EBF Spring Focus Workshop 2023

PK/PD assay strategy for a therapeutic peptide inhibitor:

Meaningful PD marker contra PK and free / total target analyte

Disclaimer:
Views and opinions expressed are those of the speaker and not necessarily Novo Nordisk

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Perspective on target assays and PD markers

For bioanalytical scientists there are three (3) critical questions:

- What are you being asked to measure and why?
- What are you measuring?
- How much cost, time and resource is relevant to invest?

Stakeholders	Free Target	Total Target
Internal stakeholdeers / KOL	Non-engaged / active Target	Target accumulation (competitor comparison)
Health Agencies (eg FDA/EMA)	Target ”levels”	

- **Free target assay challenges:**
  - No absolute measurement for LBA (equilibrium shift, sample dilution, and reagents used *in assay)*
  - The higher the K_D the less reliability of the free assay
  - The measurement of K_D may not be correct or representable (buffer/plasma/soluble)

If no meaningful value is generated from the free target assay => no knowledge building but increased cost and time to patient

Lee AAPS J. 2011); Staack Bioanalysis 2012; Staack Bioanalysis 2014; Mayer AAPS 2016; Zheng J of Clin Pharm 2015

KOL: Key Opinion Leaders. FDA: Food and Drug Administration. EMA: European Medicines Agency

PK/PD assay strategy for the peptide drug

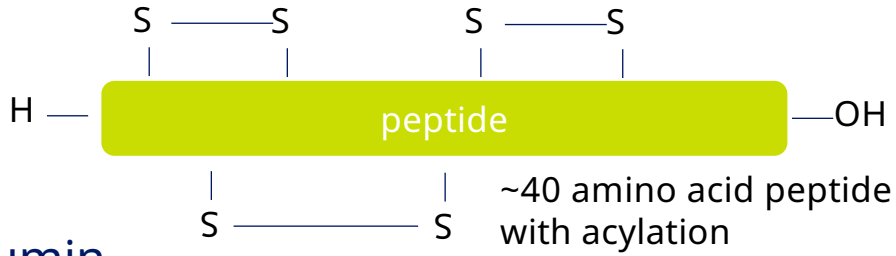
Past

Free/total assays for biotherapeutics has focused on monoclonal antibodies (mAbs) and similar modalities with low K_D

NN case

Peptide drug inhibits the enzymatic target

Acylated drug peptide for increased $t_{1/2}$ via binding to serum albumin



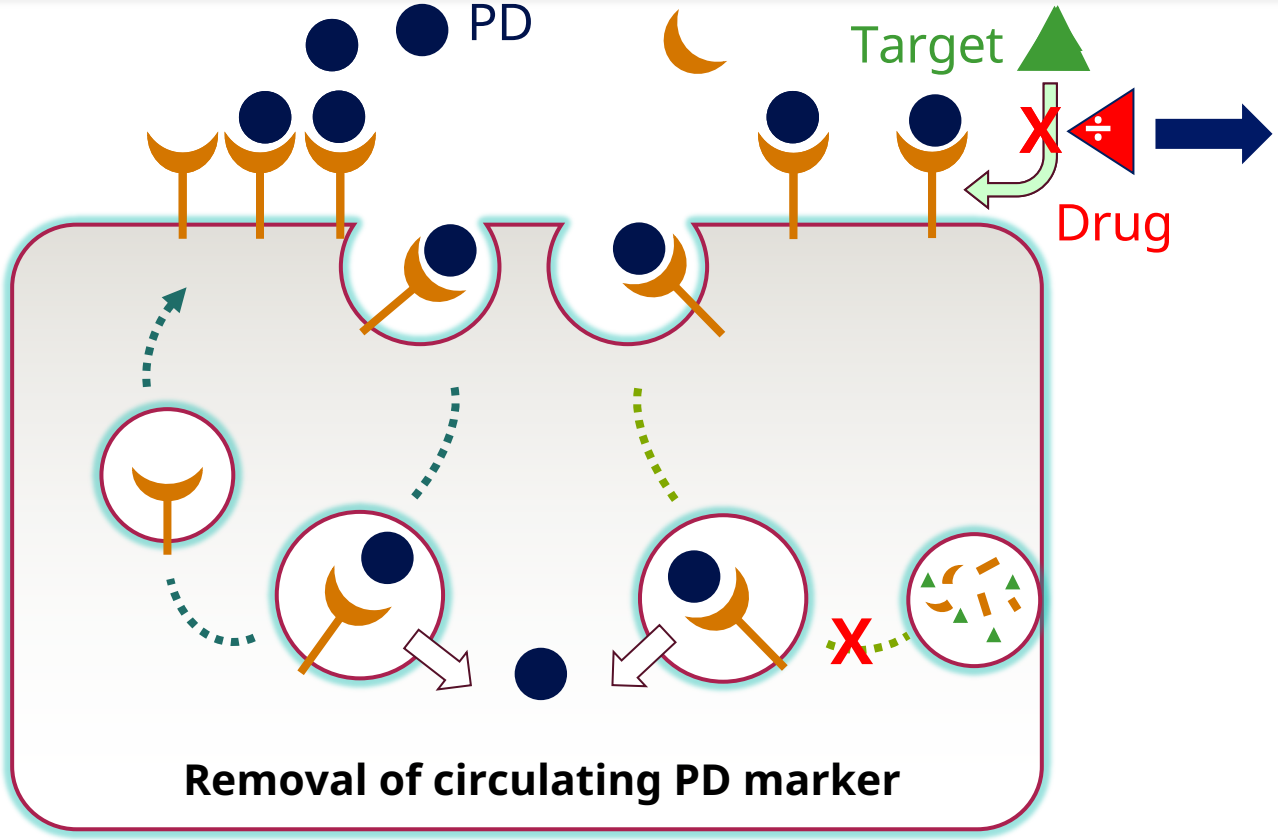
Original clinical assay strategy

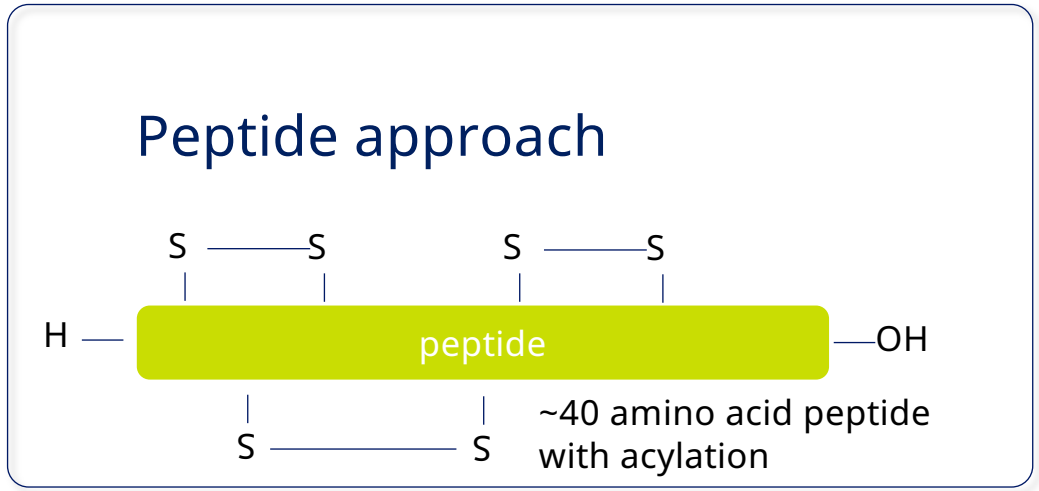
- Total PK assay (LC-MS/MS)
- Free and total target (LBA)
- Pharmacodynamic effect (PD) for a reliable response utilising diagnostic assays

Lee AAPS J. 2011); Staack Bioanalysis 2012; Staack Bioanalysis 2014; Mayer AAPS 2016; Zheng J of Clin Pharm 2015

LBA: Ligand Binding Assay. LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry PD: Pharmacodynamic

Inhibition of target receptor interaction





Drug administration

Phase 1: Subcutaneous

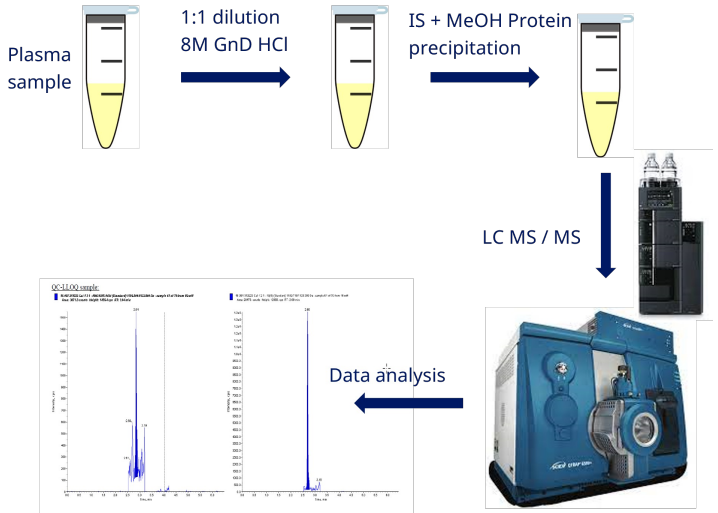
Phase 2: Oral

PD marker is measured with a diagnostic assay

PD: Pharmacodynamic

Assay formats – peptide drug

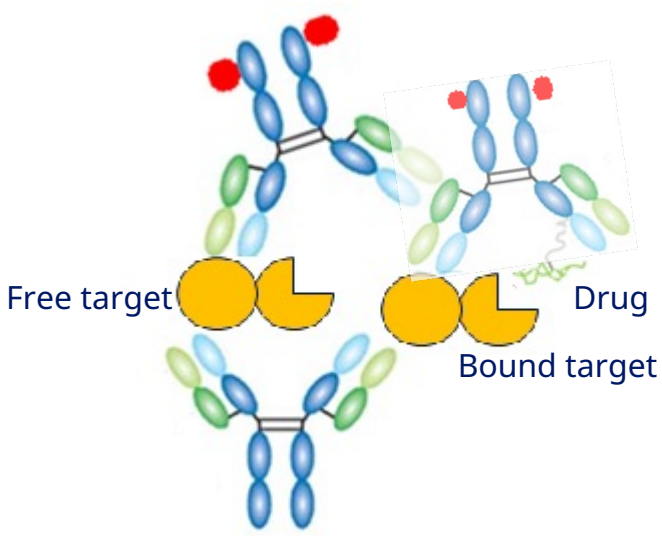
Total PK assay LC-MS/MS



No digestion

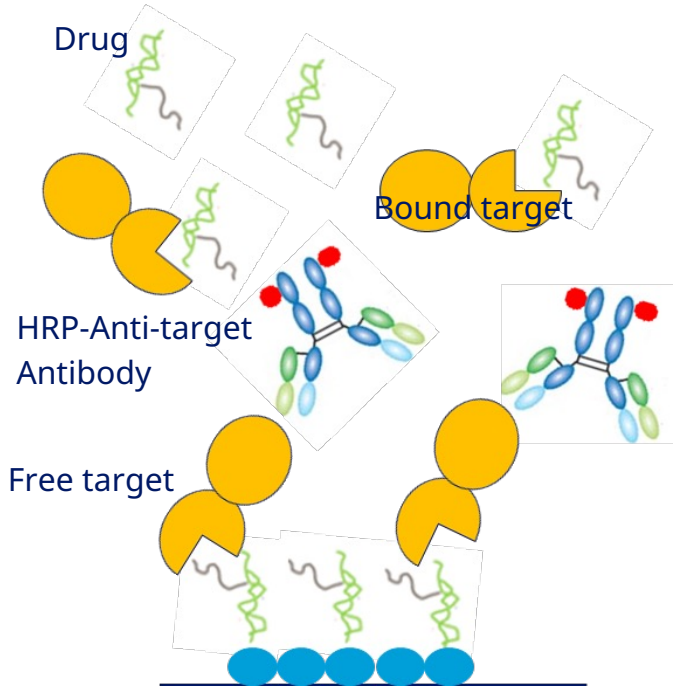
Total Target assay MSD

NN SulfoTag-Anti-Target antibody



NN Anti-Target antibody

Free Target assay ELISA



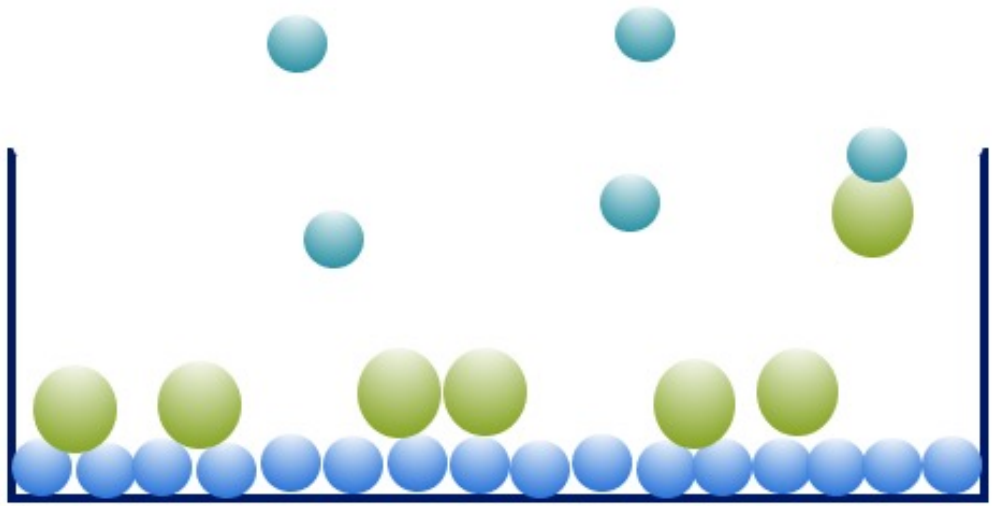
Biotin labelled drug on streptavidin coat

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry PK: Pharmacokinetics. MSD: Mesoscale Discovery. ELISA: Enzyme linked immunoassay. GnD HCl: Guanidine hydrochloride. MeOH: Methanol. IS: Internal Standard

Free assay - considerations

Target (free) + Drug (free) <=> Target-Drug (complex)

(K_D=1 nM)



Free Target Assay

Free assay dependencies

Equilibrium - shift during assay incubation

- Plasma binders of drug
- Plasma binders of target
- Sample dilution
- Assay composition

Target

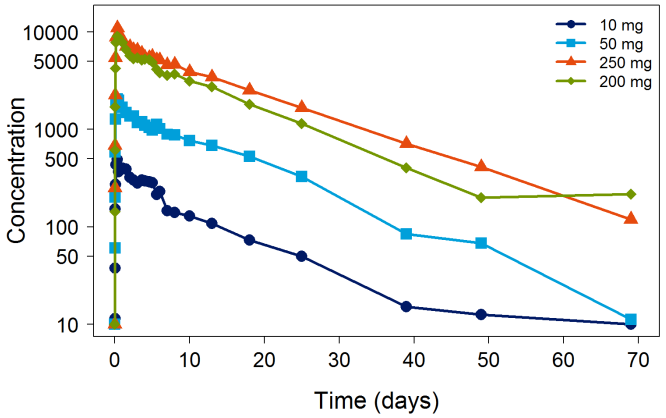
Drug

Phase 1 results – PK, free/total target and PD

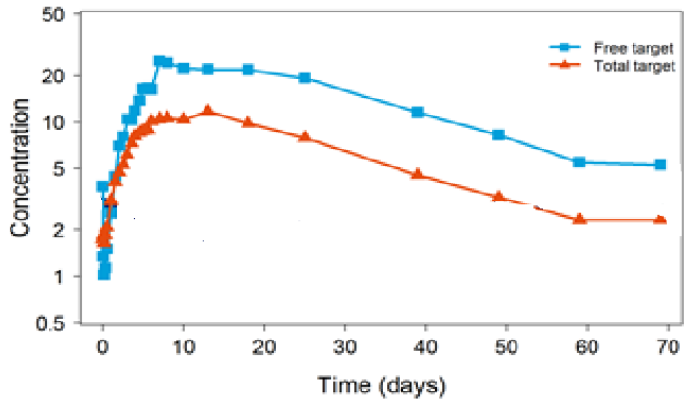
Issue statement:

- Free target increase *above* baseline in contradiction to the clear response on PD
- Free target concentration above total target

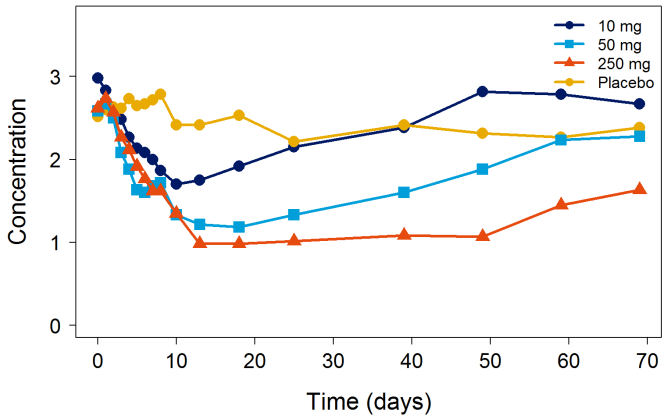
PK



Total and free target
Group 1: 10 mg dose



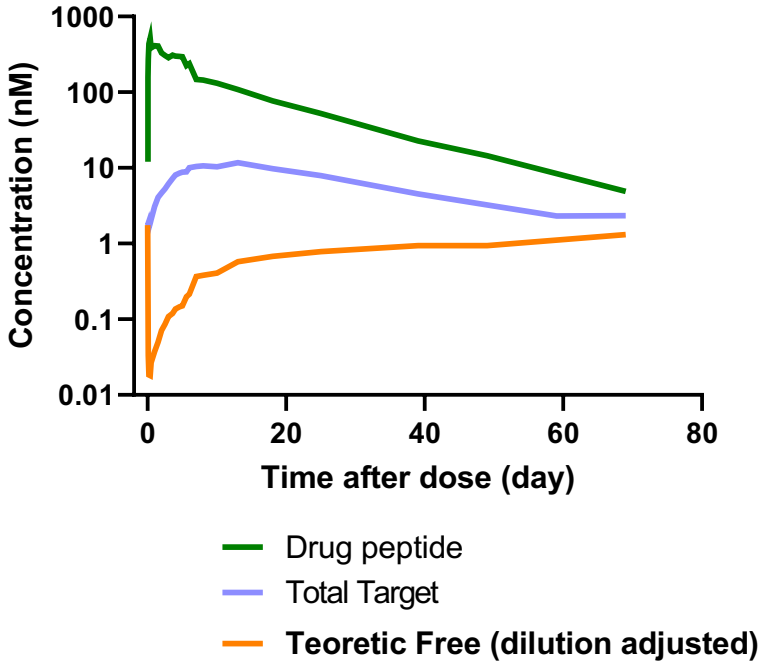
PD



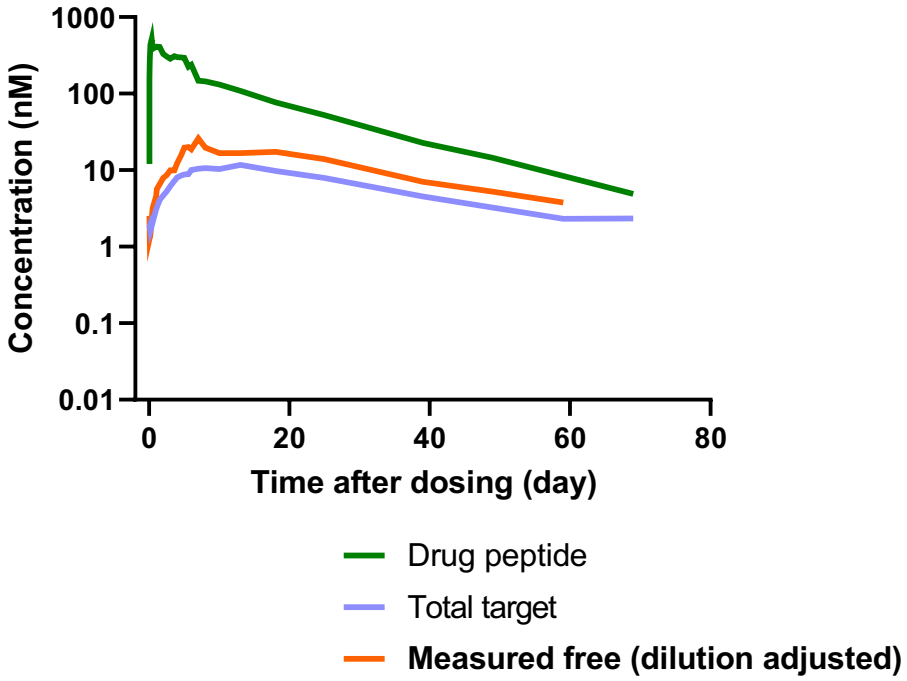
Expectations and outcome from Phase 1

Group 1: 10 mg dose

Theoretic calculations



Outcome



Back to the bench: Re-evaluation of the free target assay

- **Free target ELISA assay was adjusted**
  - Incubation time was shortened
  - Same dilution for all samples
  - Dynamic range adjusted to the expected range (affecting ULOQ)
  - Assay performance was evaluated and found acceptable in the presence of drug

- Gyrolab and Alpha-LISA was evaluated with the same reagents and showed similar performance as ELISA

What are you measuring?

(answered by assay development and validation)

Target (free) + Drug (free) <=> Target - Drug complex

K_D

$$\frac{[\text{Free Target}] * [\text{Free Drug}]}{[\text{Complex Target-drug}]} = K_D = 1 \text{ nM}$$

	Target (nM)	Drug peptide (nM)	Theoretic expected Target (nM)	Measured Target Final (nM)	Overestimation
QC high	7	25	0.36	**5.54**	15
QC medium	3	25	0.13	**2.31**	18
QC low	2	500	0.004	**0.35**	88

Dilution accounts for approx. 5 times overestimation

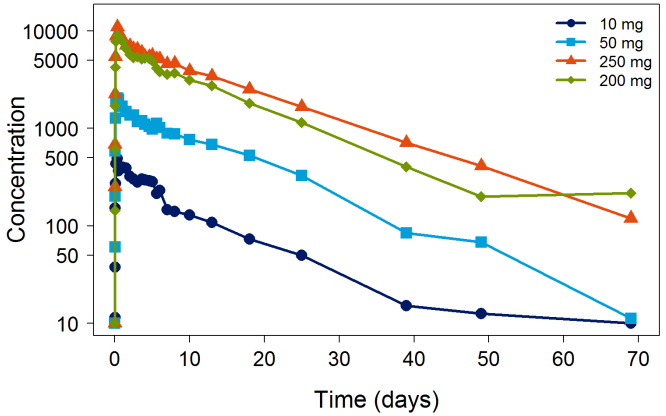
Plasma binders and assay reagents accounts for 3-18 times non-linear overestimation

Phase 1: Re-analysis with optimised free assay

Issue statement:

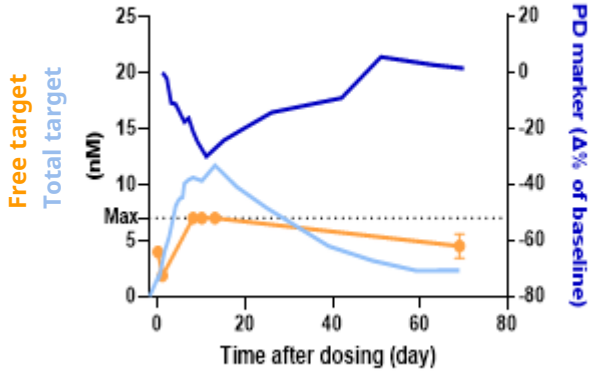
- Free target increase above baseline in contradiction to the clear response on PD
- Free target reaches ULOQ in fixed dilutions - not possible to evaluate if similar result

PK

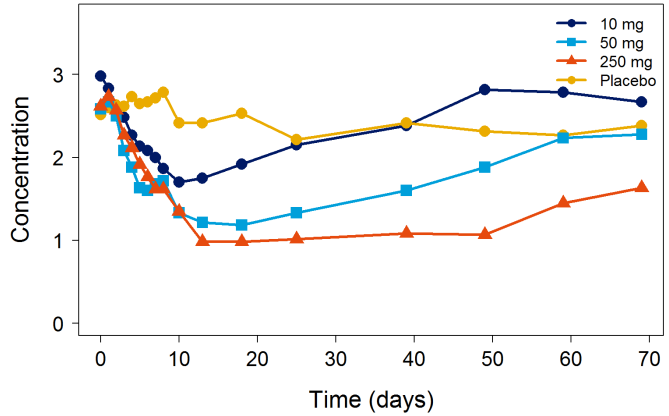


Total and free target

Group 1: 10 mg dose



PD



ULOQ: Upper limit of quantification.

Conclusion on the free target assay

Data showed the same tendency after optimization as in the initial testing.

Free target increase above baseline in contradiction to the clear response on PD.

K_D between target and drug alone cannot be used to calculate theoretic target.

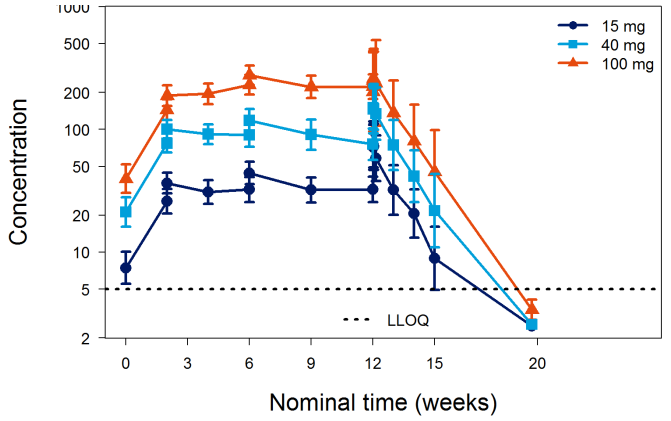
Unpredictable free drug measured.

Free target assay was excluded from phase 2.

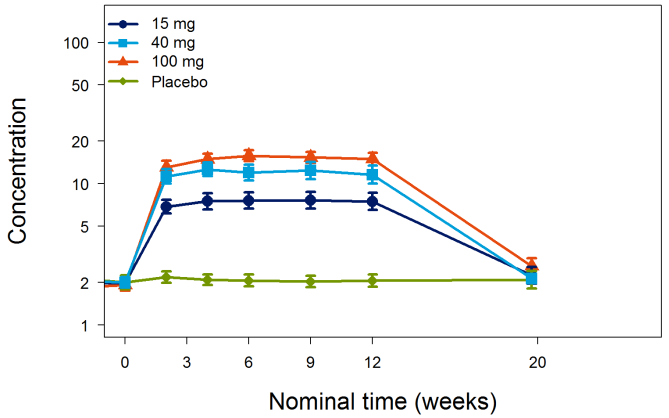
Conclusion from Phase 2 oral administration

- Total target assay showed target accumulation in line with competitors MoA.
- Reliable total PK assay and diagnostic PD marker supported MoA.

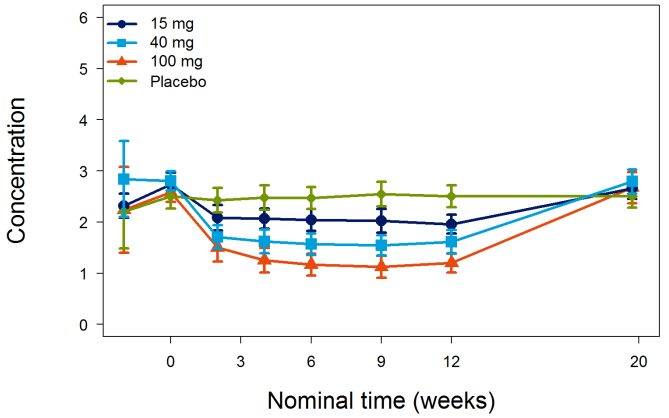
PK



Total target biomarker



PD marker



MoA: Mechanism of Action

Future Perspectives

For bioanalytical scientists there are three (3) critical questions:

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Target free / total assay challenges for peptide drugs:

- Historical data primarily based on MAbs (one soluble target with low K_D).
- A peptide drug target engagement with several plasma binders is different from previous experience of MAb therapeutics.
- Shared free assay challenges are needed to educate stakeholders.

Consider relevance of target assay(s) in phase 3 when a reliable PK assay and diagnostic PD marker is available.

Lee AAPS J. 2011); Staack Bioanalysis 2012; Staack Bioanalysis 2014; Mayer AAPS 2016; Zheng J of Clin Pharm 2015

MAbs: monoclonal Antibodies.

Acknowledgements

At Novo Nordisk A/S

- Mette Loftager
- Anna Laurén
- Charlotta D. de la Cour
- Christian Hollensen
- Line Hollesen Schefe
- Klara Tølbøl Lauritsen
- Irene Hedelund Langbakke

Thanks to our CRO’s for good collaboration