EBF

14th EBF Open Symposium

Science – Our Universal Language

Feedback from EBF discussion on NAb strategies

Robert Nelson, on behalf of the EBF

24-26 November 2021, Barcelona

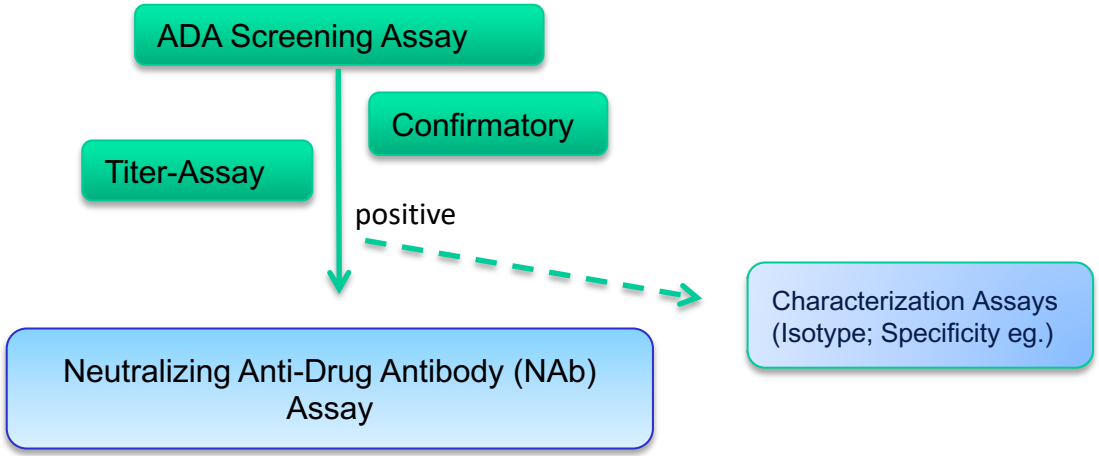
Outline

- Highlights from the Spring cyber-workshop
- Current topics of interest
- Future plans

https://e-b-f.eu/fw202101-slides/

NAb assays are part of the tiered approach

- NAb assays are part of the tiered approach for immunogenicity testing requested by authorities



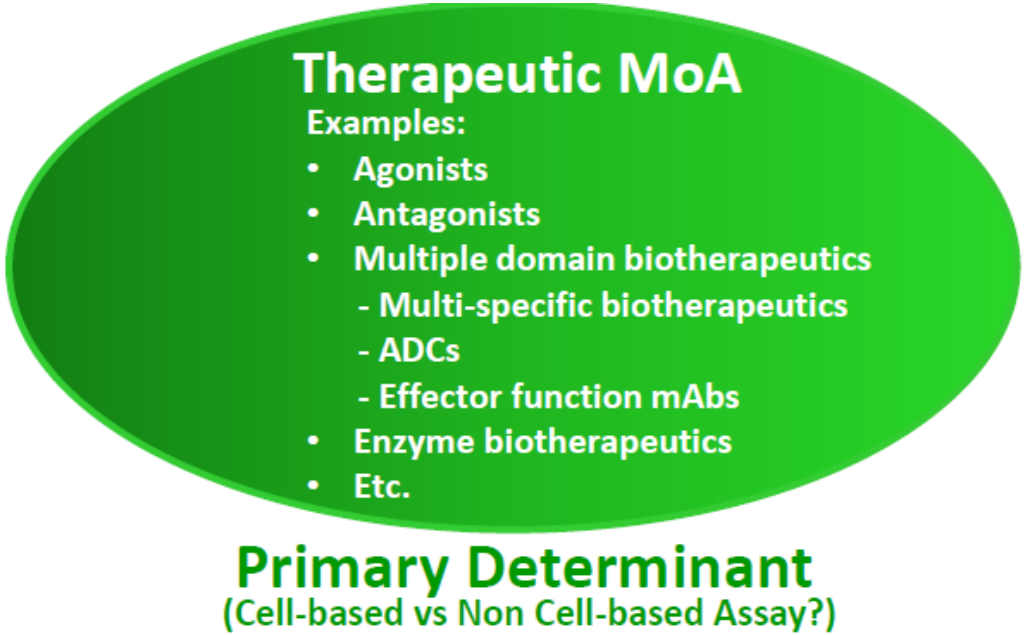
- Based on team experiences: what makes a sufficiently good NAb assay?

When to start implementing (and testing in) a NAb assay?

- Strategy is dependent on the risk associated with NAb formation

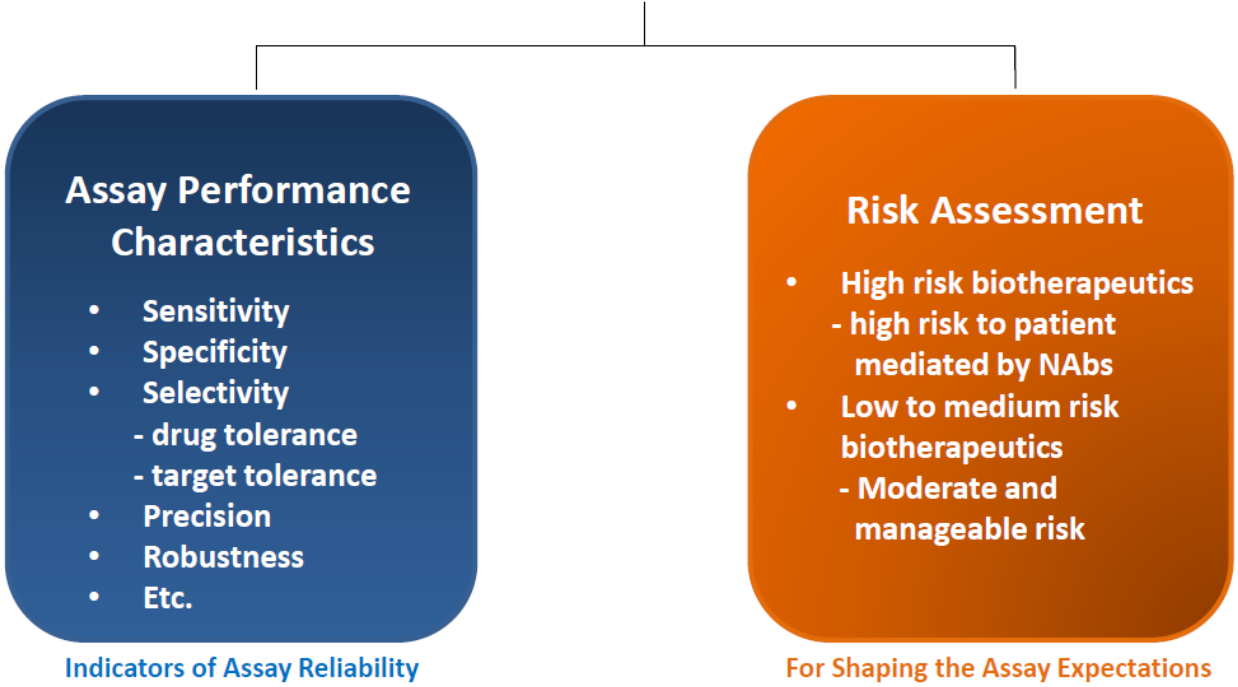
High-Risk	Low-Risk
Method development & validation before Ph I **Implement NAb testing for Ph I**	Review immunogenicity data from early clinical studies Method development during Ph I/II Validation before Ph II/III **Implement NAb testing for pivotal trials** Bank Ph I/II samples for potential NAb analysis
Real-time NAb sample analysis might be needed during study	**Batch analysis of NAb samples at the end of study**

Which format to select?



- **Therapeutic Mode of Action** is the primary driver for implementation of NAb testing

Which format to select?



- **Assay Performance** and **Risk Assessment** are the secondary drivers for selecting the assay format

How to set the cut-point

- CP: Validation
  - ~30 individuals representative of study population
  - Healthy matrix individuals when assay used in Ph I or rare target population
- CP: In Study
  - In study CP with ADA negative pre-dose samples
  - For high-risk project: In study CP should be determined as soon as the first 30 individuals are screened and included in the study

Sensitivity

- **Sensitivity** dependent on the characteristics* of the **Positive Control (PC)**
  *Affinity of PC to drug; proportion of NAbs in polyclonal preparation
  - Any type of PC (monoclonal, polyclonal) can be used that has neutralizing activity

- For CBAs: sensitivity dependent on various factors: receptor density, cell density, drug affinity to receptor, etc.
  - Matrix interference might require higher MRD

- **Sensitivity of 100 ng/mL (expected for ADA-assays) is not needed for NAb:**
  - **Low risk projects: 1-1.5 µg/mL**
  - **High risk projects ≤1 µg/mL**
  - **USP recommends sensitivity of 0.5 µg/mL – 2 µg/mL**

Drug tolerance

- Typically, NAb assay read-out is based on defined “assay“ drug concentration which is neutralized by NAb
  - Lower drug concentration usually gives:

    -> Better sensitivity **BUT** poorer drug tolerance

- When drug is “on board”, challenge to detect NAbs
  - Drug in sample:
    - masks the detection of NAbs by Drug/NAb complexes
    - Induces signal change in assay

Sample pre-treatments can improve drug tolerance and matrix interference

- Pre-treatments can be tested to improve matrix and drug-tolerance
  - SPEAD, BEAD, acid dissociation, PEG precipitation, ACE, etc.
  - Experiences from EBF companies show that drug tolerance can be improved in both CBA and C-LBA

- Examples in the training day slide deck:
  - https://e-b-f.eu/fw202101-slides/

Current topics of interest

- Currently running a survey amongst NAb experts in EBF community
  - Preliminary results presented in the next slides
    - 20 responses, including different experts at the same company
- If you are a NAb expert and would like to contribute, please reach out
  - robert.nelson@labcorp.com

Cut-point setting

- When assessing NAb assay **validation** cut-points, do you typically use healthy matrix or commercial disease-state matrix?

Other:
- Both normal and diseased
- Multiple disease states

Healthy

Commercial disease-state

Cut-point setting

- How do you determine whether your validation cut-point is appropriate for the study population?
  - Use range of false positive rates
    - Is this suitable if targeting 1% FPR?
  - By performing in-study population-specific cut-point analysis using pre-dose samples to compare with the validation cut-point
  - I always analyse pre-dose samples for cut point evaluation
  - Statistical comparison with pre-dose study samples

Cut-point setting

- Do you routinely assess **in-study** cut-points for your NAb assays?

Other: If the assay is used for new disease indication

Only if the validation cut-point isn't appropriate

Yes

No

Cut-point setting

- How many pre-dose samples do you test/include to evaluate an in-study cut-point?
  - minimum 20 samples
  - at least 30 samples
  - minimum 20, but preferably at least 30
  - 30 or more
  - 25-50 samples if possible, but will use fewer if that's all that's available
  - 30- 60
  - Approx. 100

Cut-point setting

- Do you use a balanced design for evaluating the in-study cut-point?

Other
- if possible, sometimes sample volume does not allow
- Mostly not due to informed consent constraints

Assay validation

- Do you apply different levels of validation when NAb is included in different clinical phases?

Other
- often do not apply nAb until phase 2/3 and then full validation

Assay validation

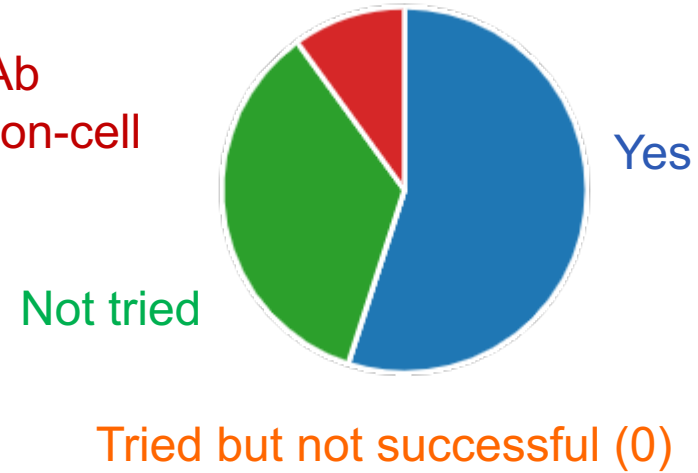
- If yes, what is included/excluded in the 'lighter' levels of validation?
  - cut point needs to be assessed for research grade assay
  - Same parameters but fewer repetitions
  - some robustness parameters

Competitive LBA vs Cell-based assays

- Have you been successful in arguments for using **non-cell based NAb** rather than a cell-based NAb assay with a regulatory agency?

Other

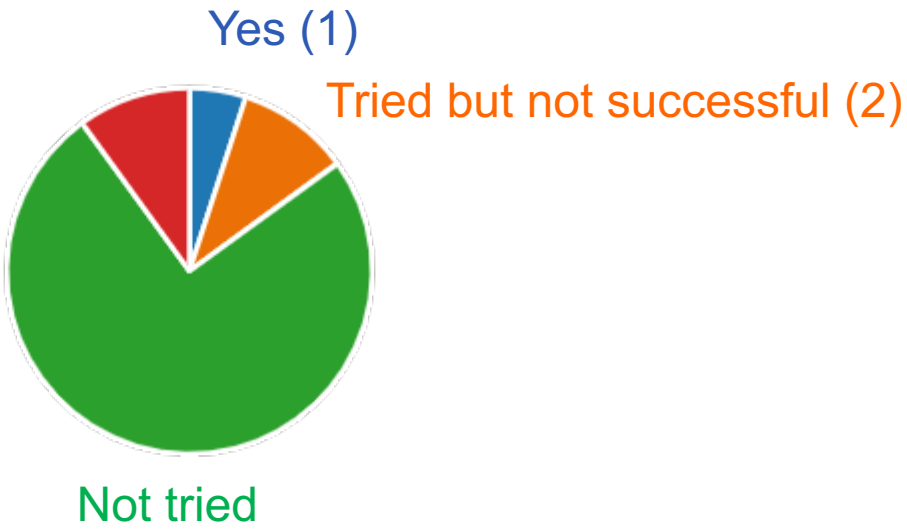
- As CRO not involved in these discussions with agencies
- non-cell based assay used for Biosimilar NAb detection in case MoA of biosimilar allows non-cell based assay (see Wu et al)



PK/PD vs NAb assay

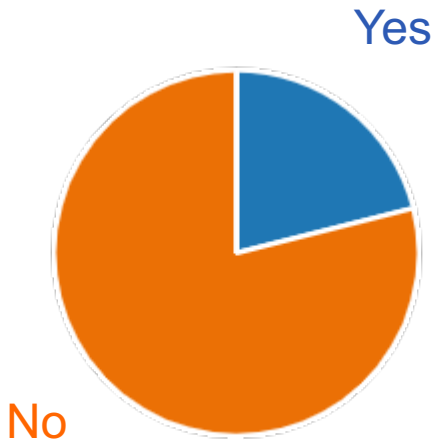
- Have you been successful in arguments for using a **pharmacodynamic endpoint** or **PK/PD** as the NAb read-out with a regulatory agency?

Other
- We always look for the opportunity but have not encounter the case
- valuable approach but not yet tried



Cross-validation of NAb assays

- Have you every **cross-validated** a NAb assay (same assay, different sites)?



Cross-validation of NAb assays

- If yes, what do you use for the cross-validation exercise?

Other
- Spiked PCs or study samples

Spiked PCs

Spiked PCs and study samples

Study samples (0)

Future plans

- Manuscript currently being drafted to share the thoughts of the EBF NAb team with the wider bioanalytical community
- Finalise the survey and give feedback to the EBF community
  - Contribution to (Spring) (Cyber)workshop on immunogenicity?
- Partner events? (e.g. AAPS, JBF)

EBF NAb Team

- Nicoline Videbæk, Novo Nordisk
- Ingeborg Dreher, Abbvie
- Maija Pfenniger, Celerion
- Martin Schaefer, Roche
- Bonnie Wu, Janssen R&D
- Per Holse Mygind, Ascendis Pharma
- Bernd Potthoff, Novartis
- Regina Bruyns, Nuvisan
- Weifeng Xu, MSD
- Richard Weaver, Labcorp Drug Development
- Joanna Grudzinska-Goebel, Bayer
- Marcel van der Linden, Genmab
- Rodolphe Gravier, Charles River

Acknowledgements

- EBF Immunogenicity Experts

To be continued…

Cybermeeting

02 DECEMBER – DAY 2

15:00	**17:20**	**Strategies on nAb – parallel**
15:00	15:10	Welcome – Introduction to the session – Robert Nelson, Labcorp
15:10	15:30	Robert Nelson, on behalf of the EBF NAb team Feedback from EBF discussion on NAb strategies
15:30	15:50	Nicoline Videbæk, NovoNordisk Recent Developments in the PK, PD, ADA Integrated Approach versus in vitro NAb Assay, New Case Studies and Evolving Trends
15:50	16:10	Weifeng Xu, MSD Novel idea to overcome Drug Interference in Immunogenicity Testing with Much Reduced Acid Treatment and Biotin-conjugated Drug Usage
16:10	16:30	Todd Lester (presenting)/Heather Myler – AAPS FB from AAPS nAb team
16:30	16:50	Joao Pedras-Vasconcelos, CDER A regulatory perspective
16:50	17:20	Panel discussion Panelist: Session presenters


Arigato
Mahalo
Grazie
Spasiba
THANK YOU
Thoinks Moite
Gracias
Merci
Danke
Shukran

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