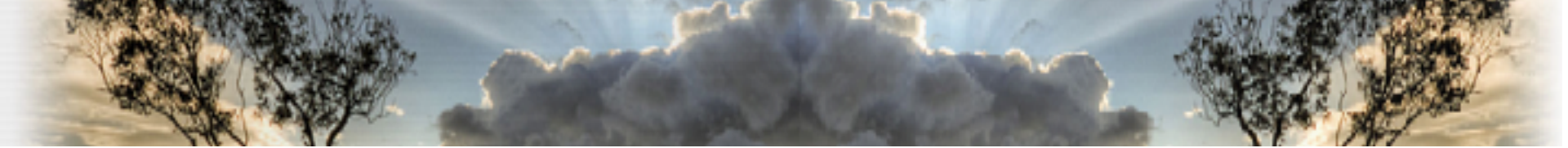


EBF

EBF Cyberconnect Events

Training Day: Managing the Practical Aspects of Immunogenicity

23-24 March 2021

A strategic approach to nonclinical immunogenicity assessment: a Recommendation from the European Bioanalysis Forum

Anna Laurén

Nonclinical ADA Assessment and ICH S6

- Current immunogenicity guidelines focus on clinical phases
- The need of multi-tiered analysis for ADA responses in pivotal Clinical studies is well understood
- When assessing ADA in nonclinical species a leaner and minimal approach can be acceptable and in line with ICH S6 addendum from 2011:
  - ADA in nonclinical studies are not predictive of human immunogenicity
  - Measurement of ADA in nonclinical studies should be evaluated if
    1. evidence of altered PD activity
    2. unexpected changes in exposure in the absence of a PD marker
    3. evidence of immune-mediated reactions (immune complex disease, vasculitis, anaphylaxis, etc.)

EBF and considerations for nonclinical immunogenicity testing

- Realisation in 2016-2017 that a high number of companies included nonclinical ADA testing as a tick-box exersize – majority followed the clinical Immunogenicity guidelines

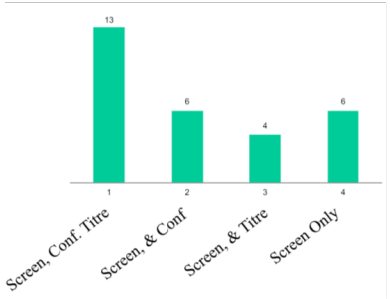
- EBF recommendations in 2016-2017:
  - *D. Stoellner EBF Open Symposium 2016:*
  - PK/TK summary statistics should be reported in two scenarios:
    - Scenario 1 with all animals
    - Scenario 2 with ADA positive animals excluded
  - *J. Munday EBF Open Symposium 2017:*
  - Minimise the different tiers for nonclinical ADA testing and a recomendation to publish a decision tree

EBF companies willingness to change

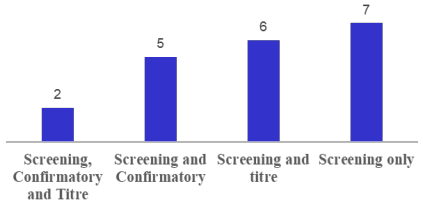
- Similar surveys betwen 2017-2019 showed that companies had started to simplify nonclinical ADA assessment:
  - Less tiers included
  - Screening cut-point set at 0.1% to 1%

Which tiers to include

2017

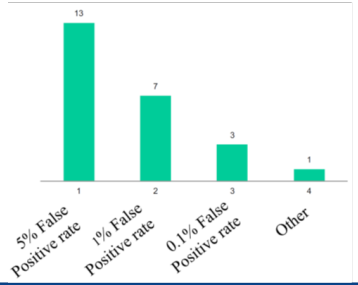


2019

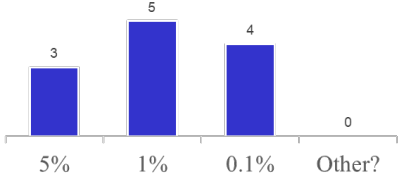


How to set cut-point

2017

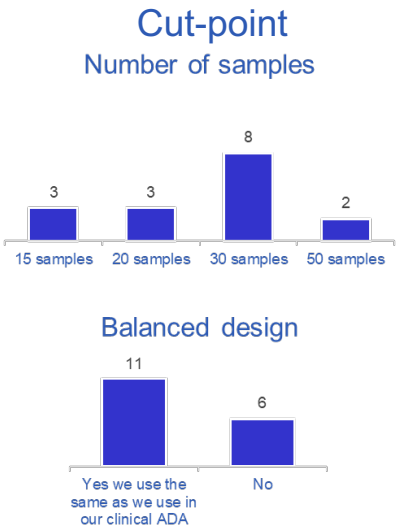


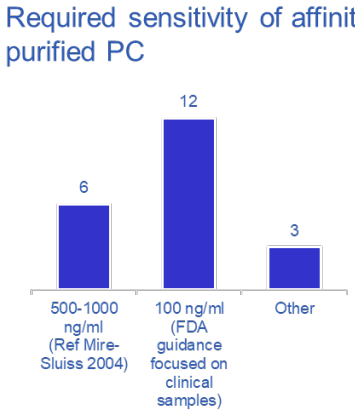
2019



In 2019: still a few tick-box exercises

- Many EBF companies still did a lot of work on ADA assays for nonclinical purposes
  - Eg focus on sensitives around 100 ng/mL, large amount of samples for cut-point assessments, balanced design, testing appropriate LPC in studies





Time to change: A minimal approach can be used

Recent EBF surveys with experience from >300 studies showed that:

- Nonclinical ADA results ***had not*** changed the dose setting for the FHD study
- Member companies shared that they had used a minimal lean approach with success in submission
- Only a minor number of studies could not explain the unexpected TK by an ADA response
  - Assumed reasons:
    1. Unknown
    2. Drug tolerance was believed to be the problem
    3. Concluded to be pharmacologic (circulating target/receptor or similar)

- Agreement to publish an EBF recommendation paper

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Bioanalysis

A strategic approach to nonclinical immunogenicity assessment: a recommendation from the European Bioanalysis Forum

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Relevance of nonclinical immunogenicity assessment and scientific discussion health authorities

- The EBF publication was written based on experience in EBF as well current guidelines.
- However, the dialogue will continue…

- A Chinese NMPA draft guideline published 24 August 2020: "… immunogenicity studies are always an important part of the chain evidence for nonclinical safety studies of therapeutic protein drugs."

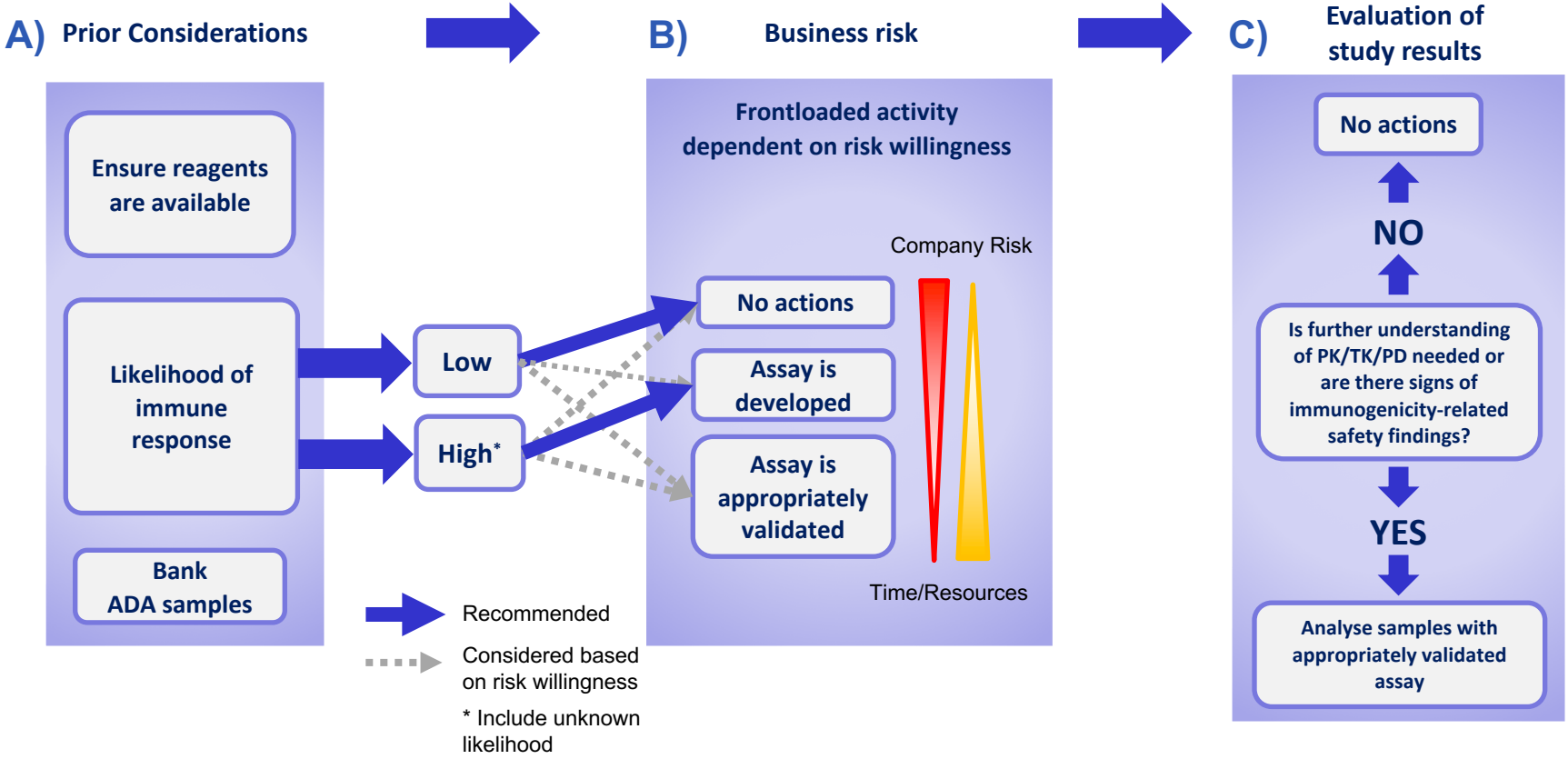
- Implications for the NMPA guideline of similarities and differences to existing EMA and FDA Guidelines:
  - EBF have considerations regarding the risk of increasing the nonclinical package for submissions in China and have addressed this in the EBF publication
  - Hopefully the challenge will turn out positive with a chance of harmonization within ICH

Points to consider for nonclinical ADA strategy

- In the context of **nonclinical studies**
- **A foreign protein administered to an animal**
- Molecule Characteristic
  - Fully human
  - Chimeric
  - Replacement protein
  - New modalities
  - Homology in tox species
  - Bispecific or multidomain
  - Other alterations
  - Glycosylation patterns
  - Potential aggregation
  - Half life
- Dosing regimen
  - Delivery system
  - Dose
  - Length of study
  - Formulation

Likelihood of immune response

ADA decision-making tree



Recommendation for lean minimal ADA method validation

- Develop assay with established concentrations of critical reagents with a mind-set that this is **not a clinical assay**
- Consider the Context of Use for the assay: number of samples from nonclinical studies will be low and analysed in limited runs
- In majority of new drug projects:
  - No confirmatory assay needed
  - No titration needed => S/N ratio is an option
  - Titre assay may be valuable when you do not have an assay with good drug tolerance
  - Neutralization assay is not needed

- Set the validation acceptance criteria a priori
- Document the results for the parameters in a validation report

Suggested nonclinical ADA method validation parameters

Parameter	Minimal Number of Runs and Samples	Comment
Screening Cut Point (SCP)	2 runs of 30 individuals or 4 runs of 15 individuals	Minimum 60 data-points from individual samples. May be generated from multiple analysts. 0.1 to 1% FPR and no confirmatory assay.
Sensitivity	1 run	At least 1000 ng/mL ≥SCP. No need for statistical analysis.
Selection of Low Positive Control (LPC)	Tested as part of precision	LPC is predefined during assay development and confirmed during validation. The concentration is selected at a reasonable range close to sensitivity (e.g. 2-3x to the signal of SCP).
Drug Tolerance	1 run	LPC (or for more sensitive methods at least at 1000 ng/mL positive control) in presence of appropriate drug concentrations should remain positive.
Precision	3 runs	Ensure that the LPC and the High Positive Control (HPC), if used, is tested ≥SCP and Negative Control (NC) is <SCP in each run.

Optional nonclinical ADA method validation parameters

- When needed

Parameter	Minimal number of Runs	Comment
Titration	Case by case	May be relevant to explain differences in the TK profiles in cases where a positive/negative or a S/N approach is not appropriate.
Selectivity	1 run	In cases where assessed (e.g. small MRD): samples spiked at LPC. Hemolytic/lipemic samples are not needed.
Specificity	1 run	Specificity against multimeric epitopes of biotherapeutic, target antigen or other interfering endogenous proteins.
Sample Stability	1 run	Include only short-term stability analysis and freeze thaw Long term stability is not needed
Changes in Critical Reagents	Case by case	Include only on a case-by-case basis as partial or in-study validation, e.g. to assess the performance of critical reagents or minor method parameter changes

Collection of ADA samples

- According to ICH S6 collect ADA samples for banking and analyse when required
- Collect ADA samples minimum at predose and at the end of the study
  - For studies with longer duration, the suggestion is to include sample collection during the study as well
- Consider the 3Rs: Reduce, refine, replace
- Rodents:
  - Limited sample volume and often sparse sampling
  - Collect samples from both main study and PK/TK animals
- Non-rodents:
  - Sufficient volume for collection of full PK/TK profiles in main study animal

Analyse the ADA samples?

- Samples should only be analysed if the PK/TK/PD profiles or safety findings indicate a potential impact due to the presence of antibodies aligned with ICH S6

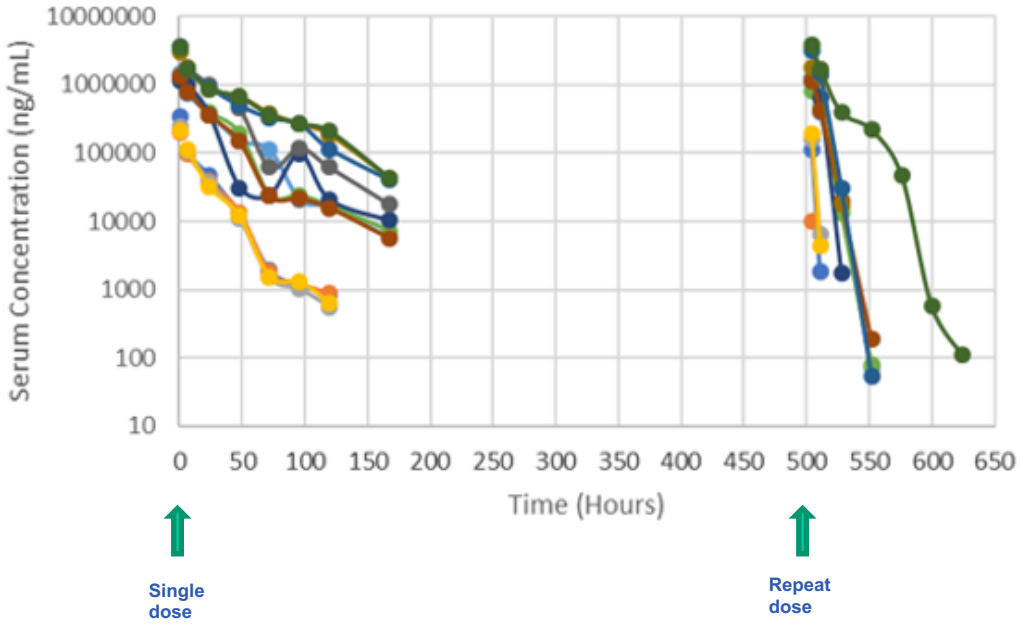
- Important to ensure a good communication/planning within the nonclinical study to inform responsible scientist for ADA analysis, if the analysis is required or not
  - If possible, do the PK/TK/PD and safety evaluation on interim results to reduce the potential delay

- Consider singlicate well sample analysis
- No need to confirm FPR among base-line samples or control animals and establish in-study CP
- No need to confirm LPC failure rate

Evaluation of nonclinical ADA

- Consider if studies have a full TK profile or sparse TK sampling
- Impact on TK/PD or safety parameters:
  - Number of animals with impacted profiles?
  - Decrease or increase in the TK response?
- Reporting the evaluation of TK study data:
  - Including (all animals or with and without ADA positive animals)
  - Excluding (all ADA positive animals or only excluding ADA positive animals if impact)
  - Apply a case-by-case approach and *a priori* criteria

Example 1:



Questions:

- Should the samples have been analysed for ADA?
- How to report TK?
- What to use for exposure ratio?

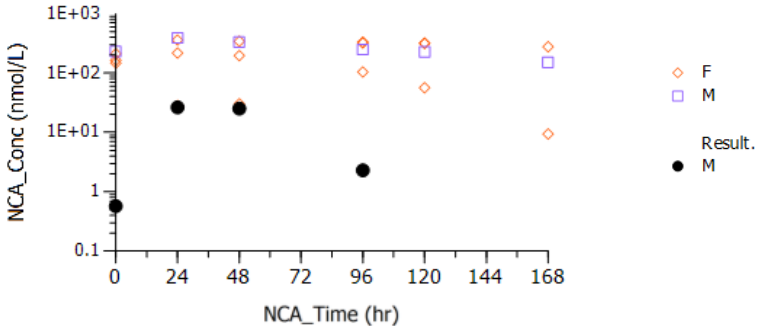
Observation and discussion:

- TK exposure level was significantly reduced in most animals
- TK exposure reduction was most likely due to ADA formation
- Samples were analysed for ADA

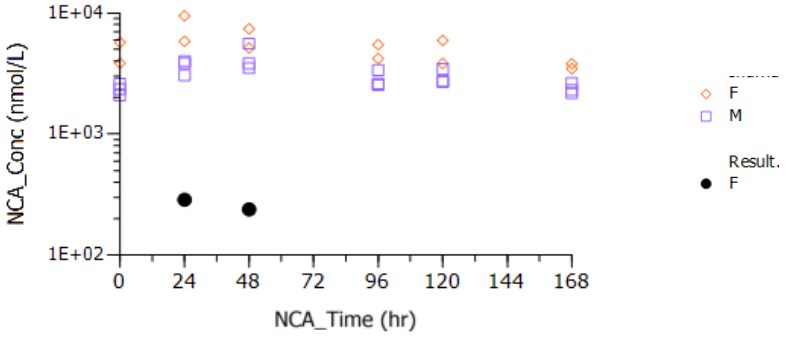
Overall Results: ADA data supported the TK analysis. The impact of ADA on TK was clear.

Example 2: 13 week toxicity study in monkey

Group 2 – Week 6



Group 3 – Week 13



Observations and discussion:

- Several animals with reduced or low exposure after repeated dosing
- Should the samples have been analysed for ADA?
- How to report TK and calculate human exposure ratio?

Approach:

- The impacted animals were excluded from TK calculations from the start
- Samples were not analysed for ADA
- Omitting the results did not change the conclusion of the study

Summary and future perspectives for nonclinical ADA assessment

- Keep a mindset that this is **not a clinical assay**
- Recommendations from the EBF community:
  - A biological drug can be approved without nonclinical ADA data
  - Consider the 3Rs
  - A decision tree can be used to plan ADA assessment
  - Implement a minimal lean assay validation approach and sample analysis strategy

- Continue to have a scientific dialogue with regulatory authorities on the relevance of nonclinical immunogenicity assessment
  - A recent Chinese NMPA draft technical guideline shows differences to EMA/FDA with emphasis on nonclinical immunogenicity analysis

Acknowledgment

- Deborah McManus
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- Susanne Pihl
- Jo Goodman

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