Biomarker assays in a bioanalytical environment – a case study

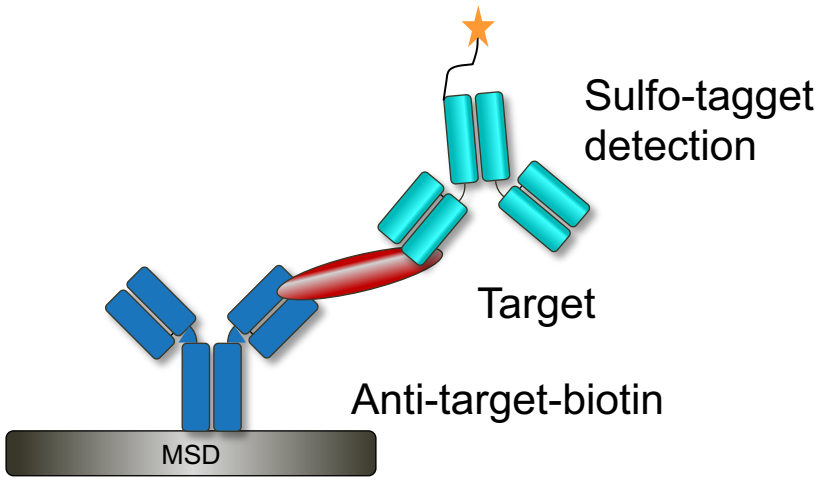
Dorte Kornerup Ditlevsen – September 2020

The project

- A monoclonal antibody targeting a protein in the brain
- Demonstration of target engagement at the site of interest (the brain) is a challenge
- As a proxy, demonstration of target engagement in plasma and cerebrospinal fluid (CSF) is desired
- mAb level in CSF is usually 0.1-0.4% of mAb level in plasma
- All bioanalytical work was outsourced

The non-clinical stage

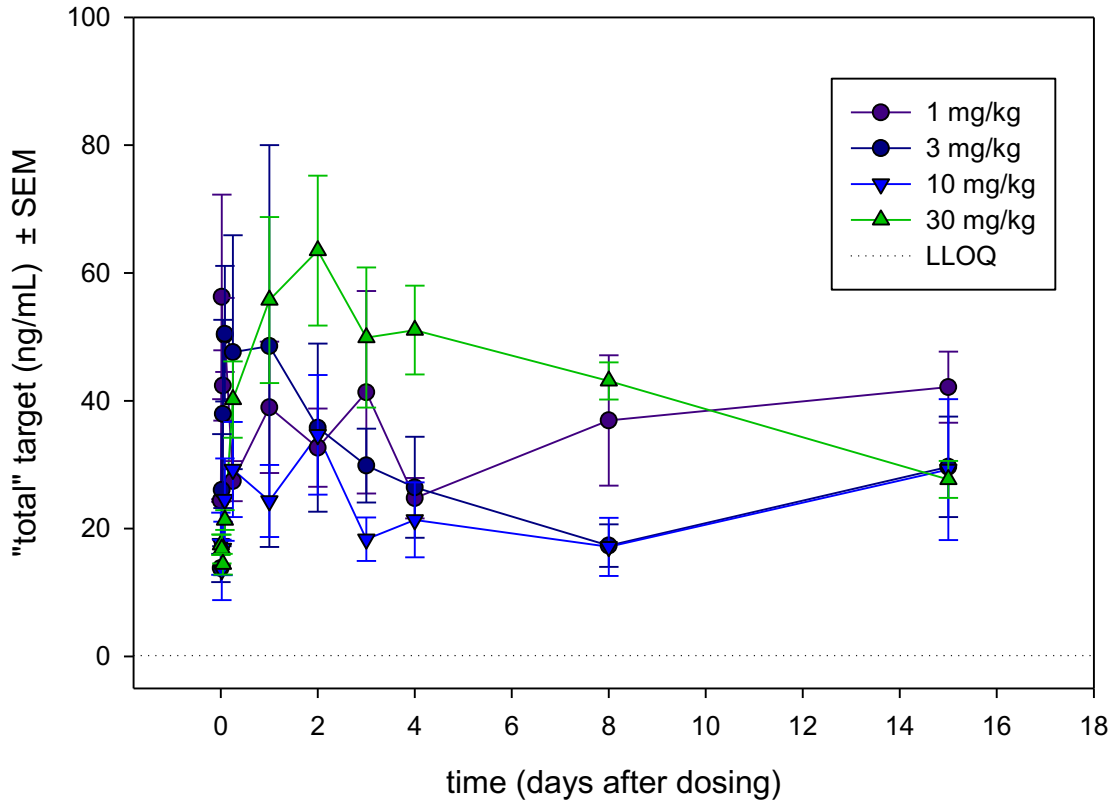
Started out with a commercially available MSD kit for quantification of the target protein to measure ”total” target



Kit developed for human target

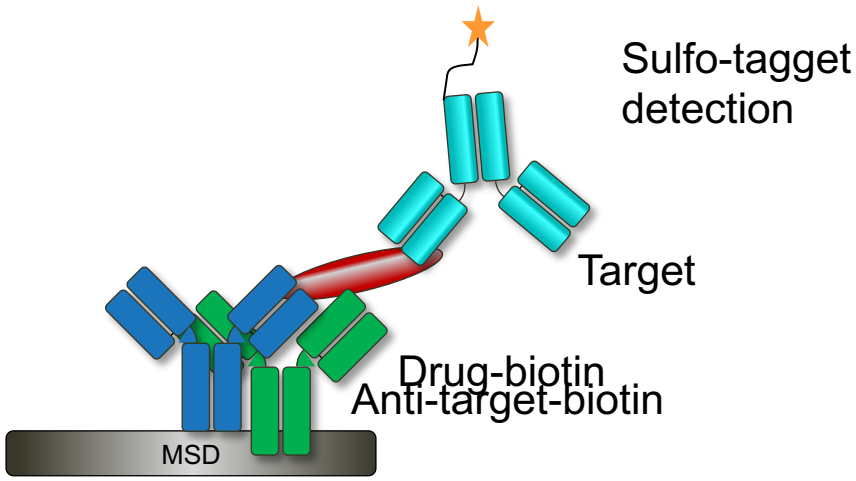
Detects monkey target but not rodent

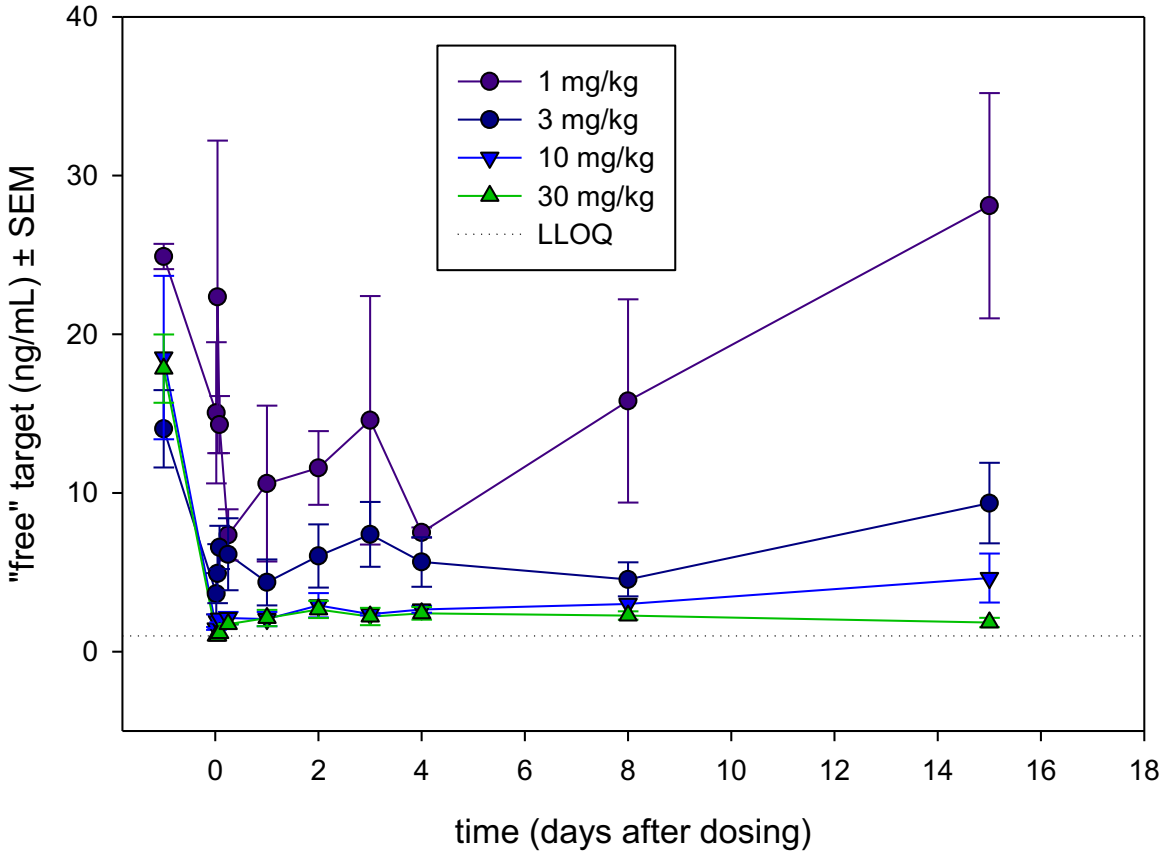
Drug interference overcome by acidification/heat inactivation



Tendency to higher levels of ”total” target in plasma from cynoomolgus monkeys dosed IV with 30 mg/kg mAb (PK/PD study)

Adjusted kit to measure ”free” target





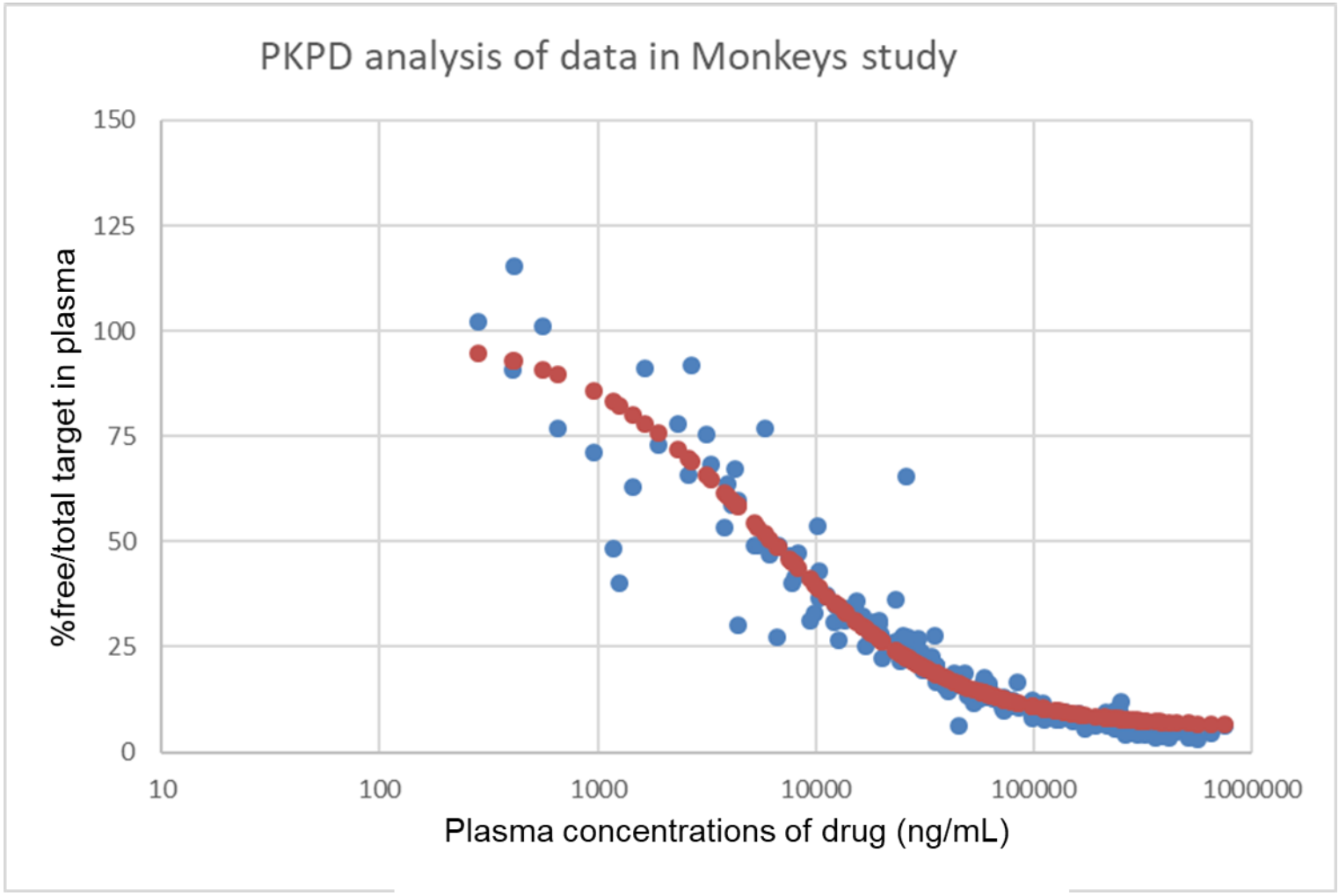
Dose-dependent decrease in ”free” target was seen in cynomolgus monkey plasma (PK/PD study)

The data were used to recommend human starting dose

Non-linear regression:

$I_{max} \approx 93.4\%$
$E_0 \approx 99.2\%$
$IC_{50} \approx 5.67$ ug/mL
≈ 5670 ng/mL
≈ 37.8 nmol/L

K_d as measured by SPR is 36 nM



Validation parameters for ”total” target assay in cynomolgus monkey

- Precision (5 individual blank samples + 1 sample with recombinant target spiked in buffer at LLOQ)
- Accuracy (1 sample with recombinant target spiked in buffer at LLOQ)
- Total error (1 sample with recombinant target spiked in buffer at LLOQ)
- Drug tolerance (unspiked and drug-spiked blank pool)
- Parallelism (1 blank sample)
- Stability (freeze-thaw; 2 individual blank samples)

- Acceptance criteria as per bioanalytical guidelines

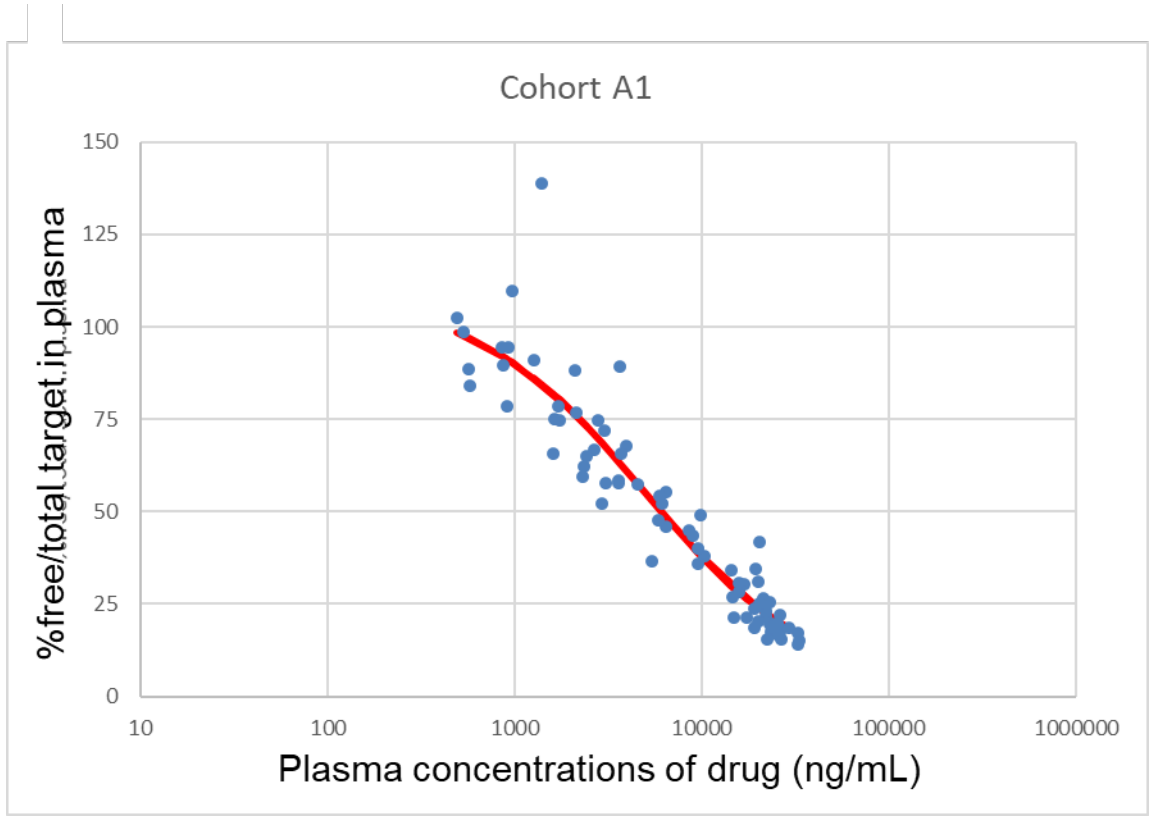
GLP claim for biomarker analyses using ”fit for purpose” validated methods?

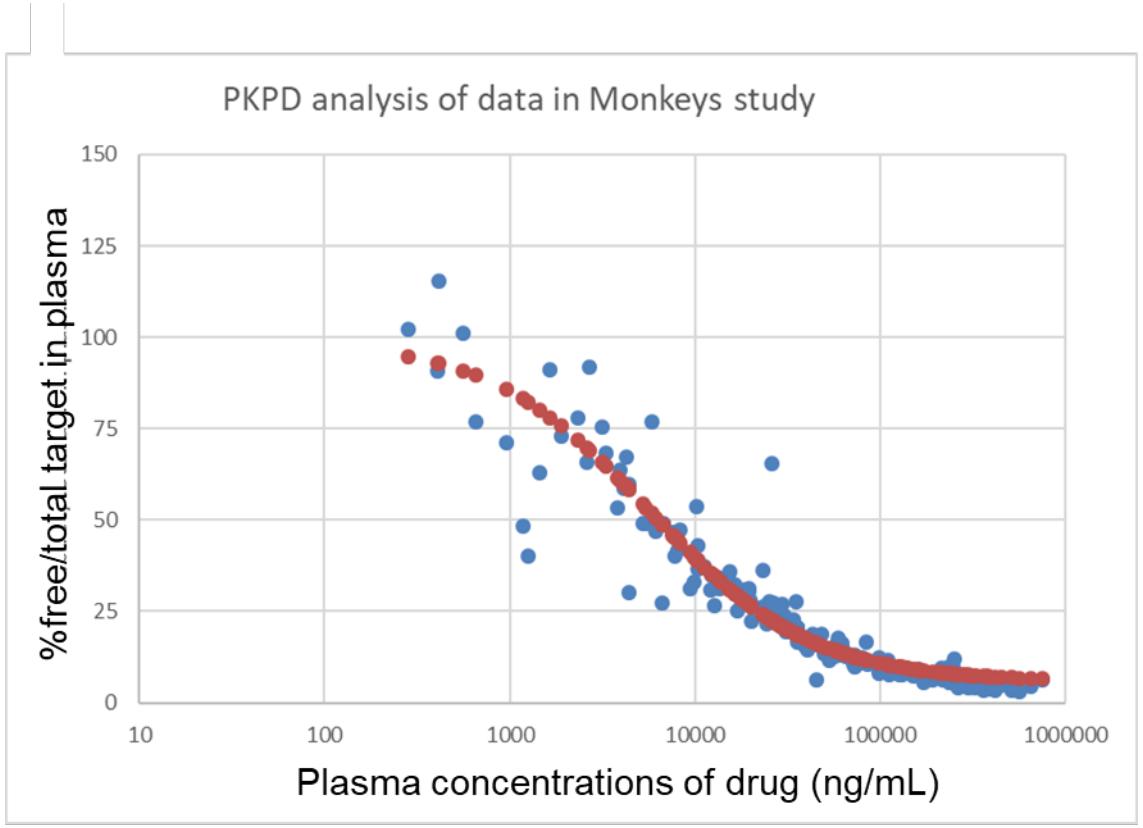
- Common misunderstanding: GLP cannot be claimed if the assay is not ”fully validated”
- ”fully validated” is often taken to mean validated according to EMA and FDA guidances on bioanalytical methods, so PK thinking
- There are many aspects of a nonclinical safety study which are not analysed according to bioanalytical guidelines. Hematology, formulation analysis etc. Still GLP is claimed

- Discussed intensively with CRO QA who ended up asking their local authorities….
- Local authorities confirmed that GLP can be claimed with a fit for purpose validation

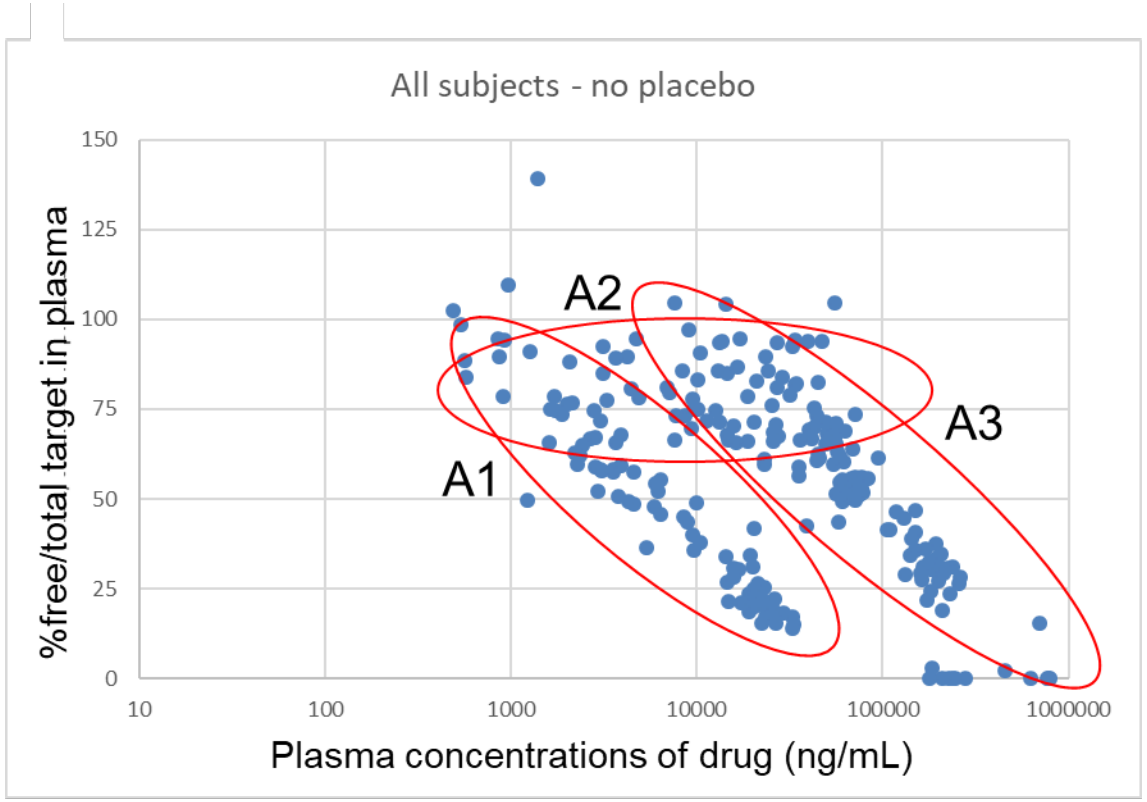
The clinical stage

Moving into clinic – first cohort data are as expected





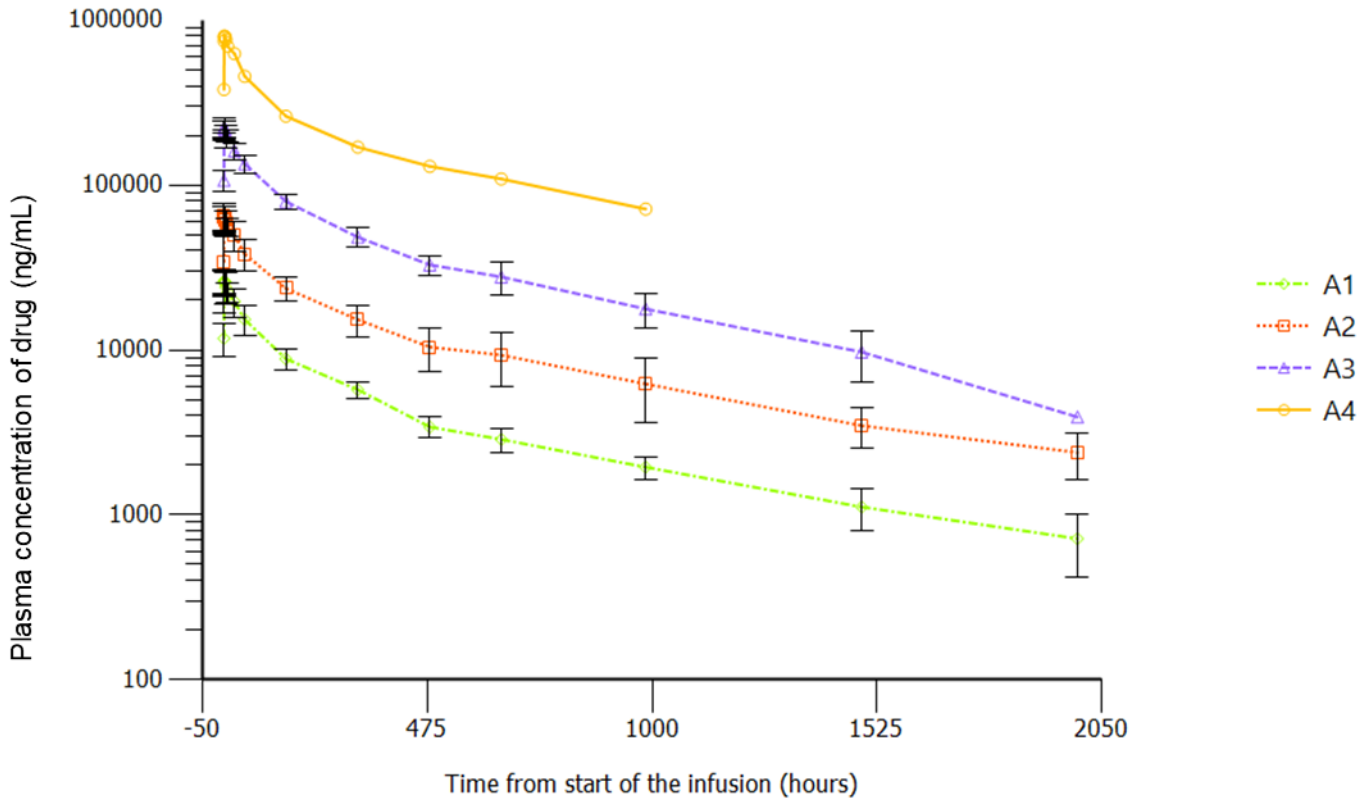
Moving into clinic – next cohort data look odd



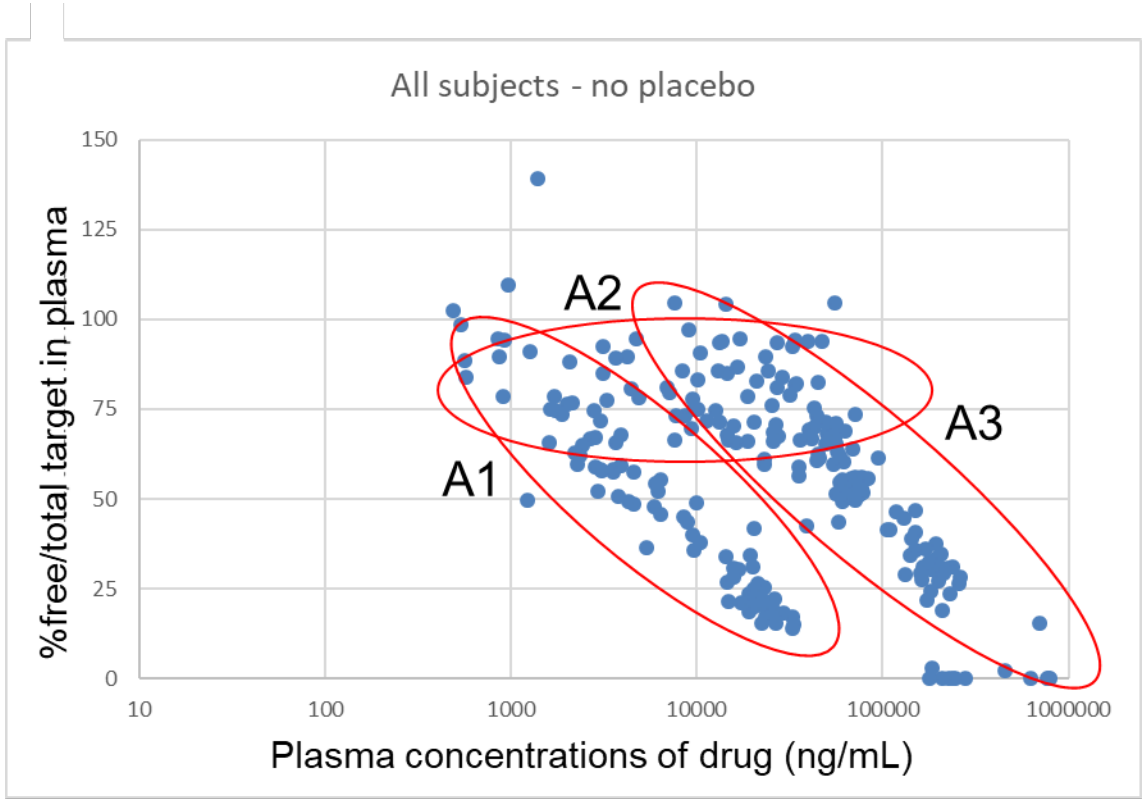
- A1 is as expected
- A2 is ”all over the place”
- A3 seems to be shifted to the right (much higher IC_{50})

- This does not make sense, so what happened?
  - Is it related to PK?
  - Is it related to ”free” target assessment
  - Is it related to ”total” target assessment

PK looks good – not the reason



A matter of dilution? (1/2)



Most of the cohort A1 samples were analysed undiluted

Almost all samples from cohort A3 were diluted 10-fold

Cohort A2 samples with high drug concentration were diluted 10-fold whereas samples with lower concentrations were analysed undiluted.

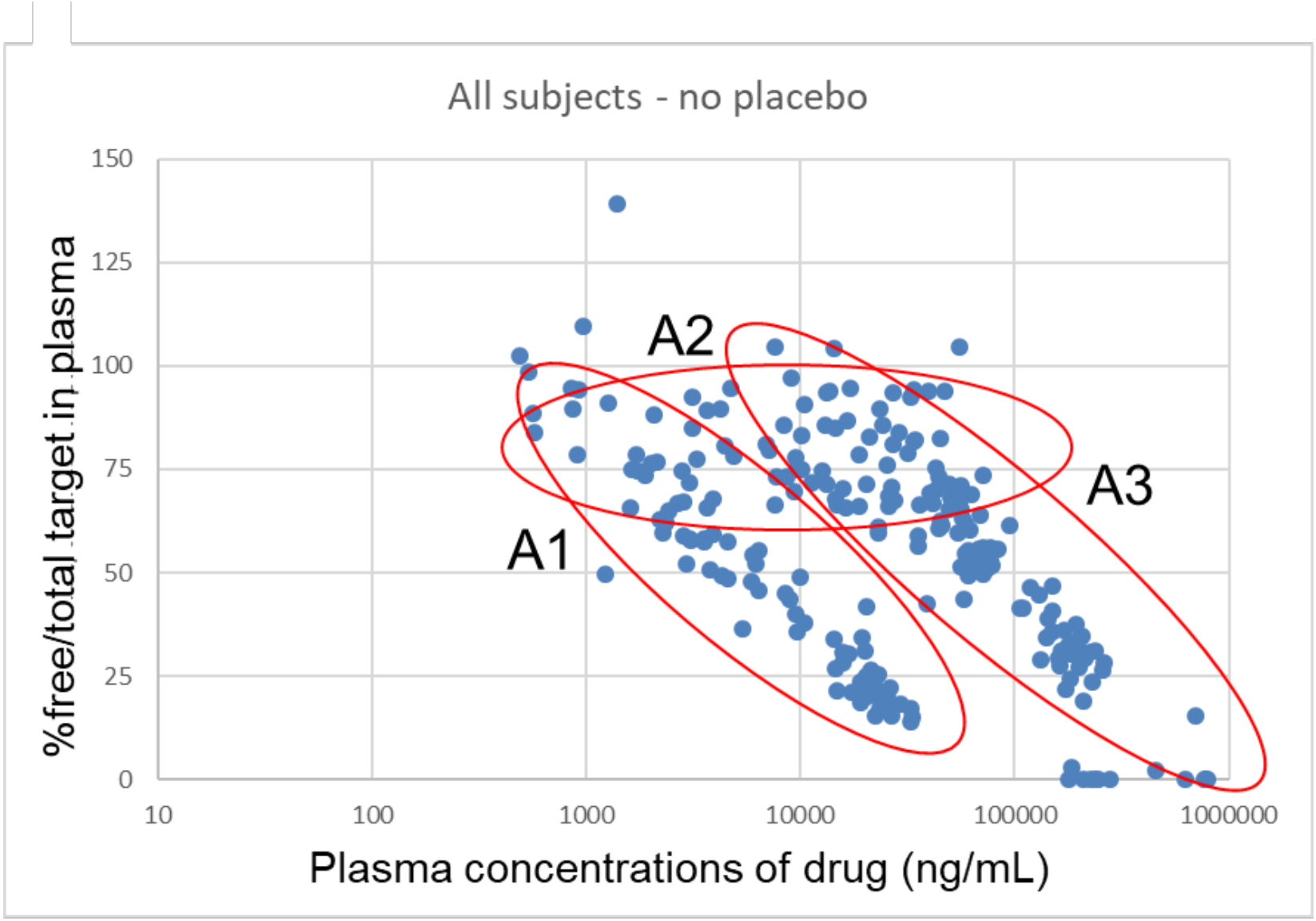
A matter of dilution? (2/2)

Parallelism assessments (”free”) in study subjects show
no parallelism when drug concentrations are high

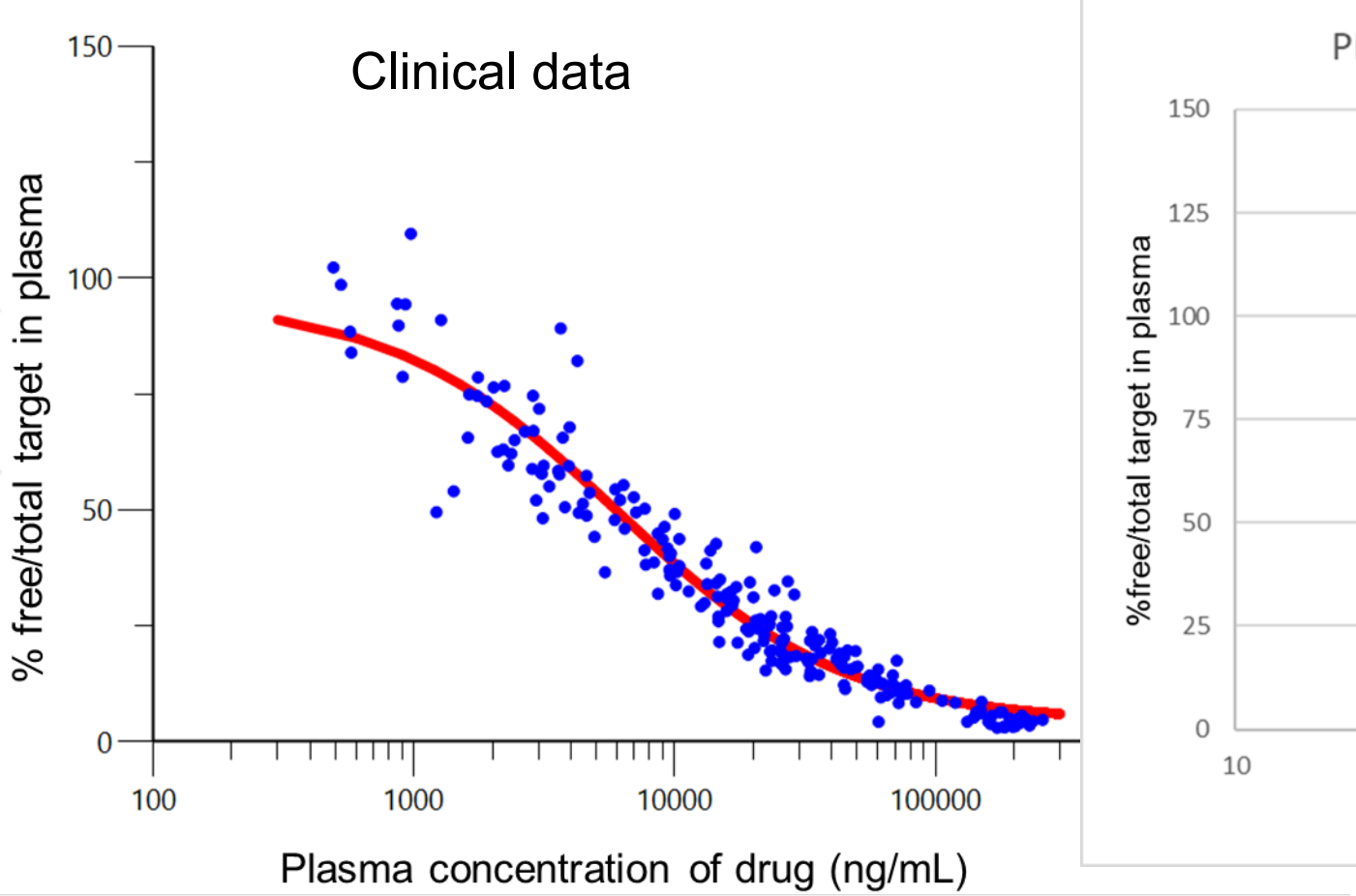
Dilution factor	A1 subject Meas. conc, ng/mL	Day 63 Back-calcul conc, ng/mL	Bias, %	Comment	A2 subject Meas. conc, ng/mL	Day 1 8h Back-calcul conc, ng/mL	Bias, %	Comment	A2 subject Meas. conc, ng/mL	Day 14 Back-calcul conc, ng/mL	Bias, %	Comment	A3 subject Meas. conc, ng/mL	Day 1 8h Back-calcul conc, ng/mL	Bias, %	Comment
1	52.4	52.4	reference	ULOQ	12.7	12.7	reference		18.6	18.6	reference		10.2	10.2	reference	
2	30.0	60.0	New ref		12.4	24.7	94.5%		14.8	29.5	58.4%		10.9	21.8	114.2%	
4	14.4	57.7	-3.9%		9.86	39.4	210.4%		10.6	42.4	127.2%		10.0	40.1	294.4%	
8	6.89	55.2	-8.1%		7.03	56.2	342.5%		6.20	49.6	166.0%		8.35	66.8	557.0%	
16	3.76	60.1	0.2%		4.49	71.9	465.6%		3.62	57.9	210.5%		6.63	106.2	943.6%	
32	1.67	53.5	-10.8%		2.57	82.2	547.3%		1.98	63.4	240.2%		4.46	142.8	1303.8%	
64	0.79	50.8	-15.4%	LLOQ	1.37	87.5	588.7%		1.02	65.4	250.9%		2.89	185.1	1719.3%	
128	0.25	32.2	-46.3%	LLOQ	0.63	81.2	538.8%	LLOQ	0.52	67.1	260.2%	LLOQ	1.66	212.9	1992.5%	
Overall mean		57.3				53.5				46.7				98.2		
Overall CV		5%				54%				38%				78%		
Drug conc, ng/ml				1390				25400				18800				182000

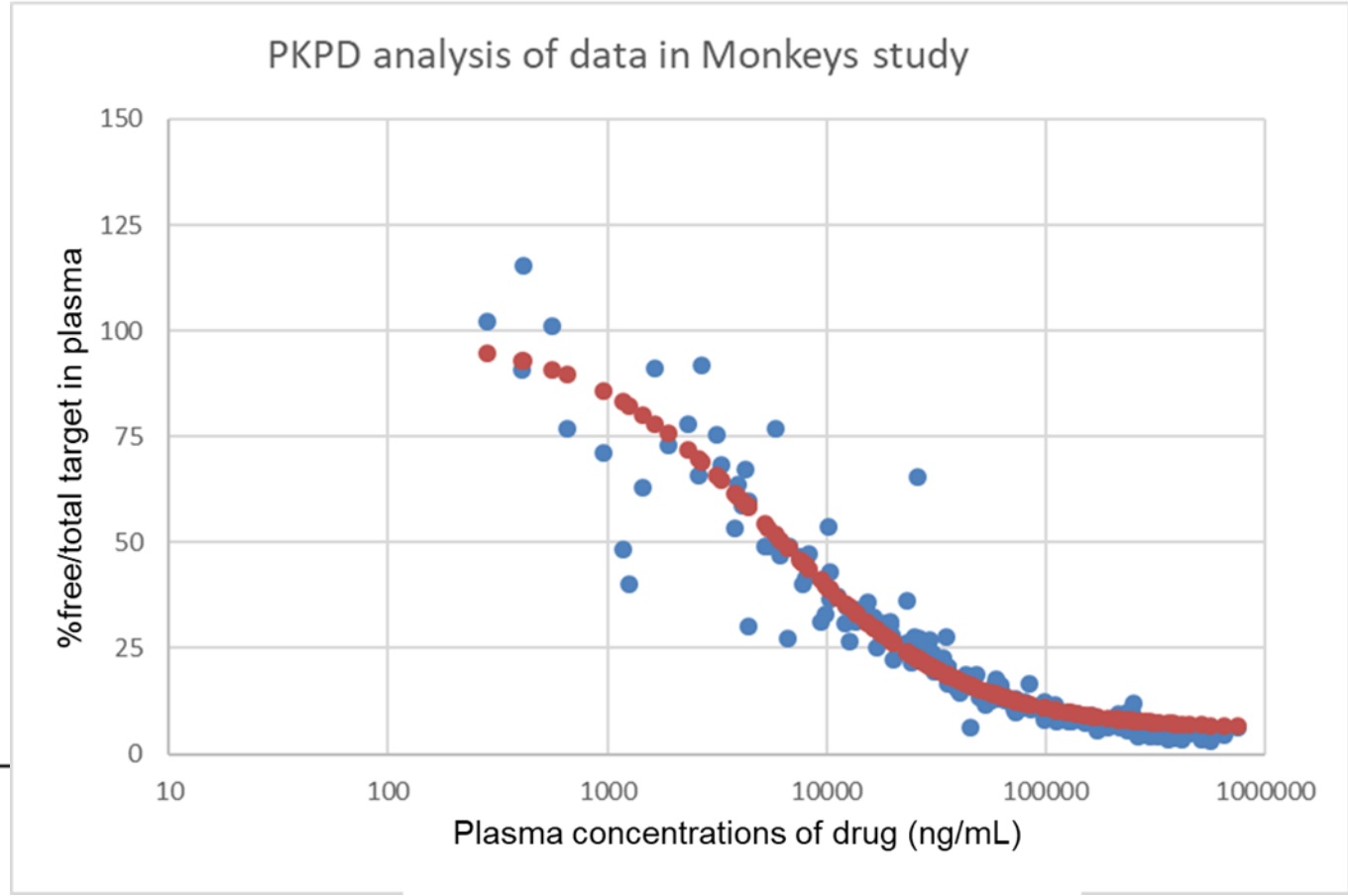
When drug concentrations are high: The more you dilute, the more you measure

Would that explain the data?



When all samples were analysed undiluted



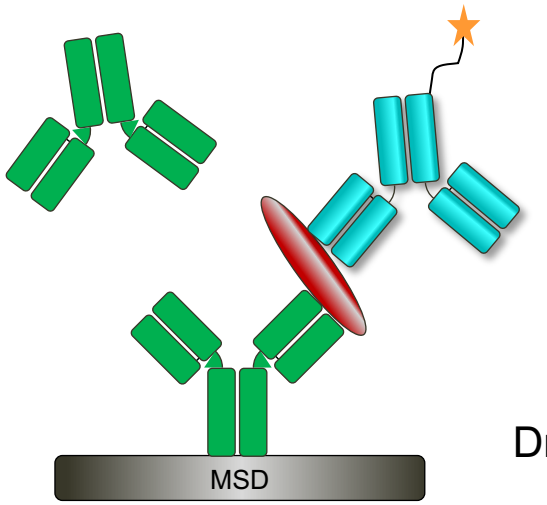


So why were the samples diluted in the first place?

- PK(classical quantitative bioanalysis) thinking: higher doses → dilute more
- Here it is opposite: drug on board → less ”free” target → no need to dilute
- Some samples were >ULOQ when analysed undiluted, but it was possible to extend the range to a 5-fold higher ULOQ meaning that all samples can now be measured undiluted
- **Learning: it is important that all involved personnel understand what is measured and are aware of the risk of falling back into PK (classical quantitative bioanalysis) thinking**

Context of use

- To assess **reduction in relative ”free” target levels** in plasma/CSF following dosing



Sulfo-tagget detection

Target

Drug-biotin

Competition between target binding to drug in solution and to drug coated on a plate will favour the drug coated on the plate

This explains the observed dilution bias

Important note to the end user:

”free” target levels are inherently overestimated in the assay meaning that **target engagement is underestimated**

Target engagement in CSF

- CSF is assessed as a proxy for interstitial fluid
- Demonstration of target engagement at the relatively low exposure levels in CSF is a challenge –especially considering the inherent overestimation of the ”free” target assay
- Therefore, a complex assay using anti-idiotypic tool antibodies specifically detecting the target-drug complex was developed.
- The anti-idiotypic antibodies appear to have a stabilising effect on the complex hence inherently overestimating target engagement
- So….the truth is somewhere in between

Take home messages

- PK assay ≠ biomarker assay
- Still, there are similarities and hence in many organisations, scientists with bioanalytical experience from PK (and immunogenicity) are expanding their role to include biomarker assessments
- This results in a need for a strong focus on the risk of slipping back into PK-thinking
- Focus on what is actually measured and the context of use

pay attention to the biology

Acknowledgements

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