Fit-for-purpose Non-clinical Immunogenicity Assessment to Support PK Data Interpretation – A Case Study

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PURPOSE

- The scope and extent of **preclinical immunogenicity** testing required to support **dose-range finding (DRF)** and **GLP toxicology (Tox)** studies remain topics of active discussion, with strategies varying based on biotherapeutic modality and risk assessment. This case study examines a recombinant human protein administered to rats and monkeys in DRF studies, highlighting the development of a targeted MSD ligand-binding assay (LBA) based pharmacokinetics (PK) method. A customized **anti-drug antibody (ADA)** assay was also developed and optimized to aid in interpreting PK data.
- During initial ADA assay development, certain challenges were encountered, including high background signals, variability in negative controls, and a low positive control (LPC) level failure rate exceeding 1%. Consequently, a streamlined, **fit-for-purpose** approach was adopted to define a screening cut point and establish pre-set LPC/sensitivity levels, with confirmatory and titration cut points excluded. ADA response levels were then estimated based on signal-to-negative control (S/NC) ratios derived from the screening assay.

OBJECTIVE

- Establishment of a **fit-for-purpose ADA assay** with pre-set sensitivity levels in Wistar Hannover Rat and Cynomolgus Monkey matrix allowing interpretation of PK data, generated with a custom assay, enabling further progression of the Drug Development program.

CONSIDERATIONS

- An **ADA response** is expected when a human or humanized biotherapeutic is administered to animals, immune responses are triggered **(Immunogenicity)**
- **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: timely results are often needed to draw fast and effective conclusions on dosing and further steps in drug development
- Animal matrix: limitation in availability (especially for NHP) and sustainability efforts **(3R principles)**
- Relevant guidelines such **ICH S6 or EMA Immunogenicity (2017)** are vague on whether it should be assessed and if so, to what level of regulation
- Companies often follow a **clinical approach** and establish fully validated methods in a lengthy process applying very high standards
- **Is this worth the effort or can a rather lean approach as suggested by Lauren et al. be followed to minimize efforts and to optimize costs and effectiveness of a Drug Development program?**
- **Is this approach still regulatory compliant with regulatory expectations and able to measure physiological relevant ADA responses?**

PK AND ADA ASSAY SETUPS

- Biopharmaceutical drug candidate
- Recombinant version of a human neurologically active protein
- Requires acidic formulation to avoid precipitation and multimerization
- Protein is challenging to label with standard approach due to biochemical properties
- Aim: Development of PK and ADA assays in rat and monkey to support non-GLP (e.g. DRF) studies and subsequent validation for the GLP repeat-dose toxicity studies

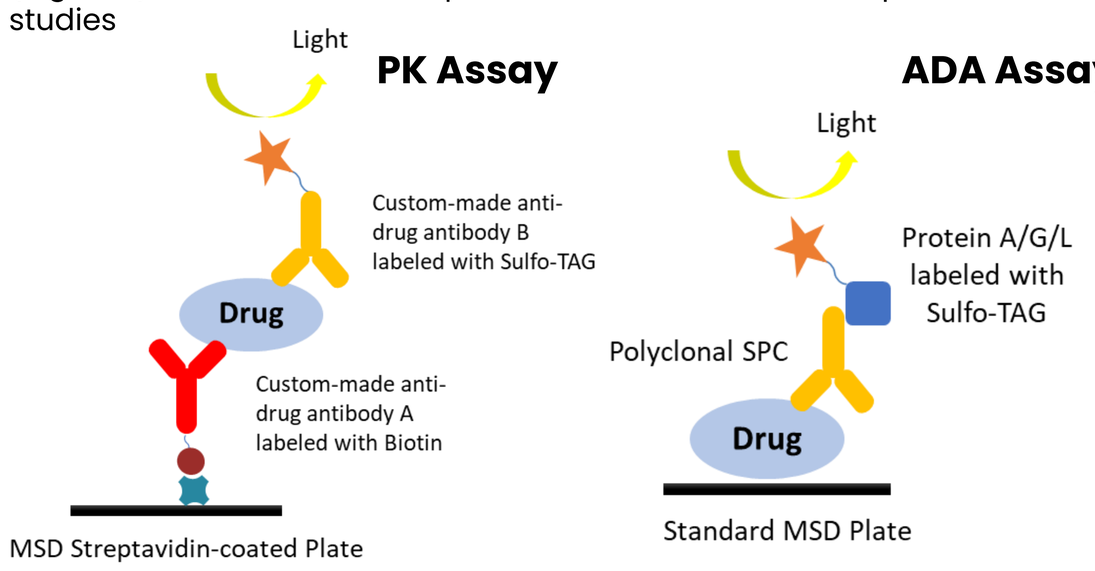


Figure 1: Custom setups for the PK and ADA assays specific to the Drug candidate
MSD: Meso Scale Discovery; Sulfo-Tag uses Ru^{2+} to generate electrochemiluminescence signals

RESULTS

DRF studies: 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)

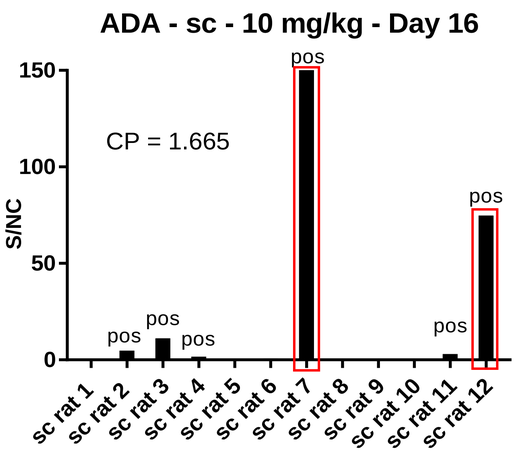
Group	Subgroup	#Animals	0 h	0.5 h	1 h	2 h	4 h	8 h
Rat sc/iv	A	4	X			X		
	B	4		X			X	
	C	4			X			X

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

Table 1: Sample Collection schedule for DRF studies in Rat and Monkey

Dose 3/Day 15 PK results upon sc injection

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



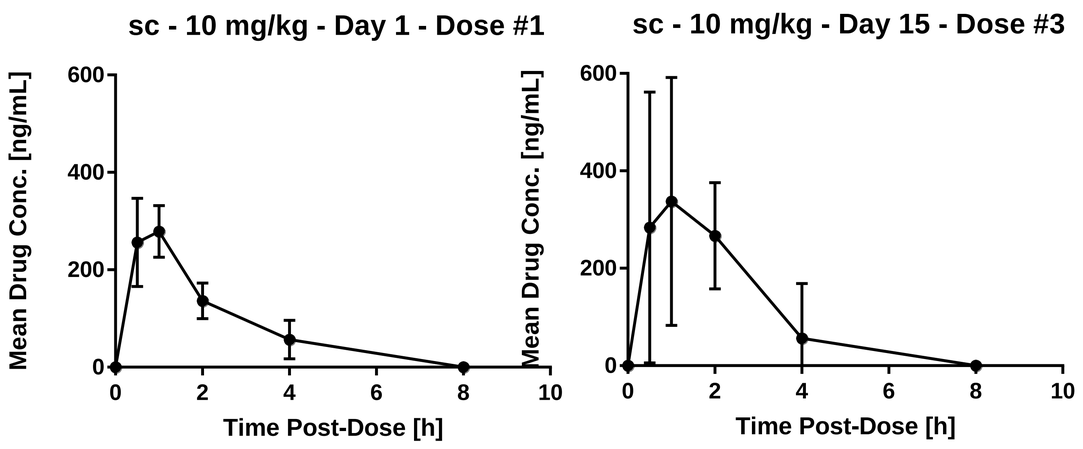


Figure 2: PK profiles at Day 1 (left panel) and Day 15 (right panel) in Rat after first and third subcutaneous administration of the Drug. On average similar exposure for all animals but with higher variation was observed on Day 15.

Figure 3: PK results obtained after the third dose in Rats upon subcutaneous injection at the indicated timepoints in all animals tested (left panel). Results for animals 7 and 12 correlate with highest incidence of Anti-Drug Antibodies (ADA) as observed by highest S/NC ratios from the simple ADA assay.

SUMMARY AND LESSONS LEARNED:

- ✓ The case study is an example of a tailored **fit-for-purpose immunogenicity** assessment strategy that was used to **support preclinical data** interpretation.
- ✓ In general, a significant reduction in hands-on time in the laboratory and costs allows quick decision making in advancing a Drug Development program
- ✓ This approach is still considered **regulatory compliant** with regulatory expectations at early preclincal stages of a Drug Development program
- ✓ A solid and well-established PK method is key in early Drug Development

REFERENCES

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