Strategy for Selecting NAb Assay Format

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Strategy for Selecting NAb Assay Formats
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White Paper

Strategies to Determine Assay Format for the Assessment of Neutralizing Antibody Responses to Biotherapeutics

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Abstract. Most biotherapeutics can elicit immune responses in dosed recipients generating anti-drug antibodies (ADAs). Neutralizing antibodies (NAbs) are a subpopulation of ADAs that can potentially impact patient safety and directly mediate loss of drug efficacy by blocking the biological activity of a therapeutic product. Therefore, NAb detection is an important aspect of immunogenicity assessment, requiring sensitive and reliable methods reflective of the therapeutic mechanism of action (MoA). Both cell-based and non cell-based assays are viable options for NAb assessment. However, the scientific approach for the selection of a suitable assay format (cell-based or non cell-based) for NAb assessment is not currently well defined. In this manuscript, the authors summarize the design and utility of cell-based and non cell-based NAb assays and recommend a NAb assay format selection approach that relies on a combination of three factors. These include (i) the therapeutic MoA, (ii) the evidence of desirable assay performance characteristics, and (iii) risk of immunogenicity. The utility of correlating NAb response with pharmacodynamic data is also discussed. The aim of this paper is to provide a consistent strategy that will guide the selection of scientifically justified assay formats capable of detecting clinically relevant NAbs for biotherapeutics with varying MoAs and diverse complexity.

KEYWORDS: assay format; biotherapeutic; mechanism of action; neutralizing antibody.

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Strategy for Selecting NAb Assay Formats
Outline

- ***Historical preference for cell-based NAb format***
- **Revisiting the rationale and providing a Mechanism of Action (MoA) based approach to select a format**
- **Potential assay formats**
- **Secondary considerations of risk assessment and assay performance**
- **Discussion**

Strategy for Selecting NAb Assay Formats
Genesis of the cell-based NAb preference

- Neutralizing antibodies are associated with potential impact to the overall risk/benefit assessment of the biotherapeutic
  - Potential efficacy impact in all biotherapeutics
  - Potential safety impact for molecules with endogenous counterparts, neutralizing both the biotherapeutic and the endogenous molecules
    - Neutralization of endogenous molecules can be life-threatening depending on the uniqueness of function and the biological effects.
- The preference for the cell-based format is based on assessing the entirety of the biological action rather than a subset of interactions that are neutralizing

- **Theoretical example:** Binding to the receptor may be necessary for the biological function, but it may not be sufficient. There may be a structural change(s) required for downstream biology that other NAb epitopes could affect.

Implement a NAb assay that makes ***scientific*** sense

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Strategy for Selecting NAb Assay Formats
Scientific basis for NAb assay format selection

Risk Based Assessment

- Both of generating an immune response and of immune response having an impact
- High rates of ADA positivity affecting sample numbers for testing
- Impact on non-redundant endogenous compounds
- Concentrations of NAb that would impact *in vivo*

Mechanism of Action

- Reflect the biology of the biotherapeutic
- Incorporates the pharmacology of the target
- Mode of drug-target interaction
- Design characteristics of the biotherapeutic for desired effect

Assay Performance

- Sensitivity
- Matrix interference: specificity and selectivity
- Drug tolerance (sensitivity in presence of C_{trough})
- Reactivity of cells to other sera components including soluble target
- Reproducibility across time course of clinical program
- Relevance of cell line receptor expression

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Strategy for Selecting NAb Assay Formats
Primary Determinant - Mechanism of Action (MoA)

MoA	Drug Modality	Drug Target	Drug-target Interaction	Examples	Recommended Assay Format
Agonist	Recombinant protein or antibody	Cellular receptor	Drug binds and activates receptor	Cytokines, growth factors, EPO agonists with no homology to endogenous protein	Cell-based assay Cell-based assay as primary choice, non cell-based assay as an alternative
Antagonist	Monoclonal antibody	Humoral target	Drug binds and inhibits the target	Golimumab, Ustekinumab, Adalimumab	Non cell-based CLB assay
	Monoclonal antibody	Cellular receptor	Drug binds cellular receptor and competitively inhibits receptor-ligand interaction	Natalizumab, Trastuzumab, Tocilizumab	Cell-based assay or non cell-based assay
	Soluble receptor	Ligand	Soluble receptor binds ligand and blocks receptor-ligand interaction	Etanercept, Abatacept	Non cell-based CLB assay recommended; cell-based assay possible with a suitable cell line
Targeted intra-cellular delivery of a potent cytotoxin mediated by antibody	ADC	Cellular receptor	ADC binds the cellular receptor and mediates the internalization of payload	Brentuximab vedotin, Adotrastuzumab emtansine	Cell-based assay(s)
Target cell lysis through antibody effector function	Monoclonal antibody	Target cell receptor, FcγR or complement	Antibody binds to target cell receptor through variable region and FcγR or complement through Fc domain	Rituximab, Cetuximab, Alemtuzumab	Cell-based effector assay recommended, cell-based binding assay or non cell-based CLB assay acceptable with justification
Enzyme replacement	Enzyme	Replace deficient protein in circulation or in target cells; may need cellular receptor for enzyme uptake	Enzyme functions in circulation or through cellular uptake	Human factor IX, Imiglucerase, Idursulfase, Galsulfase	Enzyme bioactivity assay and/or cell-based assay; two assay may be needed

"Thank you, but where do I start?"

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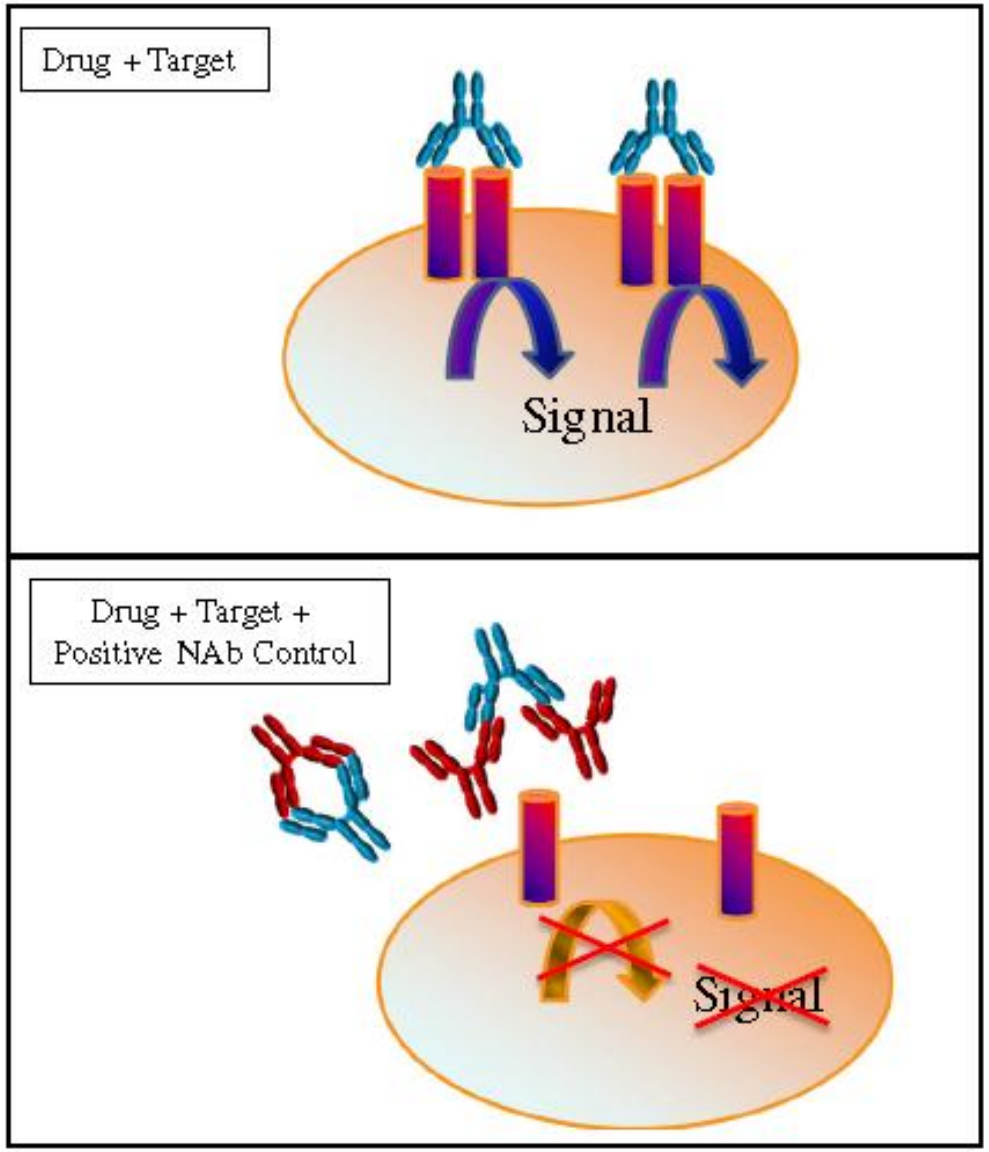
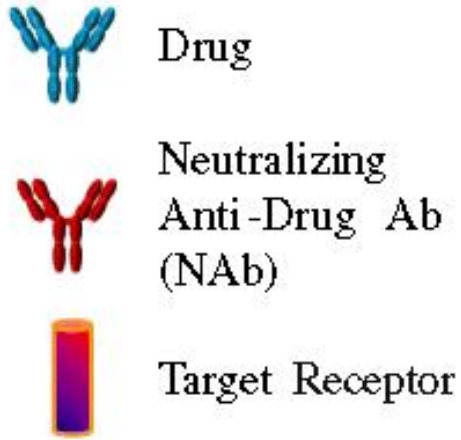
Comparison of NAb Assay to Potency Assays

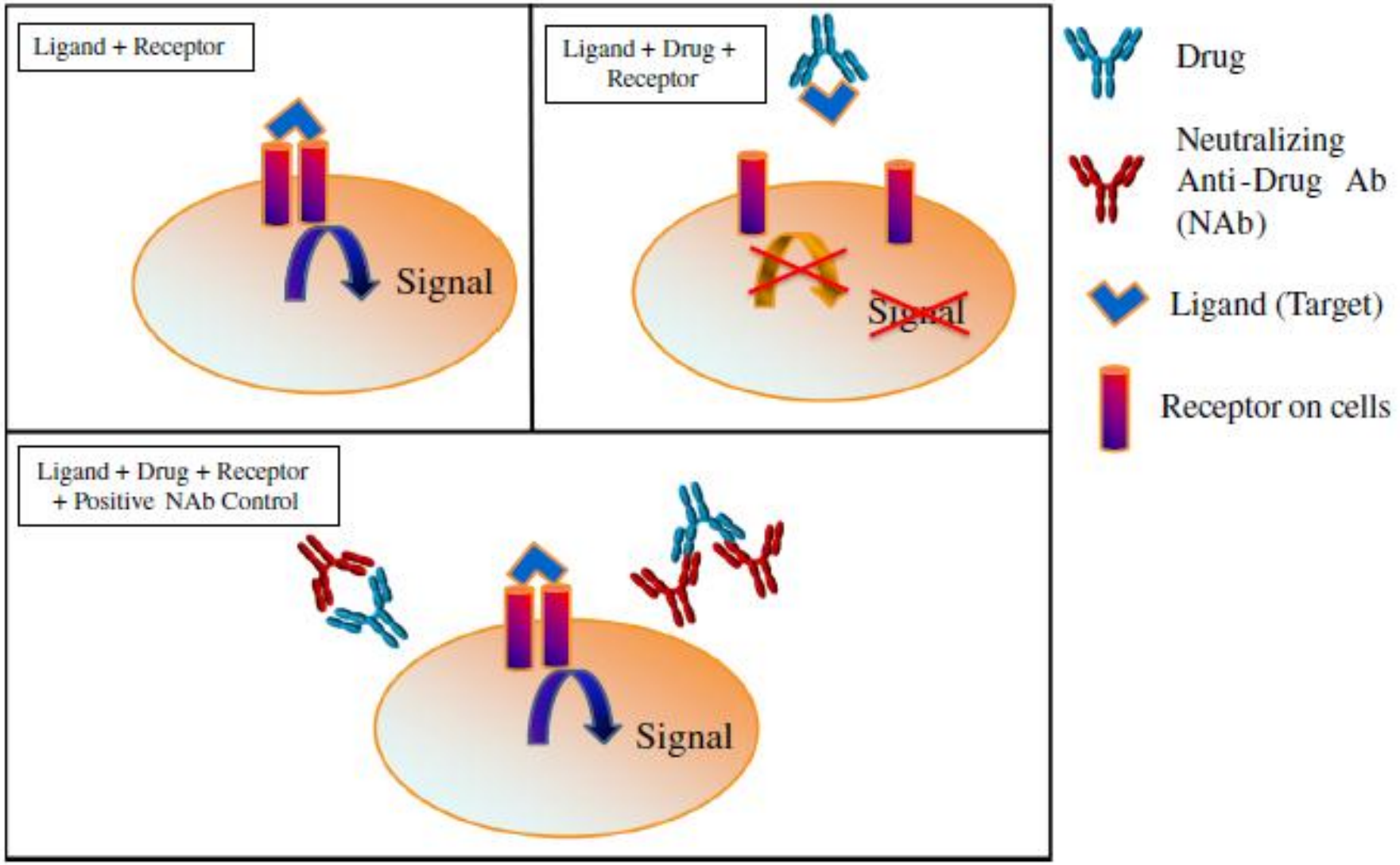
Characteristic	NAb assays	Potency assays
Purpose	Detection of NAbs	Drug lot release and stability testing
Analyte	NAbs	The active drug substance
Quantification	Qualitative or semi-quantitative	Quantitative (parallelism required)
Reference standard	No reference standard; assay controls are used	Analytical reference standard for quantification of active drug substance
Assay read-out	Inhibition of drug's biological activity	Quantification based on drug's biological activity
Sensitivity	The most sensitive assay that is available; sensitivity estimated by detection of positive NAb control	May not need the most sensitive assay; but the assay(s) should detect all structural changes important for potency; sensitivity determined by detection of reference standard
Matrix interference	Matrix sample included	No matrix sample included; use defined medium or assay buffer
Assay selectivity	Assess drug tolerance and drug-target interference	Not a concern

Strategy for Selecting NAb Assay Formats
Direct Binding – Cell Based Assay Format

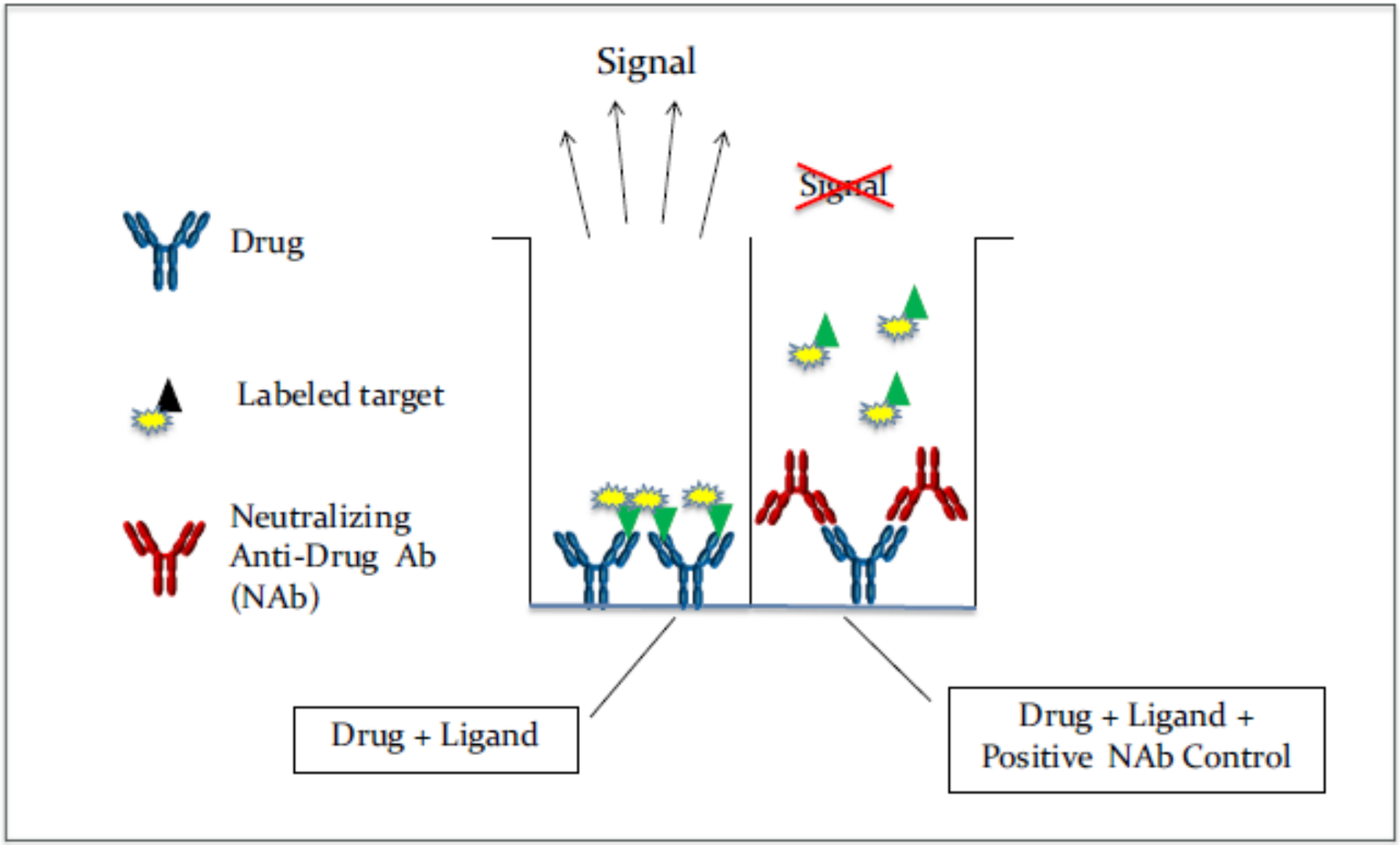




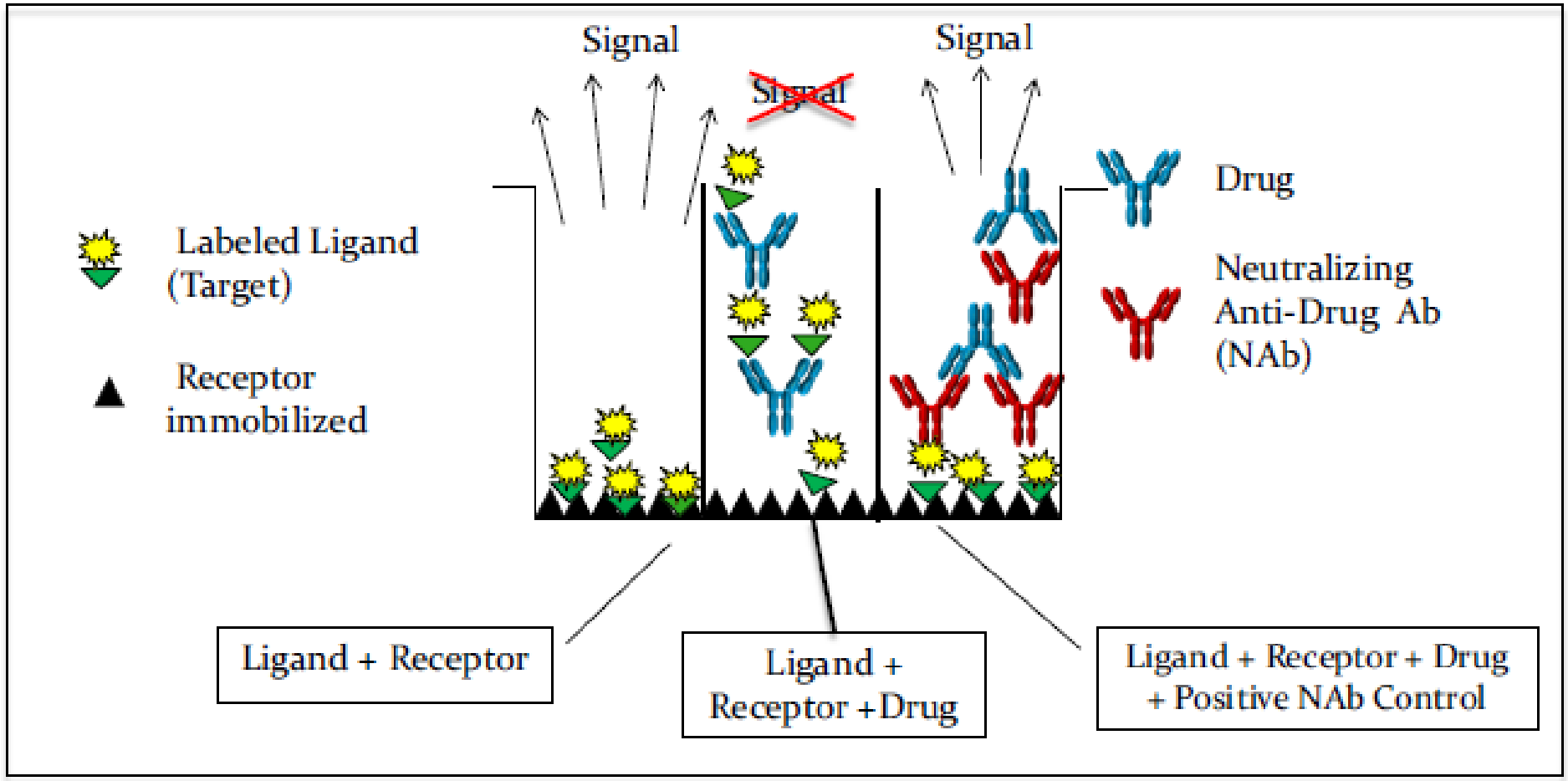
Strategy for Selecting NAb Assay Formats
Indirect Binding – Cell Based Assay Format



Strategy for Selecting NAb Assay Formats
Direct Format – Competitive Ligand Binding NAb Assays



Strategy for Selecting NAb Assay Formats
Indirect Format for Competitive Ligand Binding NAb Assays



Strategy for Selecting NAb Assay Formats
Case Study A

Antibody drug conjugate targeting Her2 receptor and carrying a cytotoxic molecule

Mechanism of Action:

- ADC, targeting cellular receptor
- Bind to tumor cells overexpressing Her2 and deliver cytotoxic payload

Proposed NAb format based on MoA:

- Cell based assay preferred
- Measure inhibition of cell death induced by ADC
- Most scientifically relevant assay format

Strategy for Selecting NAb Assay Formats
Case Study B

Antibodies against PD-1 or PD-L1 (checkpoint inhibitors)

Mechanism of Action:

- Agonist, receptor target
- Bind to PD-1 or PD-L1, inhibiting binding of the other

Normal biological activity of target:

- Inhibit the immune response: “checkpoint”

Proposed NAb format based on MoA:

- Competitive ligand binding assay or cell-based assay equally viable (agnostic assay for agonists)
- Measure inhibition of biotherapeutic binding to receptor
  - soluble receptor in CLB
  - cell-bound receptor in CBA, measure directly or through restored cell-cell interaction

Strategy for Selecting NAb Assay Formats
Case Study C

Enzyme replacement therapy

Mechanism of Action:

- Replace deficient protein

Normal biological activity of target:

- Intracellular uptake
- Activation of enzymatic activity

Proposed NAb format based on MoA:

- 2 assays:
  - Enzymatic activity neutralization where NAb could prevent conformational change necessary for activity
  - Cell based assay to assess uptake into the target cell

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Secondary Determinants - Risk Assessment and Assay Performance

Risk Assessment

- Risk of high ADA incidence yet low risk of impact may favor competitive ligand binding due to throughput and robustness
- High risk of impact on endogenous molecules provides less flexibility to choose the less-preferred option from mechanism of action
- High risk of impact places greater emphasis on assay sensitivity in presence of drug, which may favor competitive ligand-binding

Assay Performance

- Strong influence when two different formats are equally viable: test both in early assay development
- Manipulations to improve drug tolerance: more options available for competitive ligand binding formats
- Sera concentrations of both drug and soluble target
- Totality of assay performance
- Selection of cell lines and other reagents for optimal performance and relevance to mechanism of action
(one CBA is not like all others)

Strategy for Selecting NAb Assay Formats
If and When to Implement NAb assay

- Out of context for the white paper, therefore…
- ***These are the views of the speaker and not necessary the co-authors of the white paper***

- You cannot start ***developing*** the assay soon enough
- Positive control identification can be time consuming
- Identification of a cell line that will work may be difficult
- There is no telling when you will get the question – "Are the ADAs neutralizing?"

- Case study:
  - mAb – Antagonist, binds to cytokine to prevent binding to cellular receptor
  - >98% incidence of ADA in Phase I, SAD study
  - How long do you want to wait to have an answer to the question?

Thank you for your attention