Anti-drug antibody analysis in non-clinical samples

- a simplified strategy offering sufficient support for interpretation of toxicology studies

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Evaluation of non-clinical ADA analysis strategy

Purpose:

- To evaluate the level of ADA validation and ADA characterisation needed for non-clinical samples

What was evaluated:

- Regulatory guidelines
- Published recommendations in white papers etc
- Historical data from various projects

Outcome:

- Implementation of a more simple ADA strategy with sufficient support for interpretation of non-clinical studies

Guideline expectations for non-clinical ADA assays

- **EMA 2017**, Guideline on Immunogenicity assessment of therapeutic proteins :
  - Assays should be validated
  - Interference of therapeutic protein needs to be considered

- **FDA 2019**, Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection :
  - Applies to clinical development, but "some concepts discussed are relevant to the design of ADA studies for non-clinical testing"

- **ICH S6(R1) 2011**, Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals :
  - Antibody responses should be characterised (e.g. titer, number of responding animals, neutralising or non-neutralising) and correlated to any pharmacological or toxicological changes (PK/PD)
  - Assessment of neutralising potential warranted when ADAs are detected an there is no PD marker to demonstrate sustained activity

Published recommendations for non-clinical ADA assays

- Assay cut point with 1% false positive rate [2]
- Determine sensitivity, reproducibility, susceptibility to matrix effects [2]
- Evaluate cut point on pre-dose samples and derive a study specific cut point if necessary [3]
- 15 samples might be sufficient for validation cut point [2]
- Determination of sensitivity in the presence of drug is expected [3]
- Assay sensitivity of 500-1000 ng/ml is reasonable [1]
- Confirmation of specificity is generally not needed [3+4]
- Results may be reported as positive/negative [4]
- Screening cut point at the 99.9th percentile is sufficient [4]
- Titration assay is not required [4]

1: Mire-Sluis et al. (2004). Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. Journal of Immunological Methods.
2: Shankar et al. (2008). Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. Journal of Pharmaceutical and Biomedical Analysis.
3: Ponce et al (2009). Immunogenicity of biologically-derived therapeutics: Assessment and interpretation of nonclinical safety studies. Regulatory Toxicology and Pharmacology.
4: Richards et al (2016). 2016 White Paper on recent issues in bioanalysis: focus on biomarker assay validation (BAV): (Part 3 – LBA, biomarkers and immunogenicity). Bioanalysis.

Considerations related to changing practice

Validation parameters

- Screening cut point (15x4 per species)
- Confirmatory cut point
- Cross reactivity cut point
- Titration cut point
- Sensitivity (2x)
- Drug interference (1-3 drug conc)
- Drug tolerance (2x)
- Haemolysis
- Epitope shielding
- Recovery
- Drift
- Precision
- QC range

? Reproducible discrimination between positive and negative samples without tiered approach

? ADA level is important for correlation to PK/PD – can assay signal replace titre

? Better QC ranges/criteria to avoid unnecessary rejection of assays and sample re-analysis

Removal of the confirmation step - examples

			Study specific Cut Point			Validation Cut Point
			Screening 1.0% FPR + Confirmation		Screening 0.1% FPR	Screening 0.1% FPR
Species	animals	sample type	≥ screening CP	Positive samples	Positive samples	Positive samples
rat	160	predose	9	0	3	0
		postdose	9	0	2	0
rat	96	predose	5	0	1	0
		postdose	4	0	3	0

☑ Possible to avoid reporting outliers as ADA positive – more noise is not introduced

Removal of the confirmation step - examples

			Study specific Cut Point			Validation Cut Point
			Screening 1.0% FPR + Confirmation		Screening 0.1% FPR	Screening 0.1% FPR
Species	animals	sample type	≥ screening CP	Positive samples	Positive samples	Positive samples
monkey	36	predose	0	0	0	0
		postdose	22	20	20	17
monkey	34	predose	1	0	1	0
		postdose	54	50	52	48

? Lower incidence of ADA positive animals
Is that a problem for interpretation of non-clinical studies?

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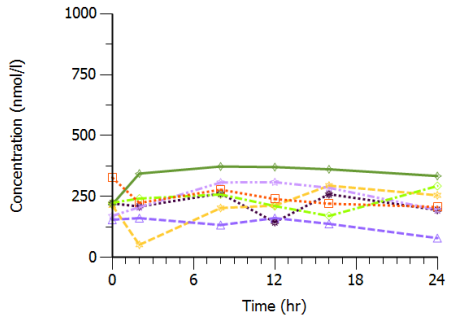
Example of unexpected changes in exposure where ADA data were used to support study

Day 14:
4/7 ADA+

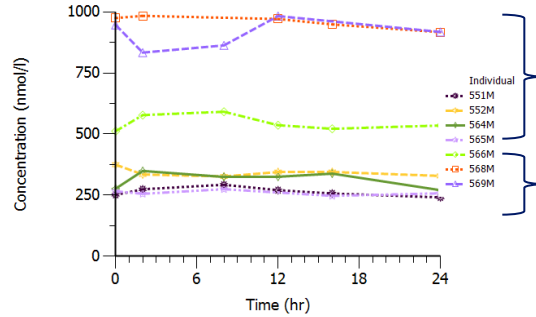


No pattern between exposure and ADA status at Day 14

- Screen and 0.1%FPR: only 3 ADA+ animals

The 3 animals with unexpected exposure is also the 3 animals with highest ADA levels

Day 112:
7/7 ADA+



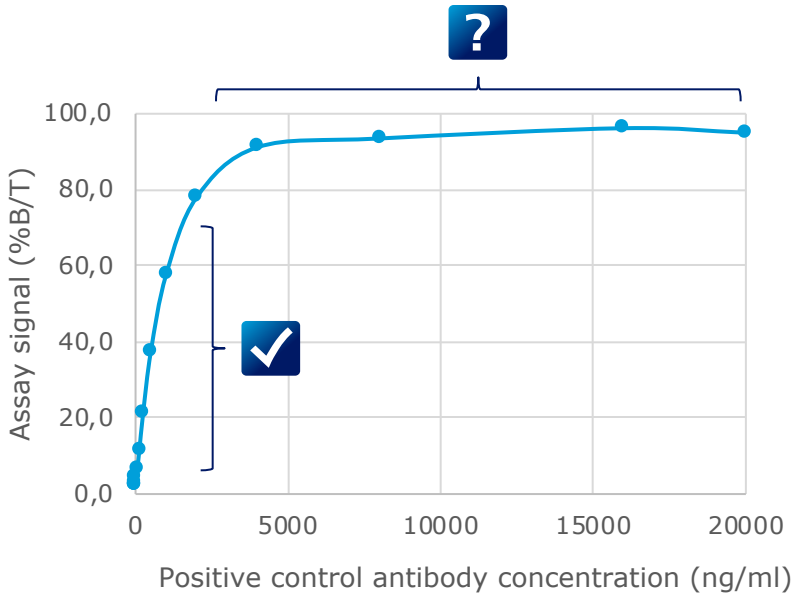
The 4 animals without unexpected exposure:

- Low ADA positive with two tiers and 1% FPR
- ADA negative using screen and 0.1% FPR

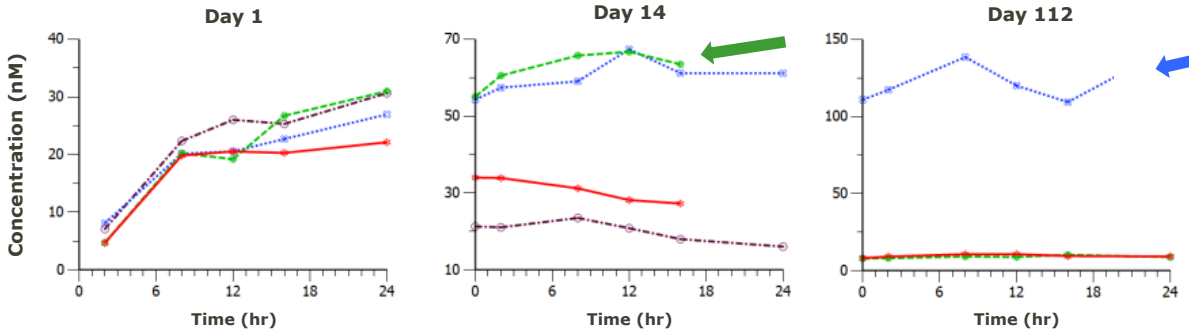
Lower ADA incidence in non-clinical study is not considered a problem

Use of assay signal as alternative to titration

- Assay signal or signal/noise ratio correlates to titre within the dynamic range of the assays
- Need for differentiation within maximum response?
- Many of ADA assays have a fair to large dynamic range where titration is not needed for interpretation of tox studies



Example of unexpected changes in exposure where ADA data were used to support study



ADA results:

Predose	Day 14	Day 112
2.7	**60.4**	**92.7**
2.9	2.8	**11.1**
2.4	**10.5**	**92.5**
3.2	**61.2**	**89.6**

Grey and bold: Positive for anti-drug antibodies, Results in %B/T.

- Simple ADA positive/negative status is not enough to explain PK changes
- But ADA assay signal levels can very often provide sufficient supportive information without titration
- Toxicological findings in the study suggested that lack of detectable exposure was caused by ADA interference in the bioanalysis assay

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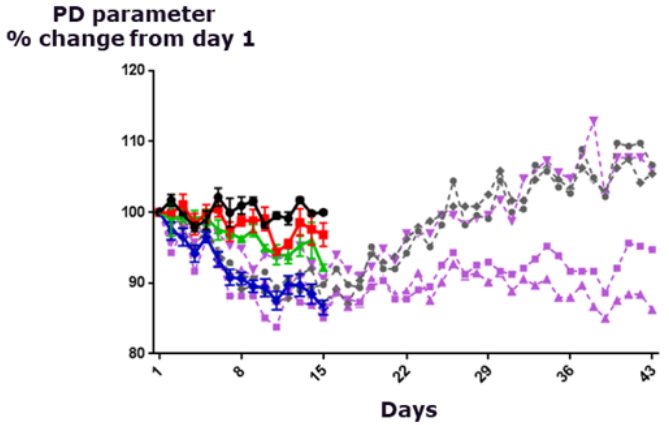
When ADA status and levels are not enough



ADA results:

Treatment	Predose	Day 15	Day 43
Recovery from day 15	3.0	3.4	**6.2**
Recovery from day 15	2.6	**5.4**	**59.3**
Treatment day 1-43	2.6	**11.5**	**63.3**
Treatment day 1-43	3.5	**17.2**	**49.5**
Treatment day 1-43	2.7	**19.8**	**26.4**

Grey and bold: Positive for anti-drug antibodies,
Results in %B/T.

- All treated animals were exposed to comparable levels
- PD marker was necessary for evaluation of neutralising potential of ADAs

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QC ranges for control of assay performance

- Recommended to use limits calculated with 1% failure rate on QCs at all levels [1]
- Not uncommon that these limits lead to rejection of assays where ADA results are considered suitable for support of non-clinical studies

- Implementation of new simple QC criteria/limits with sufficient control of assay performance:

QC level	QC neg	QC low	QC high
Purpose of QC	Sets the cut point (floating cut point)	Controls assay sensitivity	Controls the dynamic range
Simple acceptance criteria	No criteria	≥ cut point	Lower limit calculated with 0.1% failure rate

1: Shankar et al. (2008). Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. Journal of Pharmaceutical and Biomedical Analysis.

The simplified approach for non-clinical ADA

Former validation parameters

- Screening cut point (15x4 per species)
- Sensitivity (2x)
- Confirmatory cut point
- Cross reactivity cut point
- Titration cut point
- Drug interference (1-3 drug conc)
- Drug tolerance (2x)
- Haemolysis
- Epitope shielding
- Recovery
- Drift
- Precision
- QC ranges

New simple validation package

- Screening cut point (15x4 per species)
- Sensitivity (2x)
- Drug tolerance (2x)
- Precision
- QCs with simple criteria

Conclusion

- ADA results are support data required in non-clinical studies when unexpected PK/PD are observed
- ADA validation and assay control parameters were simplified with focus on what is needed for non-clinical studies
- ADA sample analysis can be performed simple and still offer sufficient support to the non-clinical studies