Fit-for-purpose non-clinical immunogenicity assessment to support PK data interpretation – a case study

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16th Open Scientific EIP Symposium on Immunogenicity of Biopharmaceuticals

26 February 2025

BioAgilytix

Agenda

1. Introduction to Non-Clinical Immunogenicity Testing
2. A Case Study
3. Summary and Discussion

Introduction to Non-Clinical Immunogenicity Testing

General and Regulatory Considerations

Non-Clinical Immunogenicity Testing Considerations

- An ADA response is expected when a human or humanized biotherapeutic is administered to animals
- Immunogenicity in animals is rarely predictive for immunogenicity in humans
- Non-clinical immunogenicity testing can be important for study interpretation (e.g. loss of exposure, ADA-related safety findings) in repeat-dose toxicity studies
- Animal matrix: limitation in availability (especially for NHP) and sustainability efforts (3R principles: reduction, refinement and replacement)

Regulatory Expectations

ICH S6:

Purpose-driven approach of whether, and when, to implement non-clinical immunogenicity testing e.g.

- changes in exposure
- evidence for immuno-mediated adverse findings

EMA Guideline of Immunogenicity Testing, 2017:

- ADA analysis may be needed as part of repeat-dose toxicity studies to aid in the study interpretation
- assays should be validated

→ In contrast to clinical ADA assays, no clear guidance on how assays should be validated

→ Therefore, clinical guidelines are often applied to non-clinical immunogenicity testing

→ However, following clinical guidelines might not be necessary due to different intention

EBF White Paper for Non-Clinical ADA Validation

Table 2. Overview of recommended anti-drug antibody validation parameters for nonclinical immunogenicity assessment.

Parameter	Minimal number of runs and samples	Comment
SCP	Two runs of 30 individuals or Four runs of 15 individuals	Minimum 60 data-points from individual samples. May be generated from multiple analysts. 0.1–1% FPR and no confirmatory assay
Sensitivity	One run	At least 1000 ng/ml ≥SCP. No need for statistical analysis
Selection of 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	One run	At LPC (or for more sensitive methods at least at 1000 ng/ml positive control) in presence of appropriate drug concentrations should remain positive
Precision	Three runs	Ensure that the LPC and the HPC, if used, is tested ≥SCP and NC is <SCP in each run Acceptance criteria defined *a priori*

HPC: High positive control; LPC: Low positive control; NP: Negative control; SCP: Screening cut point.

Lauren *et al*, A strategic approach to nonclinical immunogenicity assessment: a recommendation from the EBF, Bioanalysis (2021) 13(7), 537–549

A case study

A recombinant human neurological active protein in non-clinical development

Introduction and Considerations

Biopharmaceutical drug candidate

- recombinant version of a human neurologically active protein
- requires acidic formulation to avoid precipitation and multimerization
- protein challenging to label with standard approach due to biochemical properties

→ Development of PK and ADA assays in both rat and NHP to support non-GLP (e.g. DRF) studies and subsequent validation for the GLP repeat-dose toxicity studies

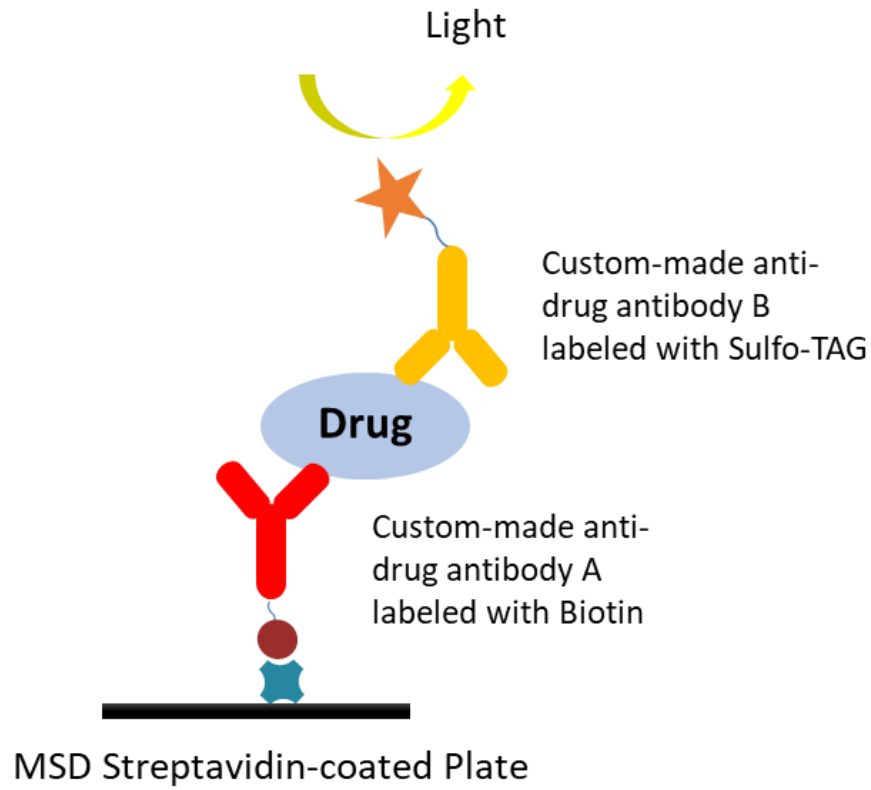
Pharmacokinetic (PK) Assay

Challenges during the development

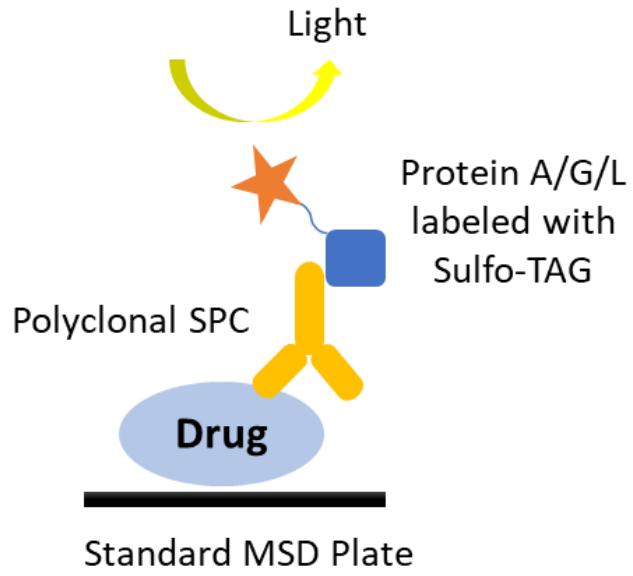
- Careful and consistent handling of drug stock critical
- Crucial to acidify plasma before spiking in drug to ensure accurate and consistent recovery of the drug
- Working on ice to increase drug stability
- Plate drift on MSD high bind plates, which was solved by using MSD Streptavidin plates
- Frozen non-serial calibration standards help to stabilize the assay

Characteristics based on development

- MRD20
- LLOQ: 50 ng/mL; ULOQ: 3200 ng/mL



Anti-Drug Antibody (ADA) Assay



Considerations

- standard labeling of drug with Biotin or Sulfo-TAG challenging → no bridging assay format
- the same assay setup should be used for both species → no species-specific detection

Characteristics based on development

- SCP: ~1.6 (1 % false positive rate)
- No confirmation and titration → use S/NC
- Calculated LPC at 1 % FR: 161 (rat) or 240 ng/mL (NHP) but failed several times in the runs → use pre-set LPC at 500 (rat) and 1000 ng/mL (NHP) to achieve a consistent positive signal
- Drug tolerance not relevant due to short half-life seen in previous studies
- Intra-/Inter-Precision <21.2% for all control level and <21.8% for NC Median

→ Can we detect relevant ADA with this assay?

Rat and NHP DRF Study - Design

Dose Route: sc and iv

Treatment duration: 3 weeks (rat)/ 5 weeks (NHP)

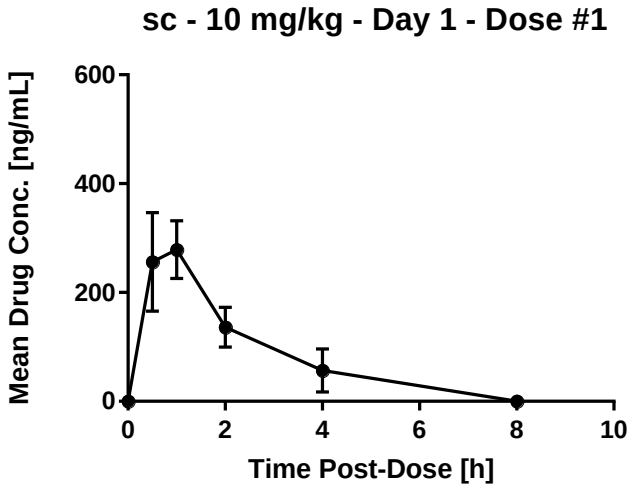
Treatment frequency: once weekly

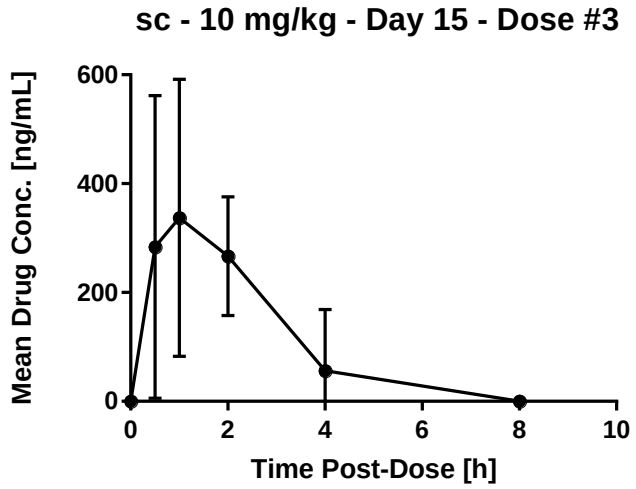
PK sample collection: 0, 0.5, 1, 2, 4, 8 h on day 1 and 15 (rat)/ 29 (NHP)

Group	Subgroup	#Animals	0 h	0.5 h	1 h	2 h	4 h	8 h
Rat sc/iv	A	4	X			X		
	B	4		X			X	
	C	4			X			X

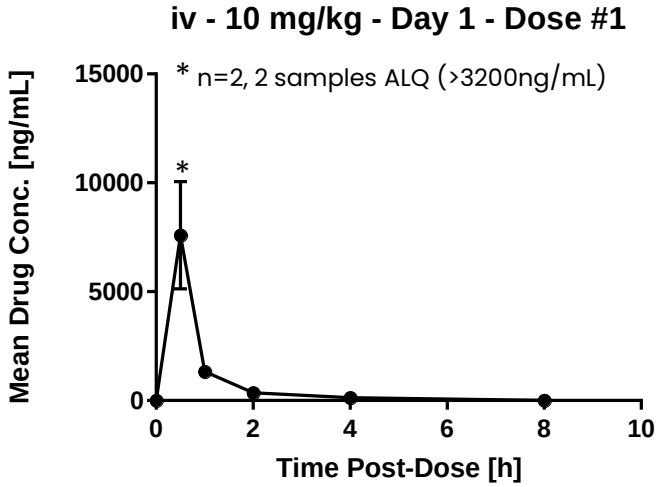
ADA sample collection: at necropsy (rat)/ baseline & at necropsy (NHP)

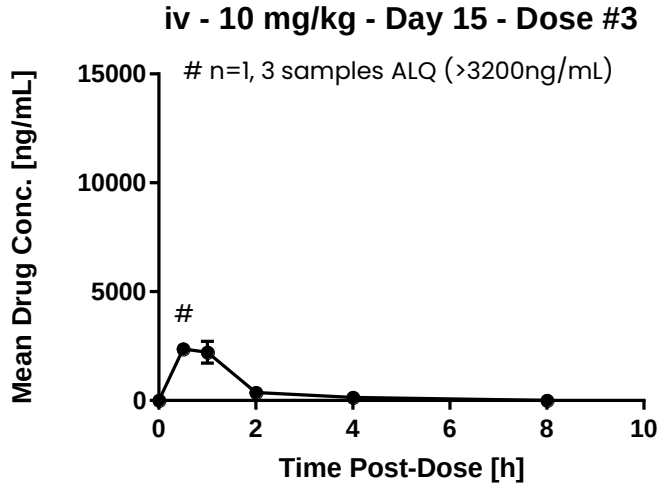
PK Assay – Rat DRF Results





On average, similar exposure for all animals but higher variation





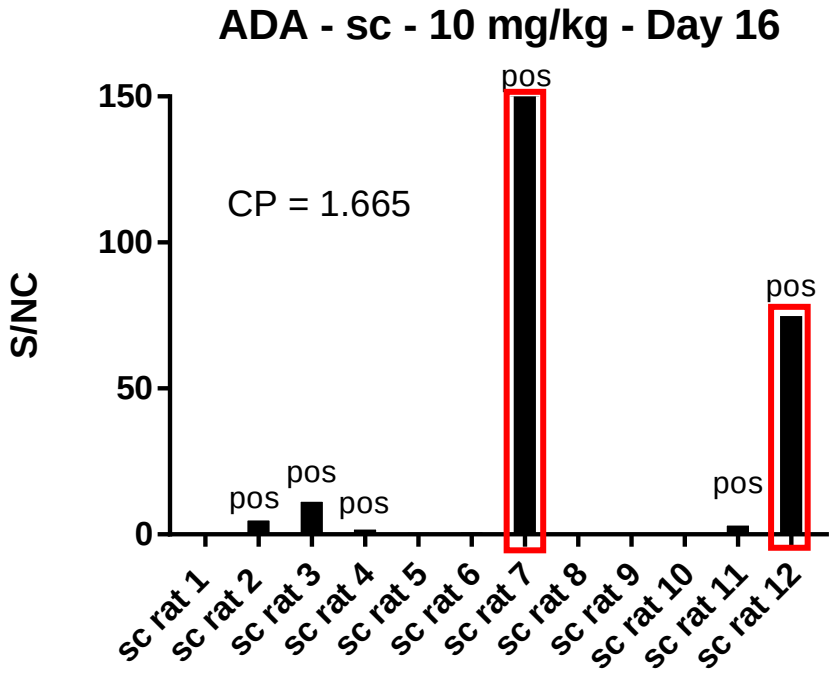
On average, similar exposure for all animals (c_{max} not representative)

Samples, which are BLQ(<50.0ng/mL), are displayed as 0. Connecting lines, no PK modeling.

PK and ADA Assay – Rat DRF Results

Drug concentration in ng/mL for sc samples at day 15 – dose #3

Animal	0 h	0.5 h post-dose	1 h post-dose	2 h post-dose	4 h post-dose	8 h post-dose
sc-1	BLQ<(50.00)	NA	NA	391.3	NA	NA
sc-2	BLQ<(50.00)			No sample		
sc-3	BLQ<(50.00)			187.8		
sc-4	No sample			221.5		
sc-5	NA	657.3	NA	NA	225.3	NA
sc-6		174.9			BLQ<(50.00)	
sc-7		BLQ<(50.00)			BLQ<(50.00)	
sc-8		303.7			BLQ<(50.00)	
sc-9	NA	NA	536.6	NA	NA	BLQ<(50.00)
sc-10			532.3			BLQ<(50.00)
sc-11			279.3			BLQ<(50.00)
sc-12			BLQ<(50.00)			No sample

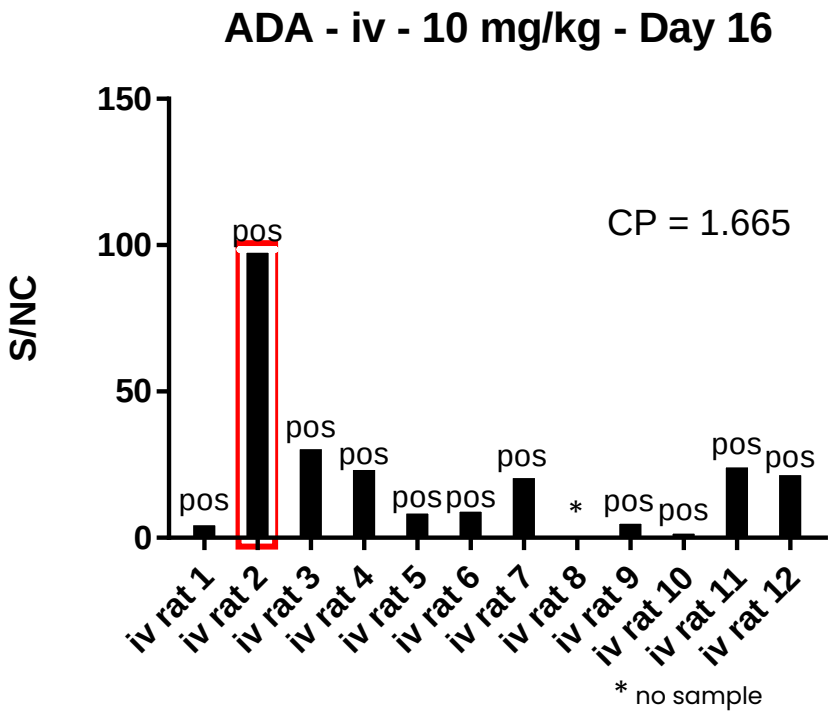


→ Loss of exposure in single animals correlates with high ADA response in those animals

PK and ADA Assay – Rat DRF Results

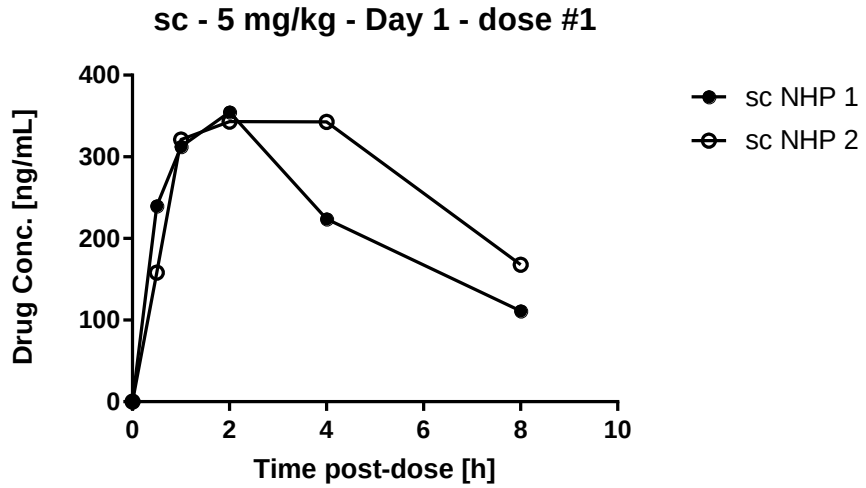
Drug concentration in ng/mL for iv samples at day 15 – dose #3

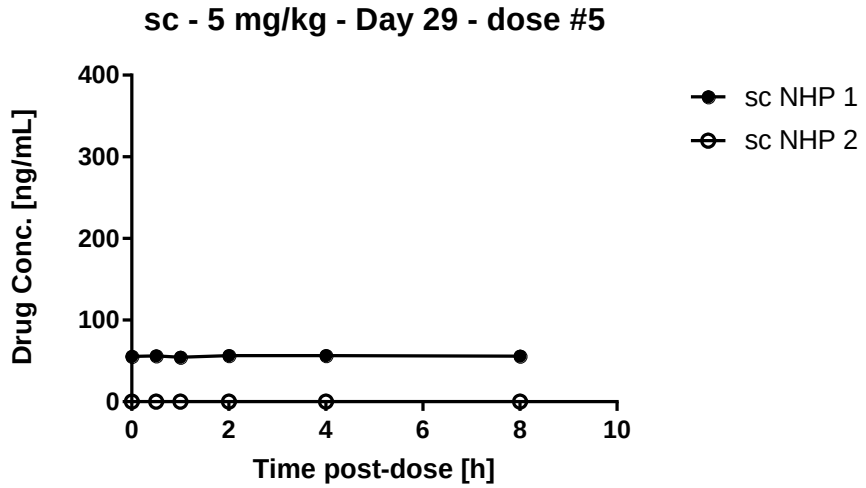
Animal	0 h	0.5 h post-dose	1 h post-dose	2 h post-dose	4 h post-dose	8 h post-dose
iv-1	BLQ<(50.00)	NA	NA	578.2	NA	NA
iv-2	BLQ<(50.00)			BLQ<(50.00)		
iv-3	BLQ<(50.00)			464.4		
iv-4	BLQ<(50.00)			420.7		
iv-5	NA	ALQ>(3200)	NA	NA	169.4	NA
iv-6		ALQ>(3200)			166.7	
iv-7		ALQ>(3200)			184.2	
iv-8		2364			74.86	
iv-9	NA	NA	2689	NA	NA	BLQ<(50.00)
iv-10			2613			BLQ<(50.00)
iv-11			1697			BLQ<(50.00)
iv-12			1877			BLQ<(50.00)



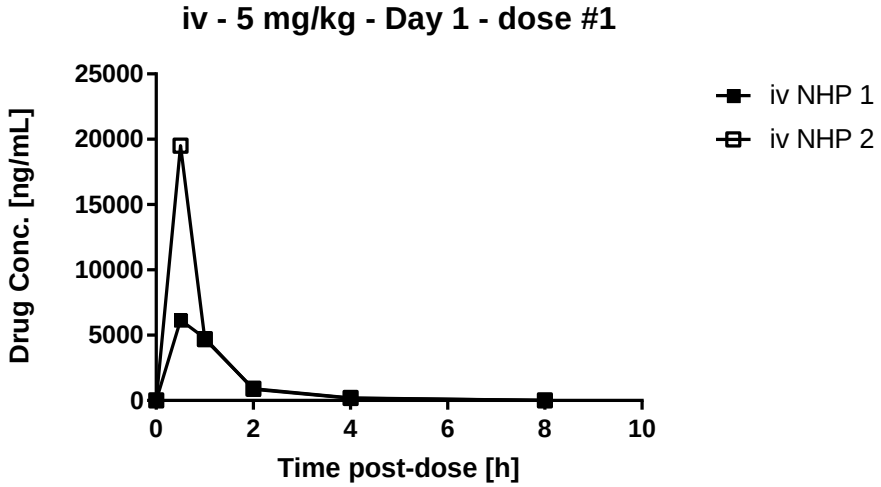
→ Loss of exposure in a single animal correlates with highest ADA response measured

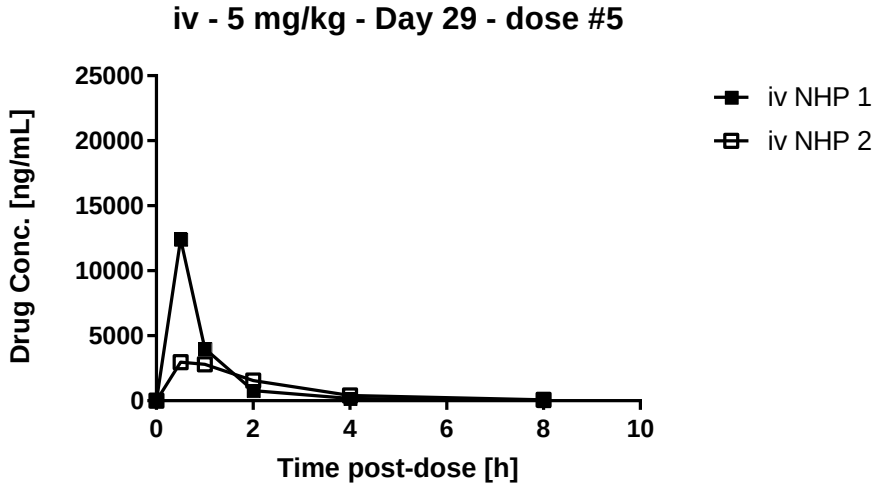
PK Assay – NHP DRF Results





Complete loss of exposure in both animals

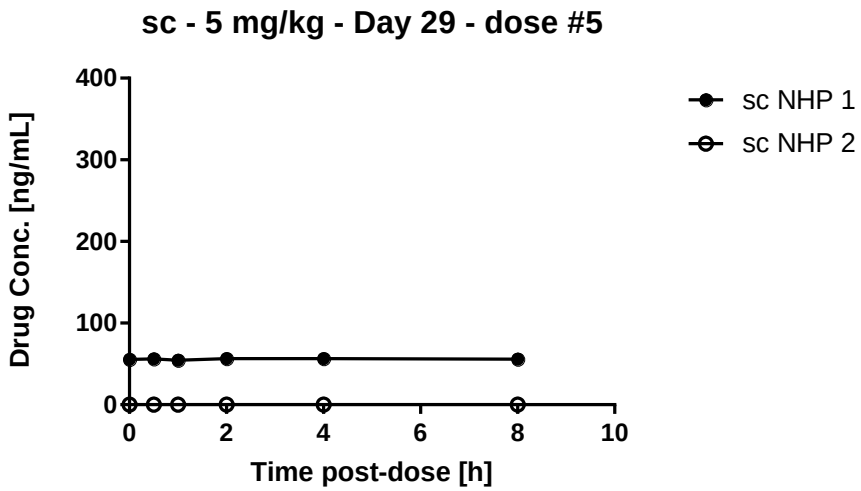


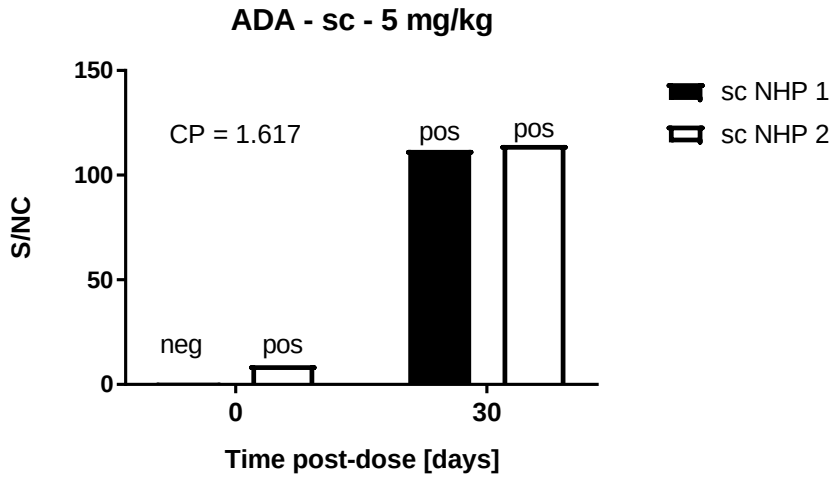


Significant loss of exposure in 1 of 2 animals

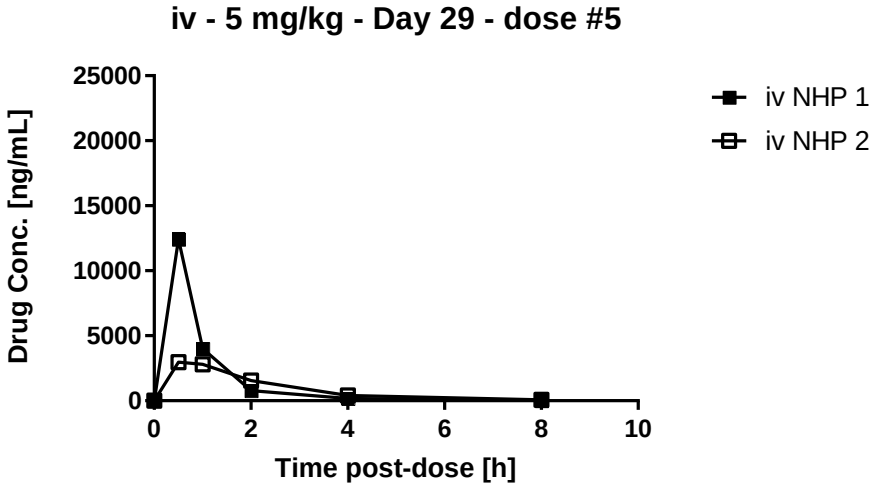
Samples, which are BLQ(<50.0ng/mL), are displayed as 0. Connecting lines, no PK modeling.

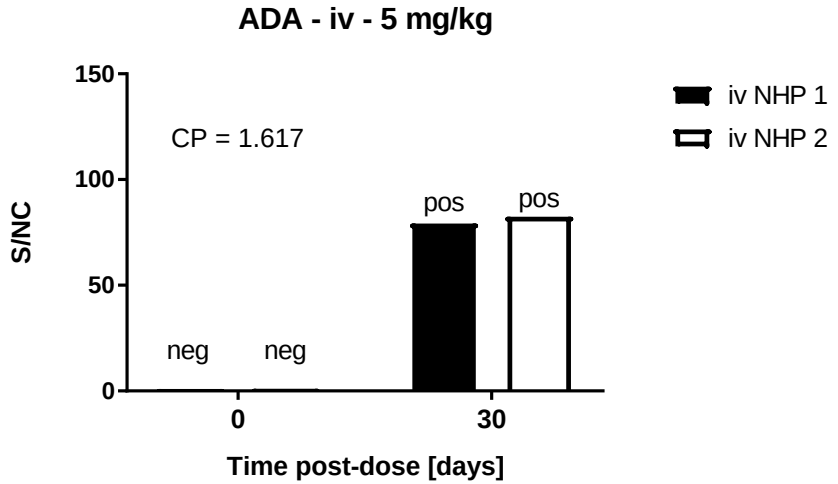
PK and ADA Assay – NHP DRF Results





High ADA response in both animals correlates with loss of exposure





Lower but still significant ADA response in both animals correlates with lower exposure at least in animal 2

Samples, which are BLQ(<50.0ng/mL), are displayed as 0. Connecting lines, no PK modeling.

Summary and Discussion

Non-clinical immunogenicity – doing less is not always wrong

Summary and Discussion

- A simple ADA screening assay, which did not fulfill all clinical regulatory requirements, allowed PK data interpretation for non-clinical studies.
- Following a leaner approach in the assay validation is an appropriate option for immunogenicity assessment in non-clinical studies.
- GLP validation of the ADA assay shown in the case study will follow the EBF White Paper by Lauren *et al.* and will be conducted soon to support the GLP repeat-dose toxicity studies.

Acknowledgements

THANK YOU…

BioAgilytix colleagues

GREAT COLLABORATION AROUND THE WORLD

SUPPORTING THIS PRESENTATION

Sponsor

INTERESTING PROJECTS

APPROVAL TO PRESENT

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ORGANIZING THE SYMPOSIUM

POSSIBILITY TO SHARE OUR SCIENCE

Thank You

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