mL
- Cytokine production: ~ 600 ng/mL
- Binding endpoint: 100 ng/mL

Selected competitive binding (inhibition of Mab X binding)

- much more sensitive
- relevant for MOA (antagonist)

Publication: clinical and nonclinical

Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 351–358

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/jpba

Comparison of competitive ligand-binding assay and bioassay formats for the measurement of neutralizing antibodies to protein therapeutics

Deborah Finco[a,*], Daniel Baltrukonis[a], Adrienne Clements-Egan[b], Kathy Delaria[c], George R. Gunn III[b], John Lowe[d], Mauricio Maia[d], Teresa Wong[c]

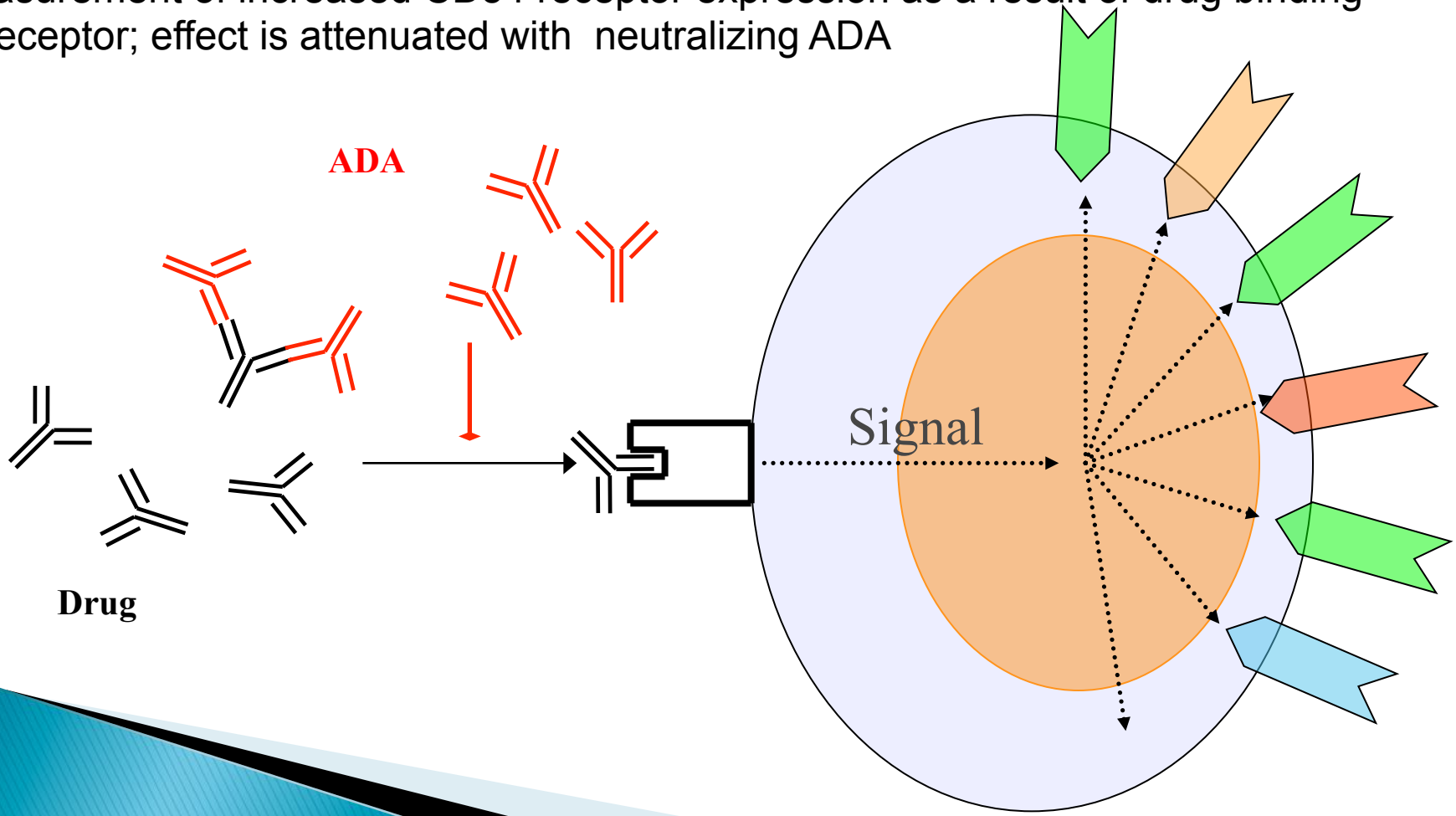
[a] *Pfizer Global Research and Development, Groton, CT, USA*
[b] *Centocor Research & Development, Radnor, PA, USA*
[c] *Aerovance Inc., Berkeley, CA, USA*
[d] *Genentech, South San Francisco, CA, USA*

Case Study 2

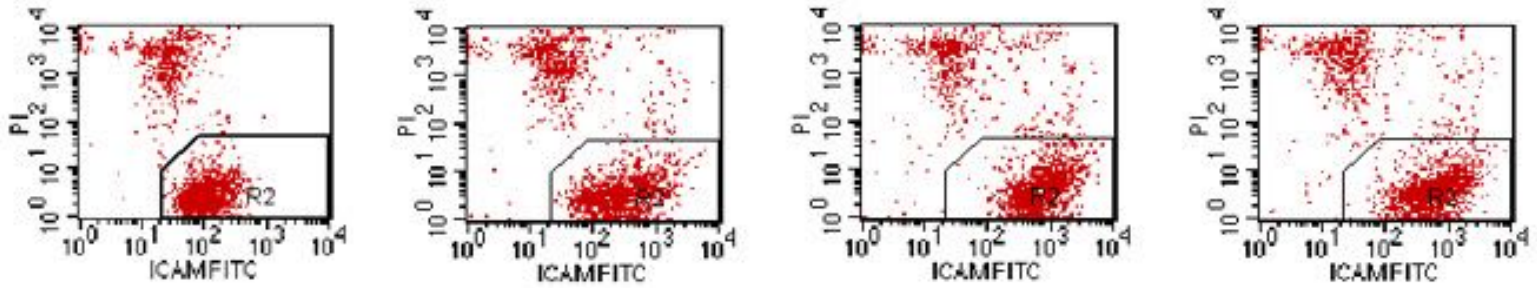
- IgG2 monoclonal antibody to CD40R
- Oncology indication
- Agonist monoclonal antibody

Bioassay format

Measurement of increased CD54 receptor expression as a result of drug binding to receptor; effect is attenuated with neutralizing ADA

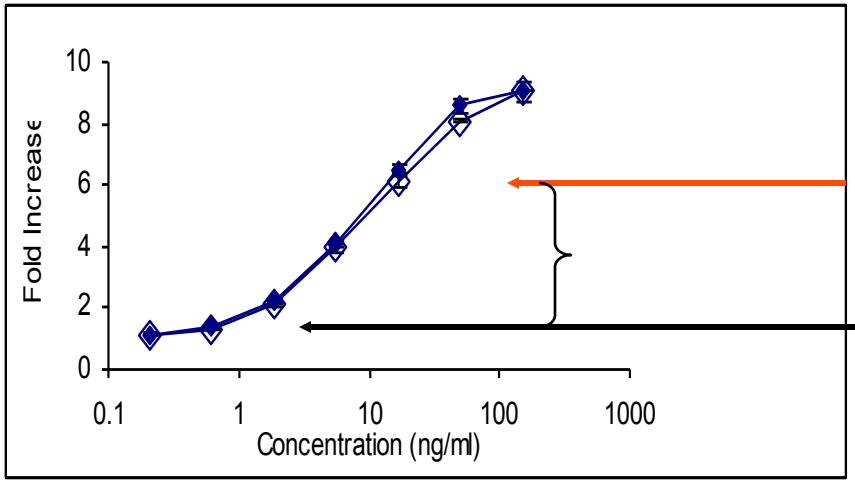


Drug Curve Example



0 10 50 100

Compound (ng/ml)

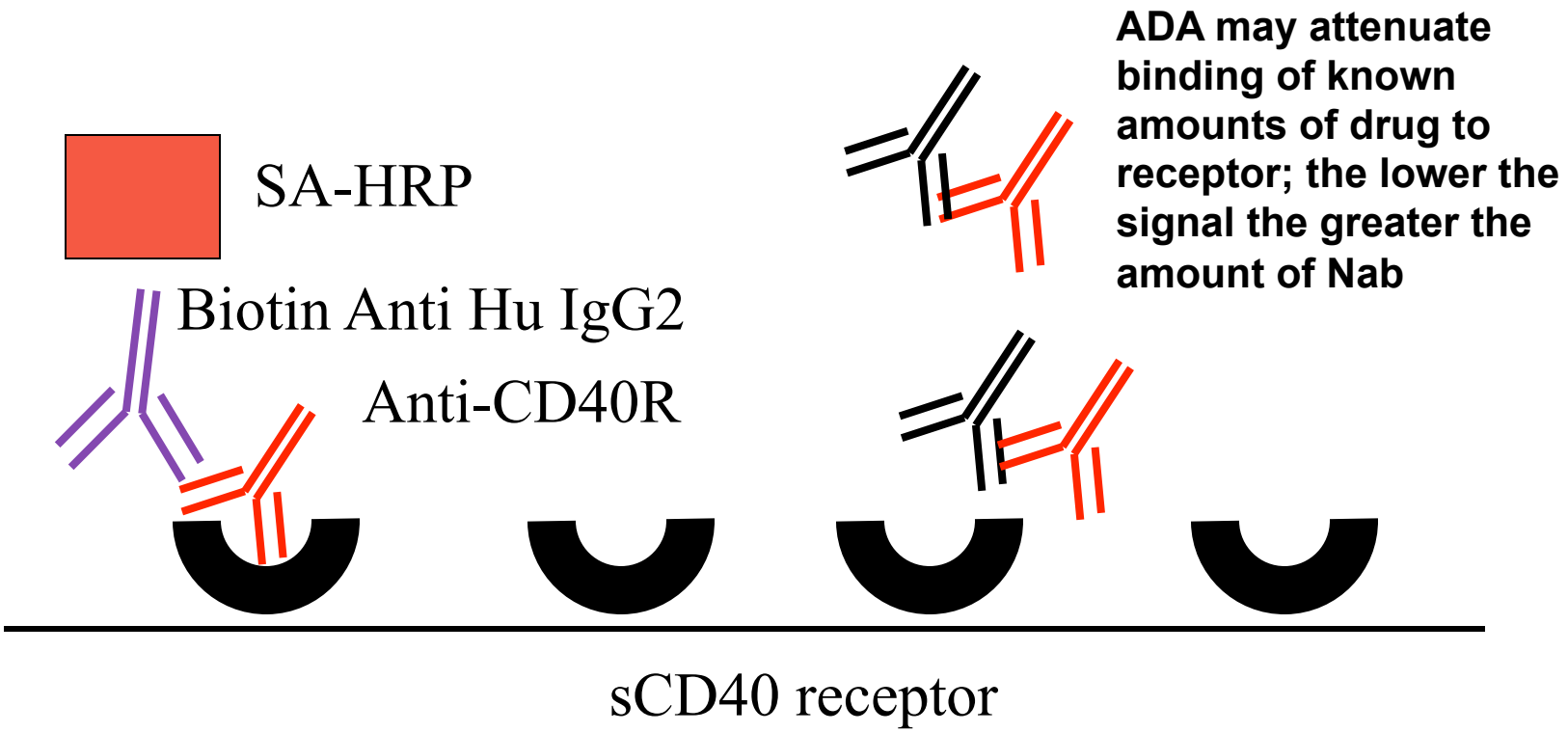


Drug added (50 ng/mL)

Sample + drug (~ 5 ng/mL; therefore ~ 45 ng neutralized)

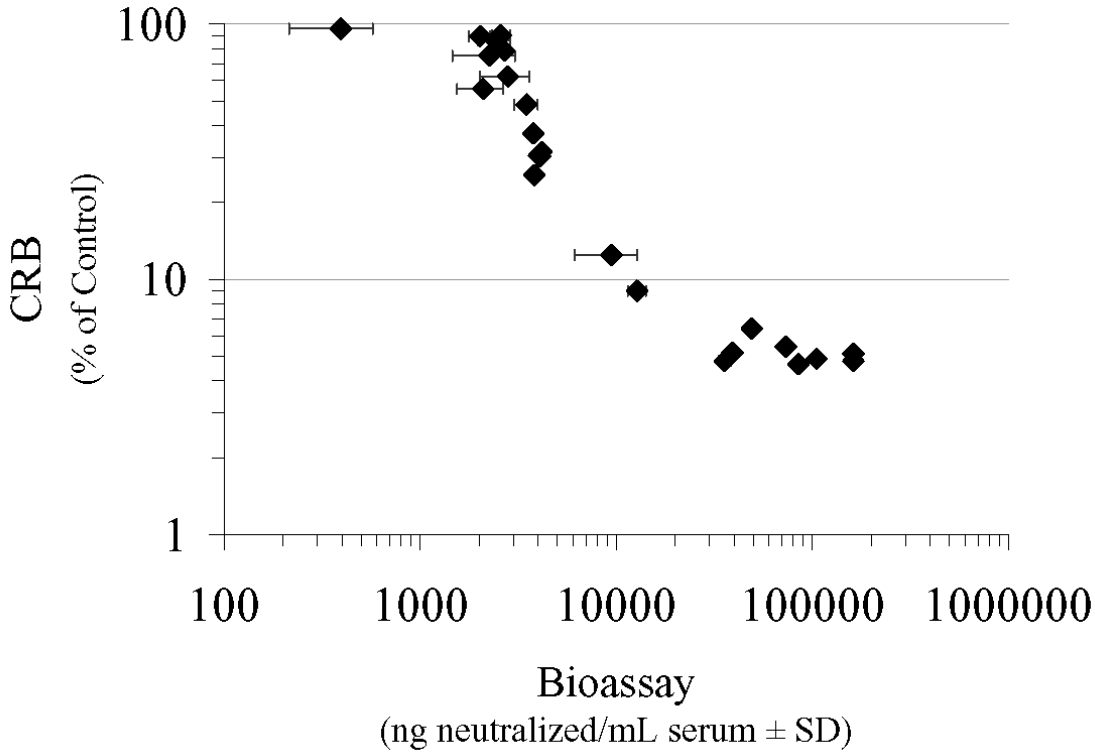
Drug Dose Curve

CLB Assay



When this assay was initially developed, data was reported as a percentage relative to normal serum. Anything less than 94% was positive for neutralizing Antibodies. Samples were only tested at MRD

Comparison Between CLB and Bioassay



Baltrukonis et al. J. of Immunotox. 2006

Case Study 2 Results

- Very good concordance between two assays; positives in one assay are positive in the other assay with one exception that was near cutpoint in CLB assay
- Both assays had same sensitivity

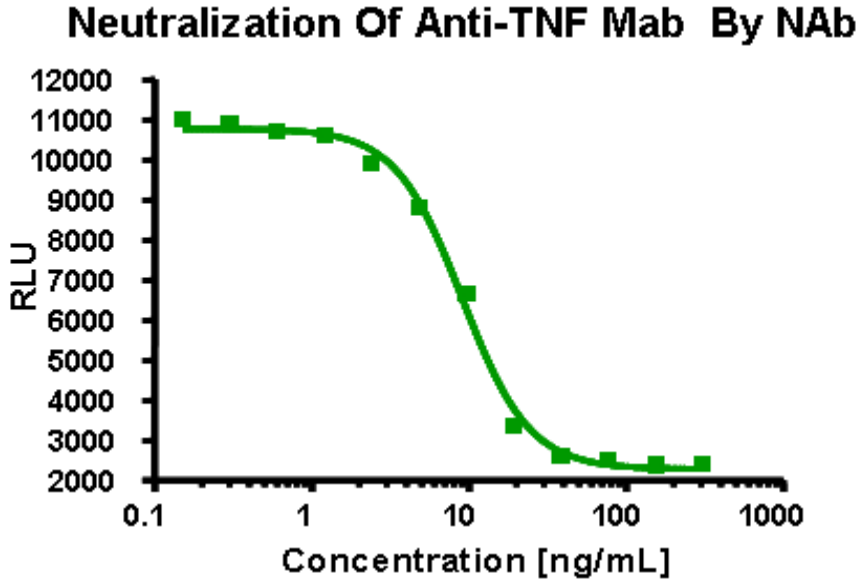
(~ 500 ng/mL)

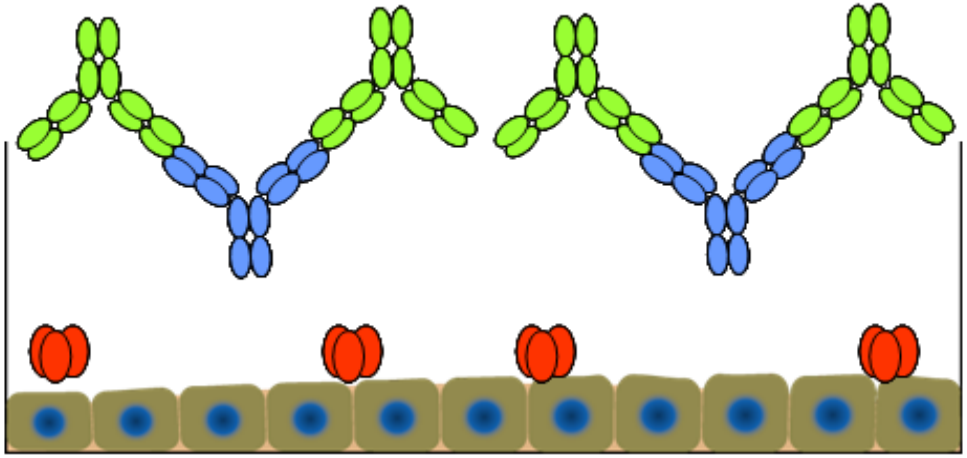
Case Study 3

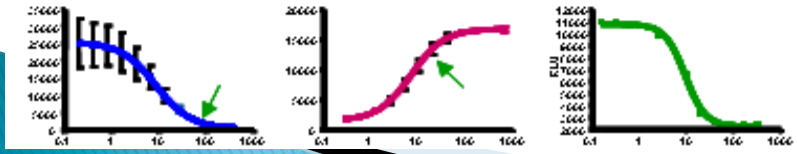
- Monoclonal Antibody to TNF: Comparison of a Bioassay and a Competitive Ligand Binding Assay

Anti-TNF Mab Neutralizing Antibody (NAb) Assay

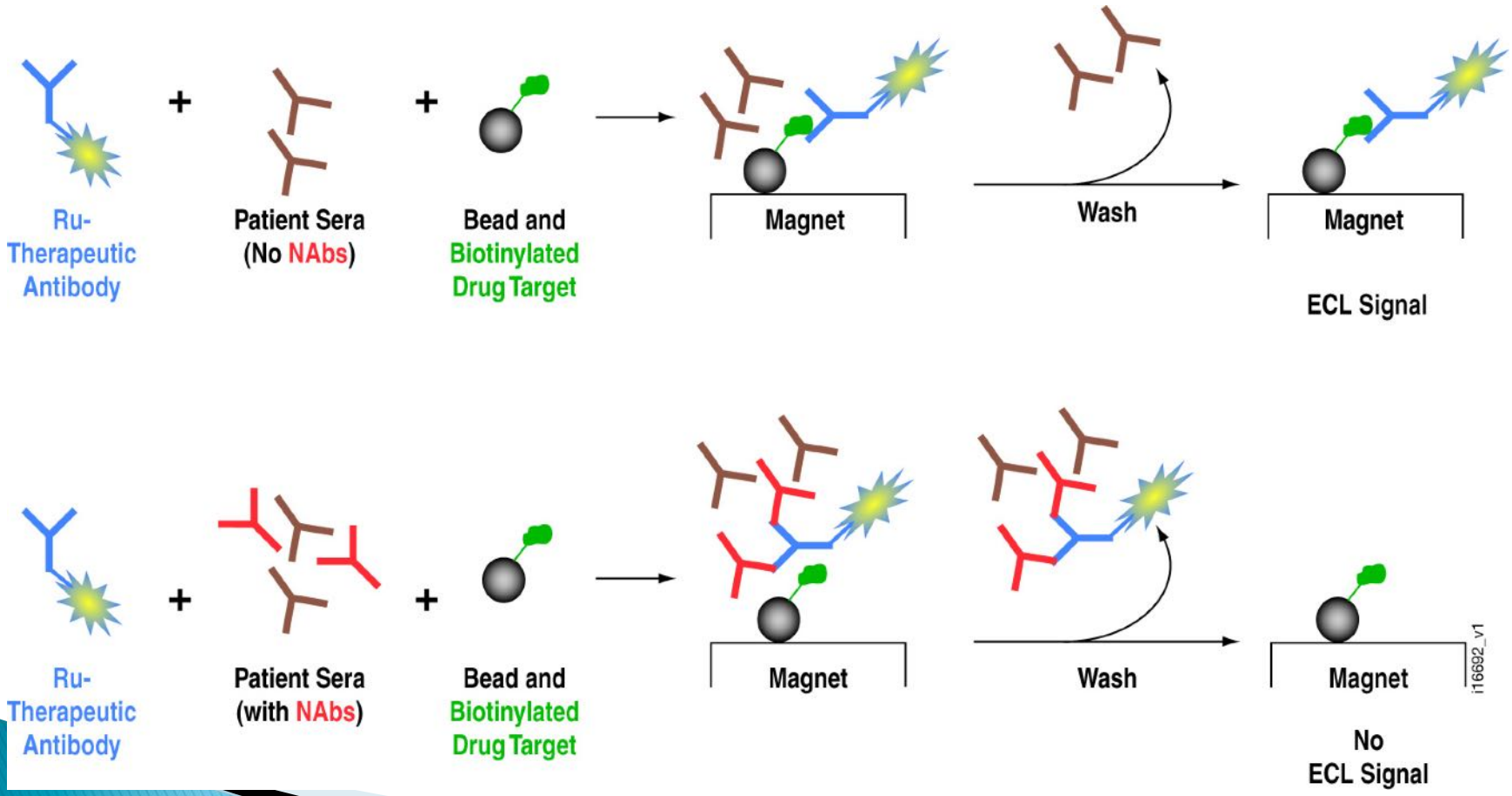
- Viable cells metabolize Cell Titer Glo
- TNF kills cells in a dose dependent manner
- Anti-TNF Mab binds to TNF and protects cells
- NAb neutralizes Anti-TNF Mab and free TNF kills cells







CLB Anti-TNF Neutralizing Antibody Assay



Comparison of Key Assay Parameters

Parameters	Cell Based Assay	Non-cell Based Assay
Validation complete	YES	NO
Sample Dilution	1/20	1/10
Sensitivity	150 ng/mL CNTO 8370	125 ng/mL CNTO 8370
Drug Tolerance	1.43 μg/mL	780 ng/mL
Assay Variability	8.68 % CV Inter-assay	10.5 % CV Inter-assay

Comparative Analysis with +ve Clinical Samples

Trial ID	No. of EIA +ves tested	Cell Based	Non-Cell Based
Trial A	5	5/5	5/5
Trial B	20	20/20	20/20
Trial C	18	8/18 **(2 IE)**	11/18

IE = Inevaluable due to serum matrix interference

Trial C Clinical Analysis

No. Patients	EIA Titer range	Cell Based	Non-Cell Based
6	10-20	-	-
1	20	-	+
1	40	-	-
2	40	IE	+
8	40-2560	+	+

IE = Inevaluable due to serum matrix interference

Case Study 3 Observations

- Cell based and non-cell based assay have comparable sensitivity
- Cell based assay can be more drug tolerant
- Non-cell based assay may be able to detect the “Inevaluable” samples in the cell based assay
- Labeling of reagents in non-cell based assay can potentially negatively influence the detection of NAbs

Nab Data

- Concerns regarding the translation of an in vitro assay to in vivo impact?
  - Nab assays determine the potential to neutralize
  - Does not take into consideration:
    - Clearance of ADA-drug immune complexes
    - Pharmacokinetics in relation to ADA levels and efficacy
    - Equilibrium/affinity in vivo and in vitro between drug/ antibody/target (examples of + Nab results to endogenous protein with no impact on efficacy of drug or PD markers for protein)
      - Hence relationship of ADA to PK/PD most critical

Possible Scenarios

Not all inclusive– other scenarios possible

Assay / Results	Binding ADA	Nab	PK	PD	Nab results support PK/ PD	Considerations/Thoughts
1	+	+			Yes	Nab assay suitable- regardless of format used
2	+	-			No	Possibly missing detection of Nab; consider assay format / drug or soluble target interference/other
3	+	+	ok	ok	No	Nab result does not agree with in vivo where it appears efficacy is not impacted

Simplified for discussion, in some cases PD markers may not be available

Regulatory Environment

- Have used CLB assays for later stage development of perceived lower risk therapeutics and have not received significant pushback from regulators (Pfizer, Centocor)
- Have used CLB for later stage agonist Mabs (various companies) with no regulatory pushback since results agree with PK/PD
- In general, it appears many companies are attempting to develop bioassays for mimetic and or enzyme replacement therapies that are internalized (many use CLB for binding to receptor)

Final Conclusions

- Emphasis by regulatory agencies for use of bioassays
  - CLB and/or bioassays may be used to evaluate neutralizing antibodies and the assay choice may vary depending on risk assessment, type of drug, MOA, phase of program etc.
  - Data in concert with PK, PD etc. may support appropriateness of one assay format or another
- How Nab data is used to aid in interpretation of other study data needs to be considered for both nonclinical and clinical programs and testing for Nab should not just a “check the box” exercise

Acknowlegements

- Centocor
- PDL BioPharma
- Pfizer Inc.

LBABFG – Cell Based Assay Focus Group; Upcoming Paper

MEMBERS: Bonnie Wu, J&J, Renuka Pillutla, BMS, Joleen White, Biogen, Shan Chung, Genentech, Manoj Rajadhyaksha, Regeneron , Joao Pedras-Vasconcelos, FDA, Yuanxin Xu, Genzyme , Shalini Gupta, Amgen – (Chair), Xu-Rong Jiang, MedImmune , Jim McNally, Pfizer

Draft Outline of Paper for LBA vs. Cell Based Assay for NAb Recommendations

1. Introduction and Background
2. Risk-based Bioanalytical strategy
3. Fit-for purpose approach
4. Assay formats
5. Determinants of NAb assay format selection
6. Regulatory perspectives
7. Future development and Emerging technology
8. Conclusions and Discussion