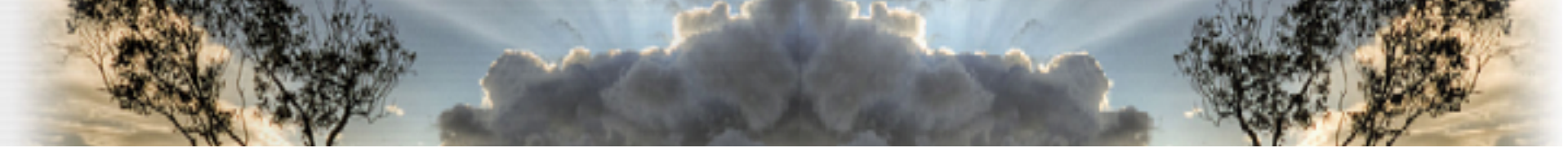


Current and future considerations for Neutralising antibody assays

Anna Laurén – on behalf of the EBF NAb project team

Objective

- The 2019 FDA guidance - “immunogenicity testing of therapeutic protein products” has more detail on NAb assays, however there are areas that are open to interpretation.

- This may be intentional by the agency to allow flexibility and sound scientific judgement depending on the assay format

- EBF NAb team (14 companies with NAb assay experts) started a discussion group:

  “Current practices within the EBF community and regulatory experience for using alternatives and competitive LBA (CLBA) versus cell-based assay (CBA)”

FDA guideline 2019

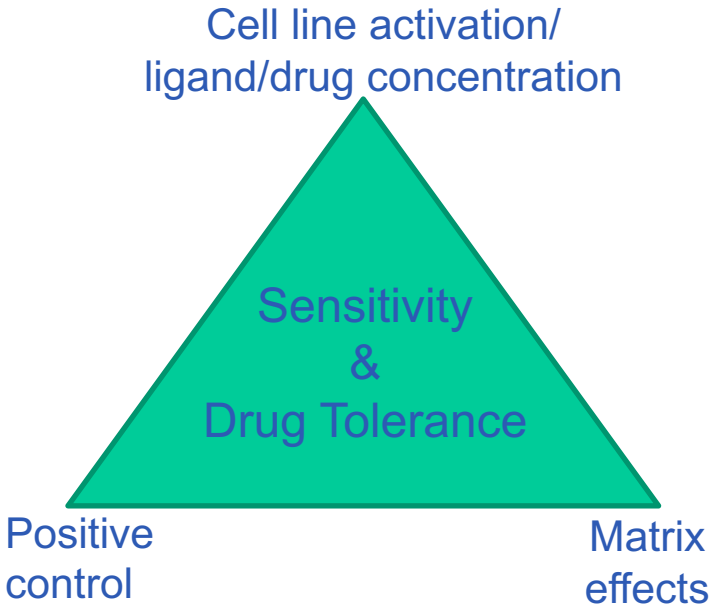
- In selected cases, a highly sensitive PD marker or well designed PK assay can be used instead of NAb assay
- Cell-based assay (CBA) is the preferred choice but competitive ligand binding assay (CLBA) can be utilized
- Titer in NAb assays primarily in high risk projects
- NAb assay CP: 30 individuals and fixed CP if possible (1% or 5% FPR), otherwise same validation parameters as ADA
- Detailed information on matrix interference, specificity/selectivity, alternative/multiple stimuli for cell lines
- Considerations for false positive results due to inhibitory endogenous molecules, use antibody depletion assays as confirmatory assay

EBF NAb team topics June 2019-October 2019

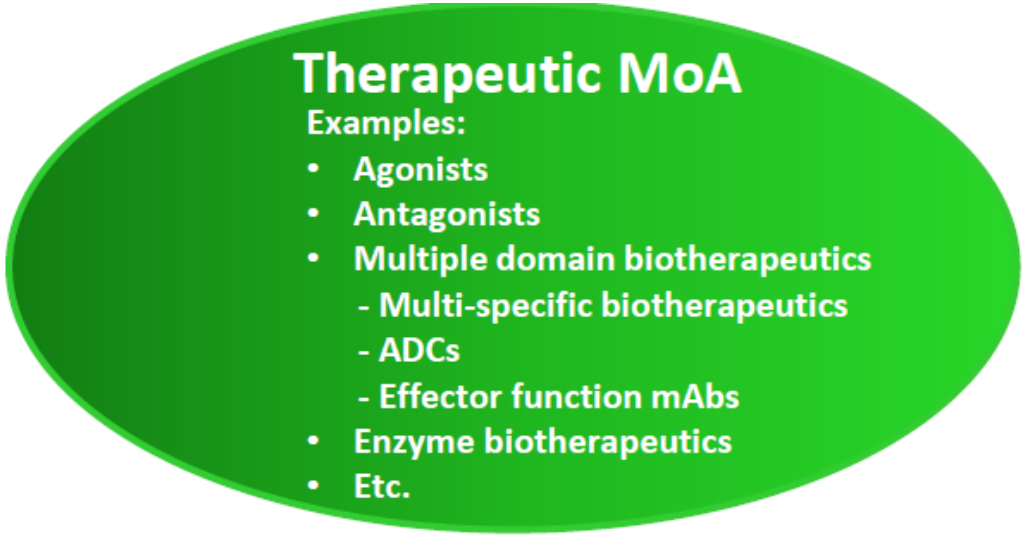
- Characteristics and Assay strategies
- Due diligence for CLBA assay when used as 1st choice
- When to start implementing the NAb assays?
- Sensitivity/drug tolerance and matrix effects from endogenous proteins
- Normalisation and CP calculation
- Is quantitative result used: titer versus %inhibition, etc.
- Experience to use other parameters than NAb assays (e.g. PD markers)?
- NAb assay data correlation to clinical data?

Characteristics of a NAb assay

- Sensitivity of a NAb assay is highly dependent on the characteristic of the PC
  - PC must neutralise mode of action (MoA)
- CBA NAb assays are also dependent on cell response to drug and sample matrix
  - drug concentration/drug affinity to target
  - cell density/receptor density
  - Interference from matrix on cell performance
- Drug interference in a NAb assay o
  - Masks the detection of NAbs
  - Induces signal change in assay
- Lower drug concentration usually gives:
  -> Better sensitivity -> Poorer drug tolerance



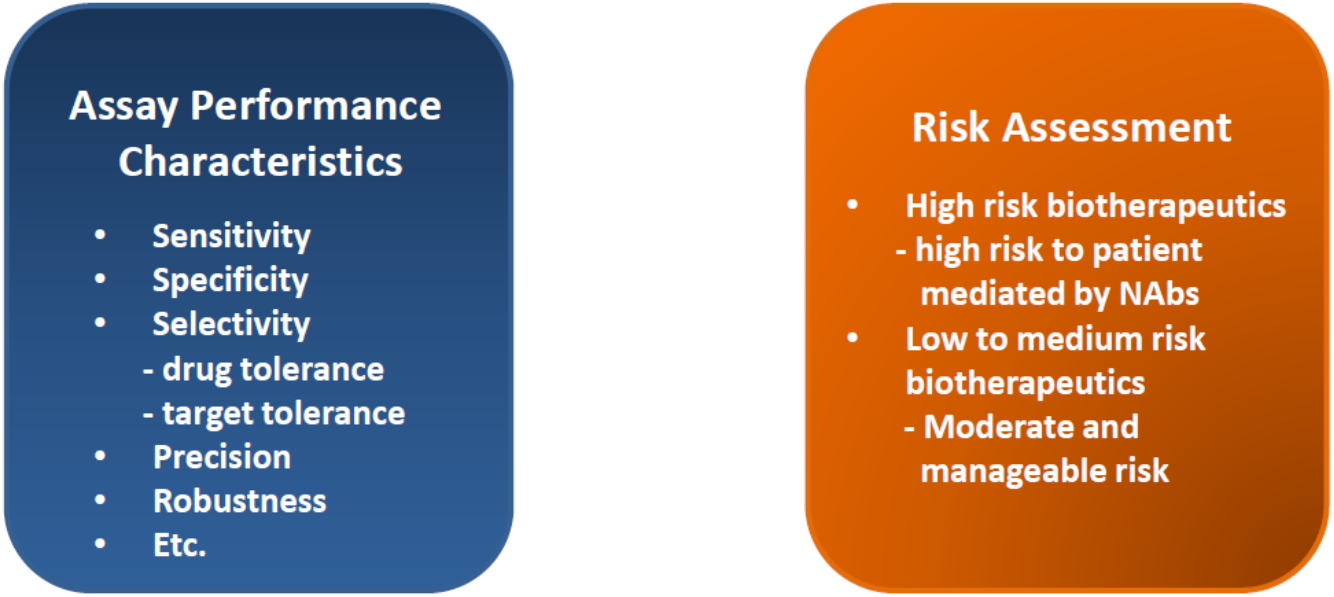
Drivers for NAb Assay Strategy



Therapeutic Mode of Action is the Primary guide for implementation of NAb testing

Wu B et al 2016

Drivers for NAb Assay Strategy



Indicators of Assay Reliability

For Shaping the Assay Expectations

Assay Performance and Risk Assessment are the Secondary drivers for NAb assay format selection

Wu B et al 2016

Experience in using CLBA as 1st choice

- CLBA method as 1st choice has been accepted for MAb drugs with antagonistic mode of action
  - Acceptance was based on a dialogue with FDA
  - Seek HA guidance at pre-IND, End of Phase 1 or 2 meetings

- CBA method have been required for drugs with endogenous counterparts

- When evaluating assay formats a head-to-head comparison was required to compare assay performance with the same NAb positive control samples :
  - Sensitivity
  - Drug tolerance

Assay strategy – Potential Scenarios
When to start implementing the NAb assays?

NAb assay for pivotal trial

Why?	- Program is **low risk**, low immunogenicity
How?	- Consider MoA and select relevant assay format - Review immunogenicity from early clinical studies - Start NAb method development during Ph I or II - Method validation before Ph II or III - Bank PhI/II samples for potential NAb analysis
When?	- Implement NAb testing for pivotal trials only

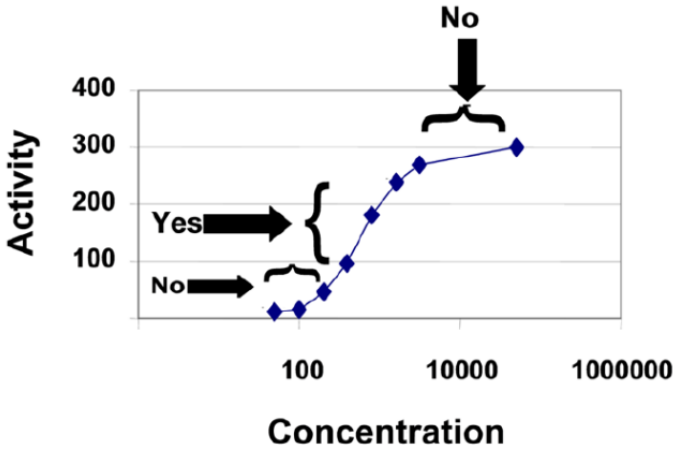
Assay strategy – Potential Scenarios
When to start implementing the NAb assays?

NAb assay in early clinical trials

Why?	- Program is **High risk**
How?	- Consider MoA and select relevant assay format - Method development & validation before Ph I
When?	- Implement NAb testing for Ph I

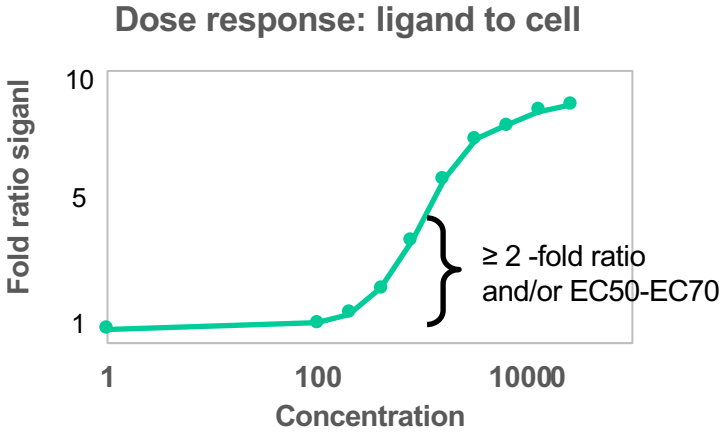
How to select your CBA critical reagent concentrations

From FDA guideline:



- Drug concentration selected at linear range while retaining dynamic response of the assay

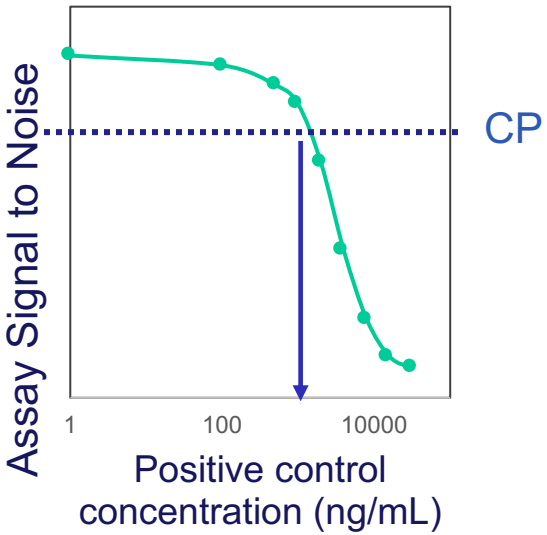
EBF NAb team experience:



- Concentration selected at ≥2 -fold signal to noise and/or EC50- EC70 with matrix present
- Important to show relevant sensitivity, drug tolerance and assay precision/robustness

Sensitivity and drug tolerance

- Any type of PC can be used that has neutralising activity
- Experiences show that sensitivity of PC as low as 100 ng/mL is not needed
  - Low risk Mab drug projects: 1-1.5 µg/mL
  - High risk and endogenous counterparts ≤1 µg/mL
  - USP recommends that NAb assays achieve a sensitivity of 0.5 ug/mL – 2 ug/mL with a “fit-for-purpose” approach to select the assay sensitivity.
- When drug is onboard it can be challenging to detect NAbs: Depending on risk of drug, testing may be acceptable only after wash-out



USP: United States Pharmacopeia, 2018, chapter 1106/1106.1: Immunogenicity assays

How to improve drug tolerance and matrix interference from endogenous proteins

- MRD can be increased for higher diluted samples – possible for high sensitive assays
- Interference from endogenous proteins may be minimised by using human serum pool instead of fetal calf serum for CBA methods
- Pre-diluting samples in human serum pool before adding samples to ligand binding NAb assay
- Rationale for using human serum pools for dilutions/assay buffers is that this will minimise matrix differences between human and fetal matrices
- The most common pre-treatment steps where Acid and Bead pre-treatment where neutralisation to relevant pH was a crucial step before adding pre-treated samples to a CBA NAb method

Assay controls

The following assay controls are commonly included:

- Background control (BC) several different BC may be needed (case-by-case)
  - For direct stimulation NAb assays:
    - drug + assay medium + matrix
  - For indirect stimulation NAb assays:
    - ligand + drug + assay medium + matrix
    - ligand + assay medium + matrix
- Negative control (NC)
- Normalisation control (NoC) - can be identical to NC or a base-line sample
- Low Positive Control (LPC)
- High Positive Control (HPC)

Normalisation and CP calculation

- Results reported as positive or negative
- Titer not needed for NAb assays (may be considered in high risk projects)
- CP calculations were based on the following type of data:
  (always case by case)
  - Direct assay signal
  - Log assay signal was not used
  - Normalisation examples:
    - Normalised as ratio to NC/NoC
    - %INH or %N* to BC and NC/NoC
    - %INH or %N* to NoC

    $e.g.\ \%INH(or\ \%N)=100*(1-((sample\ RLU-BC\ RLU)/(NoC\ RLU-BC\ RLU))$
- When high inter-individual variation: Normalise to the individual base-line samples

Clinical experience and NAb sample analysis in studies

- None in the NAb team have yet been able to use PD marker instead of NAb assay with one exception:
  - Historically NAb assays has not been required for insulin drugs => HbA1c and dose level are used to follow treatment effect
- The clinical relevance and possibility of not using NAbs or how to analyse (one batch or ongoing) is dependent on the risk assessment
  - For Low/Medium risk project, it is OK to analyse all NAb samples (samples confirmed positive for ADA) in one batch at the end of study
  - For High risk projects it may be needed to consider to include ongoing analysis of samples
- Necessity of NAb testing during treatment must be assessed in conjunction with the binding antibody analysis. If the general picture is a transient antibody response of low magnitude, and efficacy and safety is not impacted, NAb during treatment is not relevant, only after drug wash-out.
- Use Integrated Summary of Immunogenicity (ISI) for justification package

Summary

- Start with risk assessment based on type of drug and Mode of Action
  - Drugs with antagonistic Mode of Action and Low risk project – CLBA NAb method can be accepted and batch analysis of samples end of study
  - CBA method have been required for drugs with endogenous counterparts
  - High risk projects may need detection of NAb in the presence of drug in samples
- Sensitivity of PC as low as 100 ng/mL is not needed
- Bead and acid pre-treatment has been used to obtain better drug tolerance
- Titerering is rarely needed for NAb assays
- CP calculations can be based on (always case by case)
  - Direct assay signal
  - Normalised to NoC or base line samples
  - Normalised as ratio or %INH or %N

Acknowledgment

EBF community

- Team Sponsor: Jo Goodman - AstraZeneca
- Lead: Anna Laurén - Svar Life Science

- Team members:
  - Rob Nelson – Covance
  - Inge Dreher – Abbvie
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  - Maria Jadhav – Novartis
  - Nicoline Videbæk– NovoNordisk
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  - Weifeng Xu – MSD

Thank you and time for questions

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