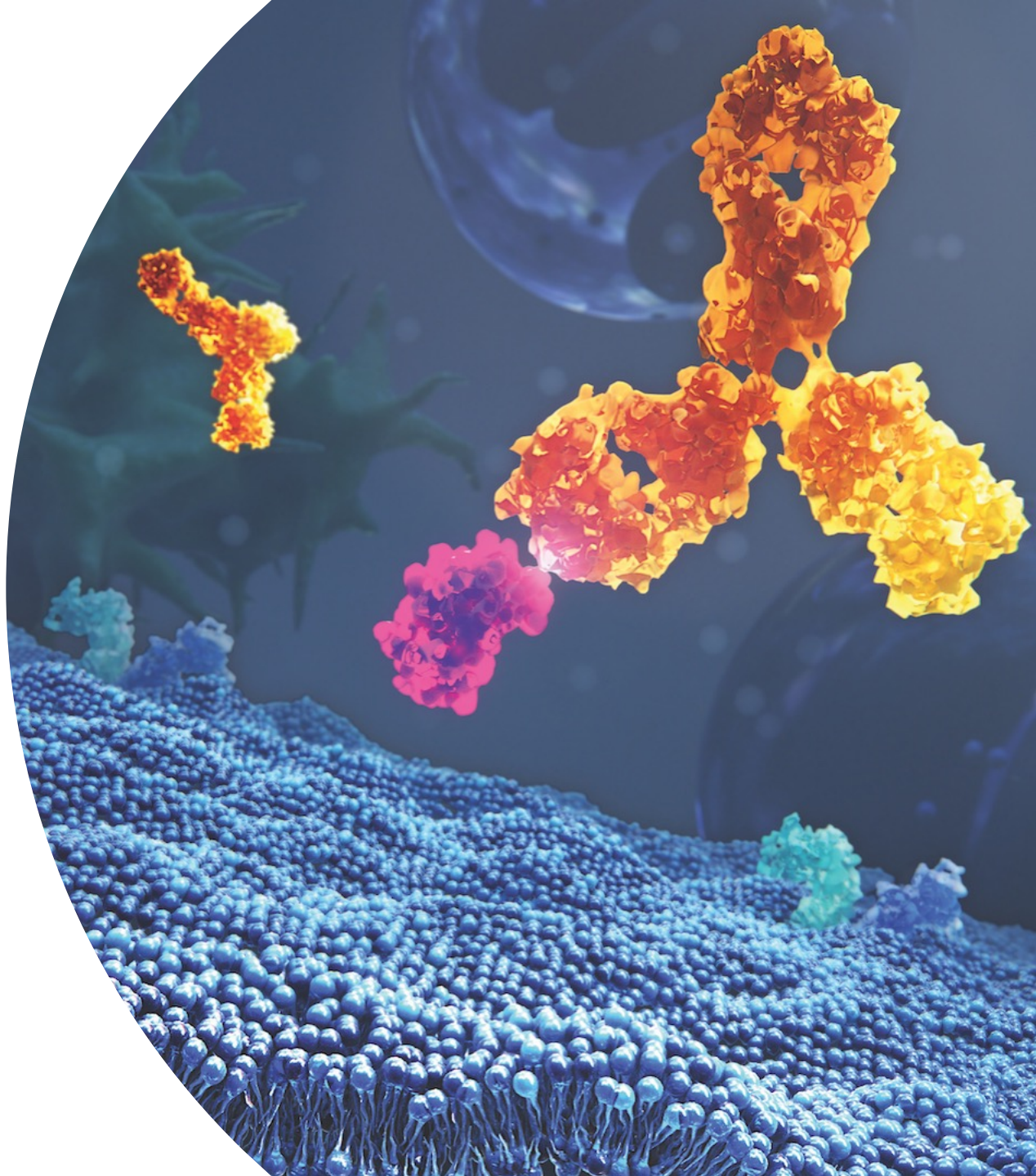
Immunogenicity assessment of incretin mimetics; a review of recently approved molecules with a view to technical, regulatory and strategic considerations

Chris Jones

Integrated Bioanalysis, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, Cambridge, UK

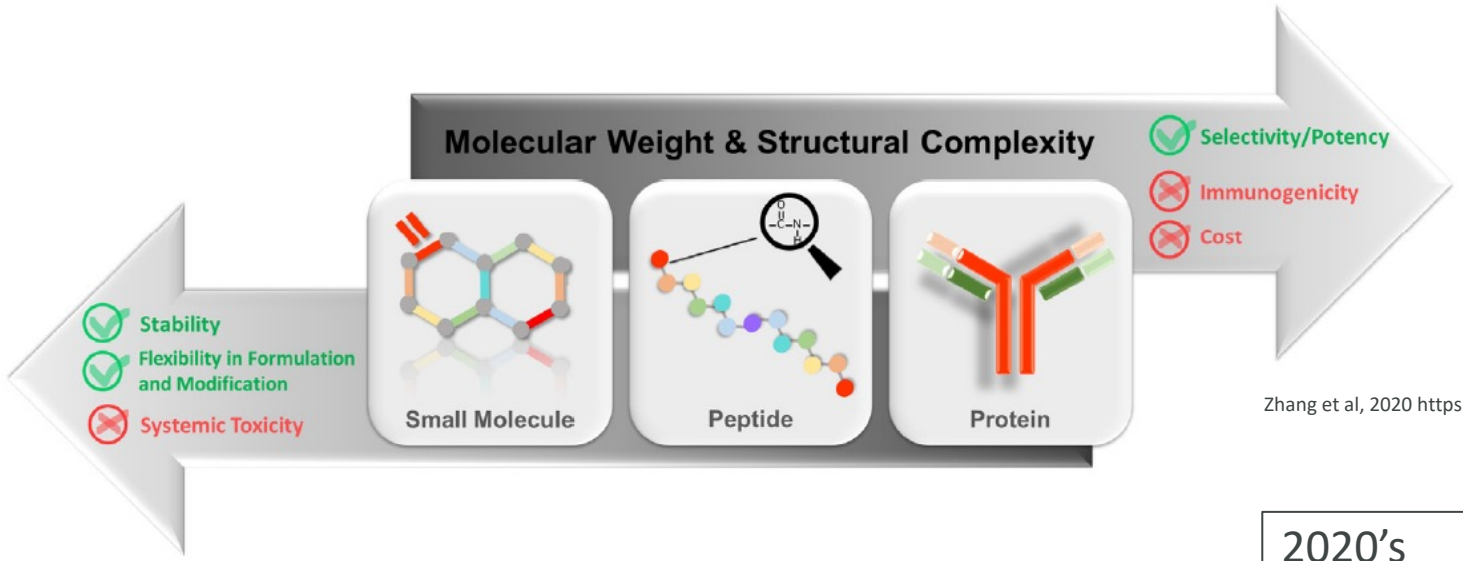


08 June 2023

Agenda

1. Introduction
2. Overview - the incretins and incretin mimetics
3. Lixisenatide, semaglutide and tirzepatide
4. Considerations and conclusions

Peptides occupy a niche between small and large molecules and have played a notable role as therapeutics for 100 years



Zhang et al, 2020 https://doi.org/10.1016/j.ijpharm.2020.11949

1923

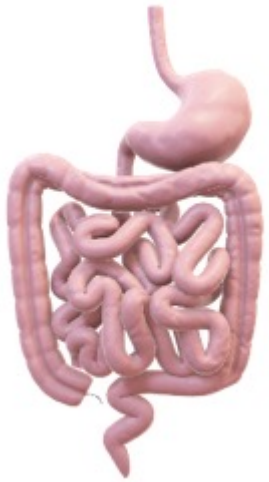
- Insulin becomes first commercial peptide therapeutic

2020's

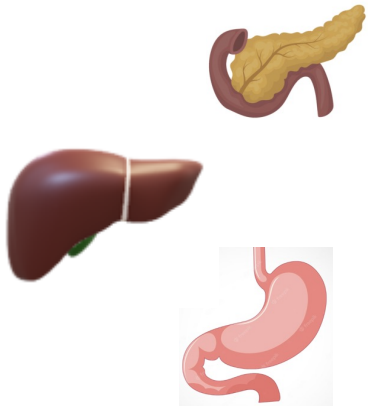
- Multi-billion dollar market for peptide therapeutics
- >70 approved peptides
- Novo Nordisk reported sales of $3.87 billion for semaglutide and liraglutide in 2022*

*https://endpts.com/novo-nordisk-notches-big-glp-1-sales-amid-rebounding-supplies-but-cautions-on-future-periodic-constraints/

Incretin hormones exert a glucose dependent insulinotropic effect and the incretin effect is a key component of glycaemic control

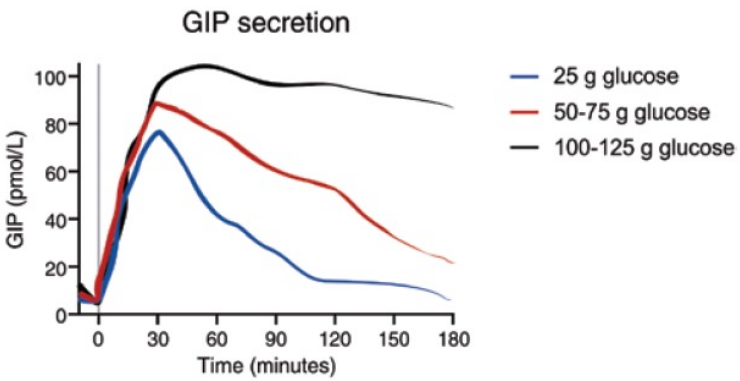


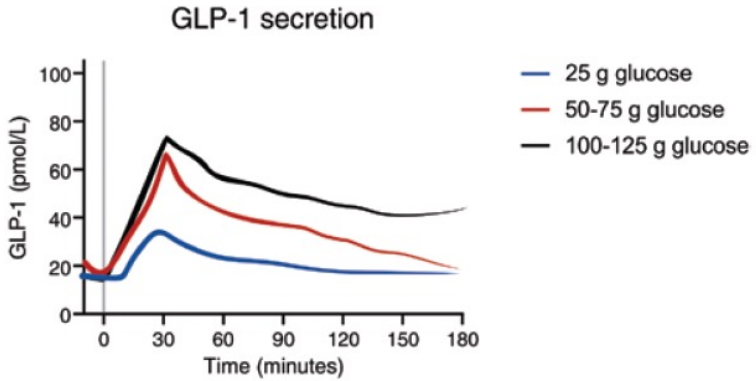
GIP and GLP-1 secretion in small intestine

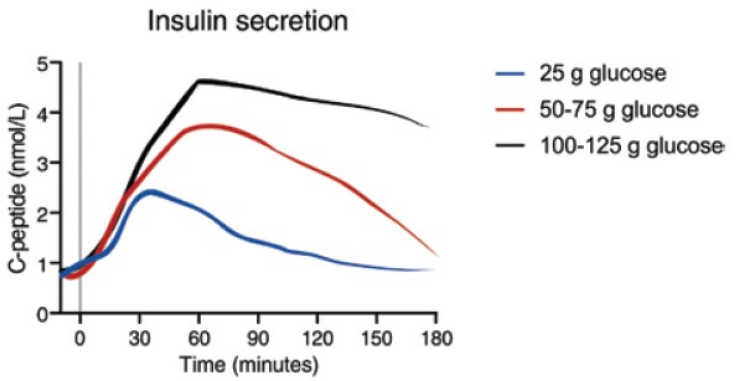


↑ Insulin production in pancreas

↓ Pancreatic glucagon
↓ Hepatic glucose output
↓ Gastric emptying

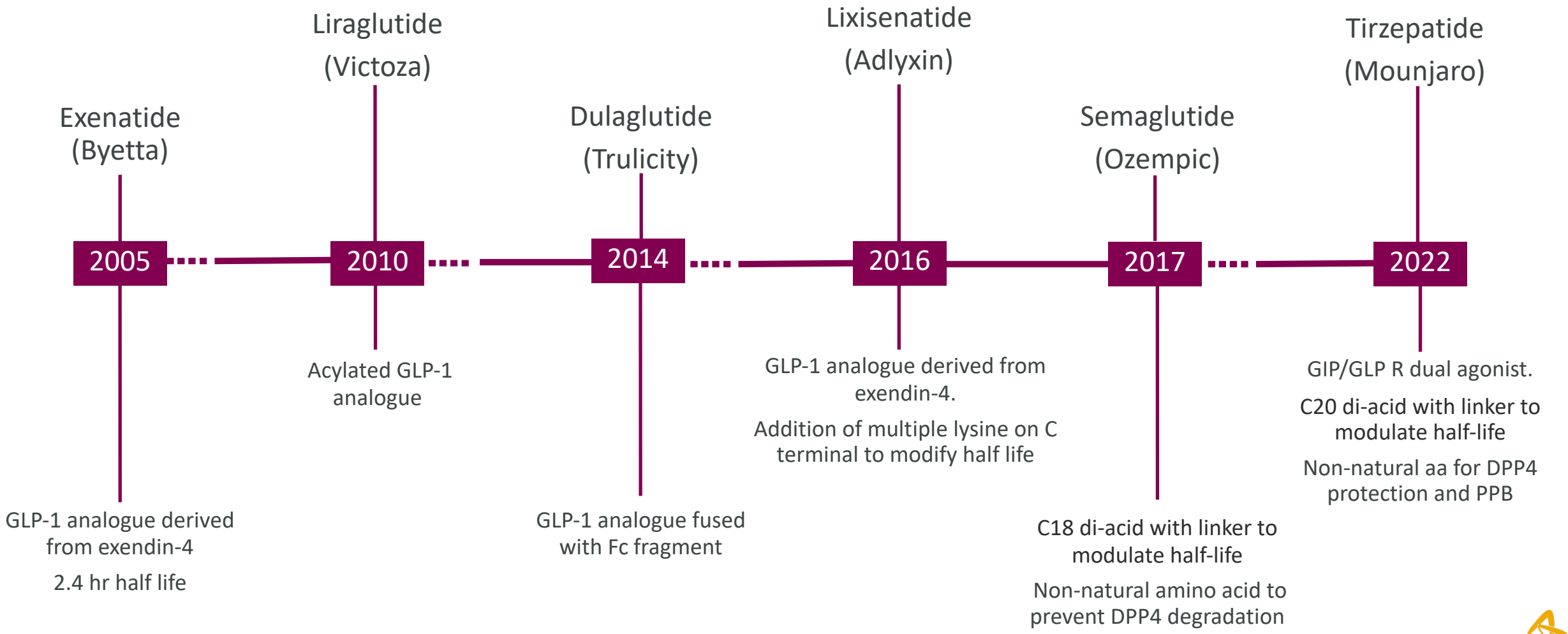






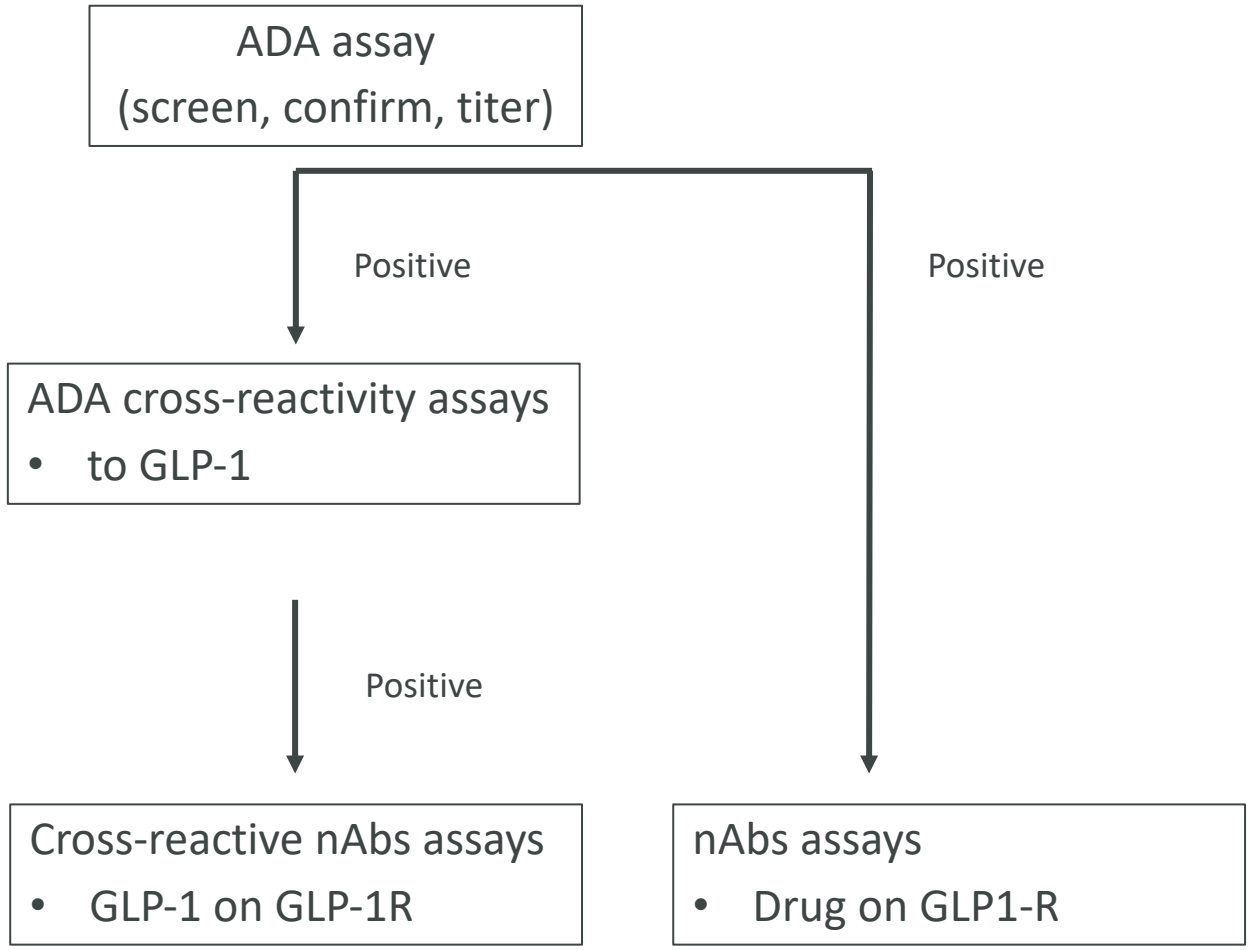
Holst *et al, Endocrinology*, 2021, Vol. 162, No. 7

Six incretin mimetics have gained regulatory approval for glycaemic control in T2DM patients



Immunogenicity risk assessment identifies incretin mimetics as higher risk molecules

Key risk: sequence homology with native peptides



Operational considerations

- Additional characterisation of ADA response required
  - Binding assays and neutralisation assays to assess cross-reactivity between ADA and native peptide
- Increased burden of testing in clinic
  - Extra blood volume
  - Associated cost
- Health agencies may request ADA follow-up required at end of treatment
- Retention of back-up samples advised

Review of approved molecules highlights differing ADA incidence but arguably limited ADA impact

GLP-1 RA	Ozempic®	Victoza®	Trulicity®	Byetta®	Adlyxin® Lyxumia®	Mounjaro®
Active drug	Semaglutide	Liraglutide	Dulagutide	Exenatide	Lixisenatide	Tirzepatide
Homology to human GLP-1	**94%**	**97%**	**90%**	**53%**	**<53%**	**<53%**
Level of ADA in Phase 3	**1-2%** (low titres)	**8.6%** (low titres)	**1.6%** (low titres)	**38% (low titres)** 6% (high titres)	**70%**	**51%**
Level of cross-reactivity to GLP-1	0.6%	4.8-6.9%	0.9%	None	28.4% (GLP-1) 4.7% (glucagon)	34% (GIP) 14% (GLP-1)
Level of in-vitro neutralising ADA	0%	1.0-2.3%	0.9%	-	-	2% nAbs to tirzepatide 0.4% GIP 0.9% GLP-1
Impact on efficacy	None	None	None	Half of those with highest titre had no glycaemic response	2.4% had attenuated or no glycaemic response	None
Impact on safety	None	None	-	Injection site reactions	Mild ISR and allergic reactions	None

- : Information not available ISR = injection site reactions

Adapted from 215256Orig1s000

Lixisenatide was submitted in 2016 and received multiple questions from FDA

- Sequence based upon exendin-4
- 53% sequence homology to GLP-1
- 70% incidence ADA

- Early bioanalysis performed by RIA
- Phase 3 bioanalysis performed by Biacore
- Data submitted from nine Phase 3 studies

- Safety and efficacy:
  - Impact on PK noted with sustaining effect up to 10-fold higher
  - Sanofi performed meta-analysis of studies to define that an ADA concentration of >100 nmol/L impacted HbA1c
  - Some increased hypersensitivity and mild ISR observed

- Bioanalytical comments
  - No detail on positive control antibodies
  - Limited cross-reactivity assessment – no validation of cross-reactivity assays
  - No nAbs assessment conducted
  - 78% of subjects were ADA negative or BLQ on Biacore assay
  - FDA requested titre data but the Biacore did not generate this readout

Source: 208471Orig1s000

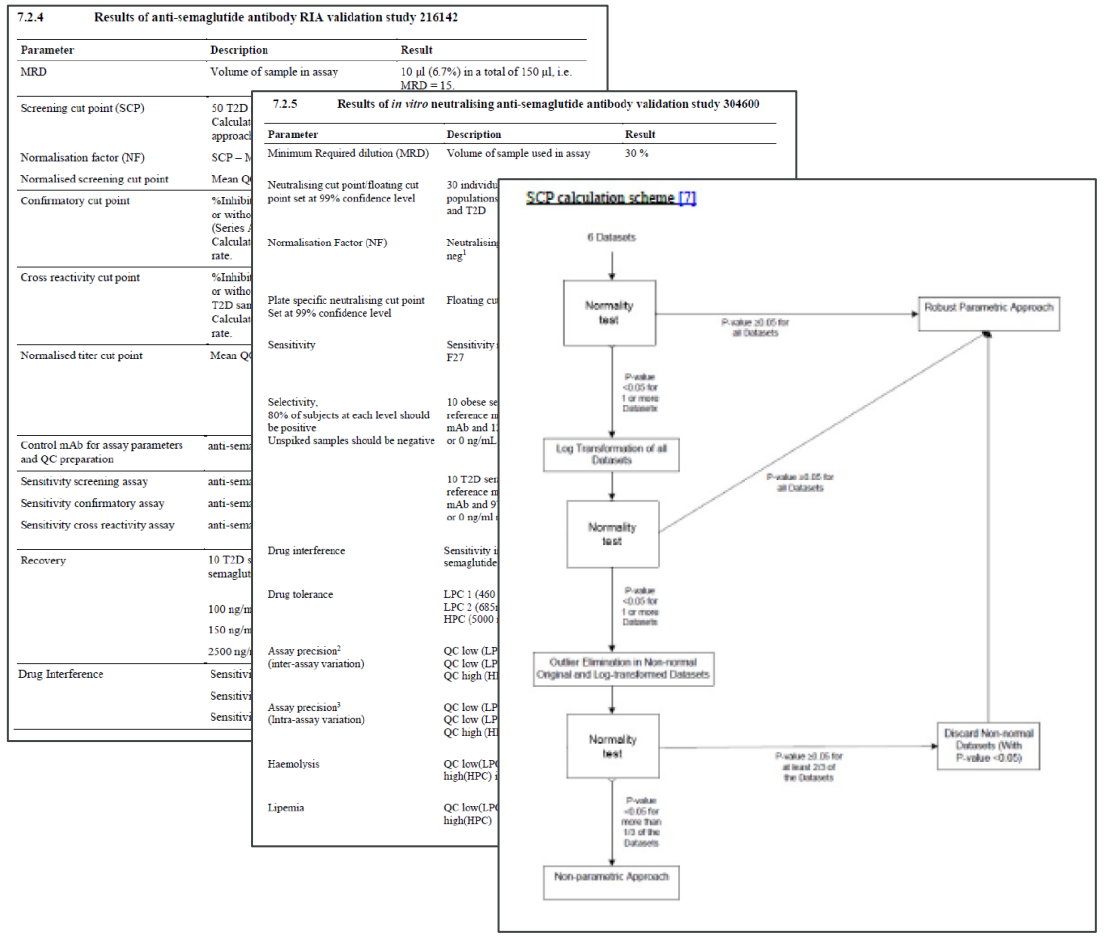
Semaglutide submission reflected evolving expectations from the agency

- 94% sequence homology to GLP-1
- 1 to 2% incidence ADA

- Phase 3 binding assay performed by RIA with Glycine/HCL and PEG precipitation
- Data submitted from nine Phase 3 studies

- Efficacy:
  - No impact on PK, PD or safety observed

- Bioanalytical comments
  - Significantly more detailed than lixisenatide
  - Granular review of:
    - Positive control selection
    - Tabulated validation data
    - Cut-point derivation
  - Cross-reactivity assay validated
  - nAb assay validated but deemed inadequate by FDA
    - Addition of PMC to redevelop and assay samples
    - See Nicoline Videbæk EBF OS 2020



7.2.4 Results of anti-semaglutide antibody RIA validation study 216142

Parameter	Description	Result
MRD	Volume of sample in assay	10 µl (6.7%) in a total of 150 µl, i.e. MRD = 15.
Screening cut point (SCP)	50 T2D Calculat approac	
Normalisation factor (NF)	SCP – M	
Normalised screening cut point	Mean Q	
Confirmatory cut point	%Inhibi or witho (Series Calculat rate.	
Cross reactivity cut point	%Inhibi or witho T2D sam Calculat rate.	
Normalised titer cut point	Mean Q	
Control mAb for assay parameters and QC preparation	anti-sem	
Sensitivity screening assay	anti-sem	
Sensitivity confirmatory assay	anti-sem	
Sensitivity cross reactivity assay	anti-sem	
Recovery	10 T2D semaglut	
	100 ng/m	
	150 ng/m	
	2500 ng/	
Drug Interference	Sensitivi	
	Sensitivi	
	Sensitivi	

7.2.5 Results of *in vitro* neutralising anti-semaglutide antibody validation study 304600

Parameter	Description	Result
Minimum Required dilution (MRD)	Volume of sample used in assay	30 %
Neutralising cut point/floating cut point set at 99% confidence level	30 individu populations and T2D	
Normalisation Factor (NF)	Neutralisin neg[1]	
Plate specific neutralising cut point Set at 99% confidence level	Floating cu	
Sensitivity	Sensitivity F27	
Selectivity, 80% of subjects at each level should be positive Unspiked samples should be negative	10 obese se reference m mAb and 1 or 0 ng/mL	
	10 T2D ser reference m mAb and 9 or 0 ng/ml s	
Drug interference	Sensitivity i semaglutide	
Drug tolerance	LPC 1 (460 LPC 2 (685 HPC (5000	
Assay precision[2] (inter-assay variation)	QC low (LP QC low (LP QC high (H	
Assay precision[3] (Intra-assay variation)	QC low (LP QC low (LP QC high (H	
Haemolysis	QC low(LP high(HPC)	
Lipemia	QC low(LP high(HPC)	

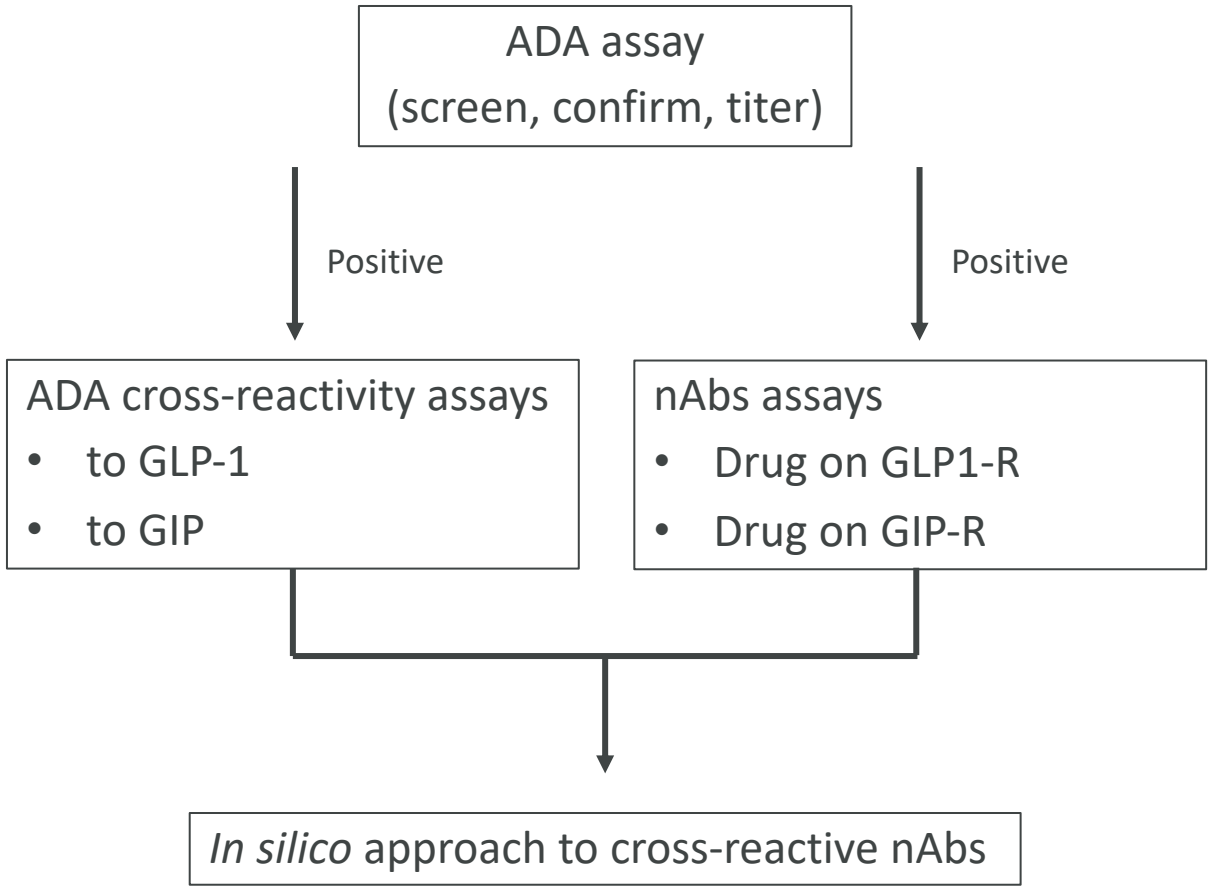
Source: 209637Orig1s000

Strategy deployed for tirzepatide acknowledged drug tolerance challenge of nAbs assays

- Efficacy:
  - No impact on PK, PD or safety observed

- Phase 3 bioanalysis performed using ACE-LBA
- Data submitted from five global and two regional Phase 3 studies

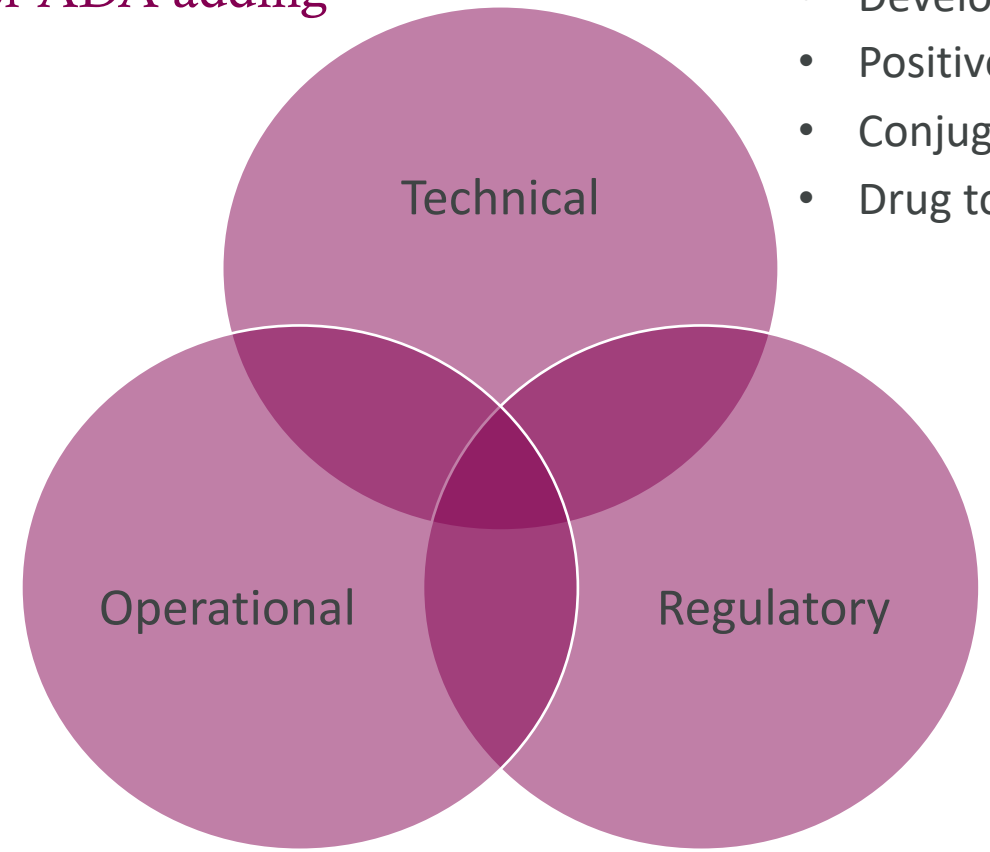
Level of ADA in Phase 3	**51%**
Level of cross-reactivity to GLP-1	34% (GIP) 14% (GLP-1)
Level of in-vitro neutralising ADA	1.9% and 2.1 % nAbs to GIP and GLP-1 function of tirzepatide respectively 0.4% GIP 0.9% GLP-1



Source: 215866Orig1s000

Multiple considerations emerge from these case studies

- Is bioanalytical strategy for ADA adding value to the programme?

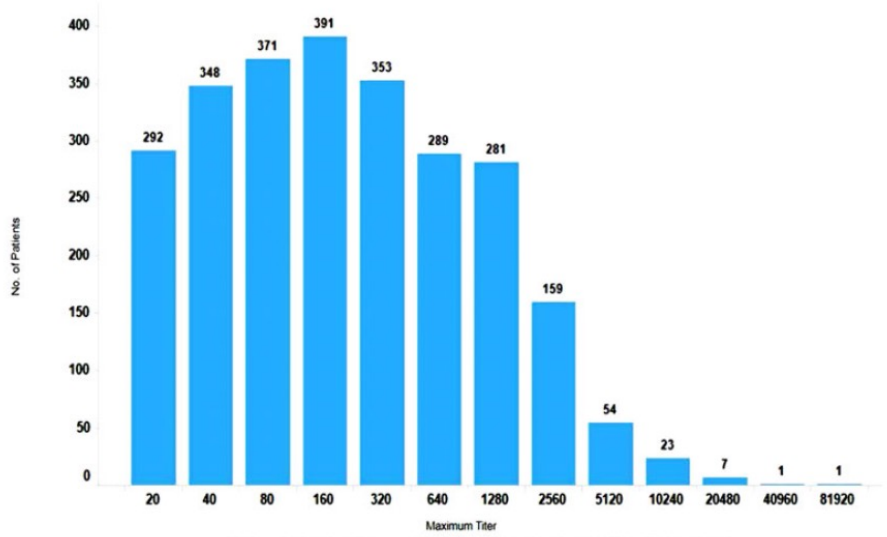


- Development and validation of multiple assays
- Positive control selection
- Conjugation of reagents
- Drug tolerance strategy

- Deployment of multiple assays
- Significant burden of testing where incidence is high
- S:N as alternate to titre
- Need for ADA+ follow-up?

- FDA may request validation reports prior to use
  - Consideration for how long this takes
- Early interaction over strategy
  - *In silico* approach from EL

Analysis of low titre ADA positive samples from highly sensitive LBA is futile on CBA where drug tolerance is a challenge



Abbreviations: ADA = antidrug antibody; TE = treatment emergent.

Figure ISI.4.2. **Maximum titer distribution of tirzepatide TE ADA+ patients during the planned treatment period.**

ACE LBA

Sensitivity 2.81 ng/mL

25 ng/mL PC detectable in presence of **250 µg/mL** drug

CBA

5000 ng/mL PC detectable in presence of **159 ng/mL** drug

2500 ng/mL detectable in presence of **65 ng/mL** drug

In study Ctrough concentrations

- 491 ng/mL (5 mg dose)
- 983 ng/mL (10 mg dose)
- 1470 ng/mL (15 mg dose)

→ Wash out samples used where 95% samples were within stated tolerance

→ 51% incidence of ADA

1.9% and 2.1 % nAbs to GIP and GLP-1

0.4% GIP and 0.9% GLP-1 cross-reactive nAbs

Source: 215866Orig1s000

Following up ADA+ subjects to baseline has large implication for conduct of trials

> "When there is a high risk of serious consequences from ADAs, sponsors should plan to collect samples from subjects until ADAs return to baseline levels."

FDA Guidance: Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection Guidance for Industry 2019

- Clinical sites have to remain open for an unspecified period
- Mechanism required to analyse samples and report ADA data
  - Monthly analysis of data?
  - Need to capture patients who discontinue early
- Unblinding challenge

- In absence of safety signals why perform this?

There is growing discussion of signal:noise as a replacement for titration data

Methodology

For reprint orders, please contact: reprints@future-science.com

Bioanalysis

Assay signal as an alternative to titer for assessment of magnitude of an antidrug antibody response

Marta Starcevic Manning*[1,2], Mark A K…
Sarah A Hoofring[1], Daniel T Mytych[2] & V…
[1]Pharmacokinetics & Drug Metabolism, Amgen, Thousan…
[2]Clinical Immunology, Amgen, Thousand Oaks, CA 9132…
[3]One Amgen Center Drive, Mail Stop 30E-3-B, Thousand…
[4]Currently Biologics & Vaccine Bioanalytics, Merck, Kenil…
* Author for correspondence: Tel.: +1 805 447 4987; ms…

Background: Titer methods are common… response. Assay S/N is an appealing alter… (*S/N*) is appropriate have not been well c… for several therapeutics. S/N correlated … per subject basis. Analysis of impact of a… using either method. Each assay demon… tolerance. **Conclusion:** Under these circu… of the magnitude of an antidrug antibo…

First draft submitted: 24 August 2017; Ac… October 2017

Keywords: antidrug antibody • immunogeni…

Key terms

- Immunogenicity: the ability of an antigen… humans or animals.
- Antidrug antibody: an antibody elicited b… therapeutic.
- Antidrug antibody magnitude: a surrogat… patient samples.
- Signal-to-noise (S/N): assay response gene… generated by the negative control analyz…
- Titer: the reciprocal of the highest sample … the dilution value derived from interpolat…

The assessment of antidrug antibody (AD… important component of the safety evaluatio… ranging from complete neutralization of dru… assess how ADA may impact pharmacokinet… and formats have been explored and the b… commonly used, especially for monoclonal … qualitative, with a positive or negative resu… there are cases where it is important to pr… availability of quasi-quantitative ADA data … This ensures that a potential impact of AD…

10.4155/bio-2017-0185 © 2017 Future Science Ltd

The AAPS Journal (2022) 24: 81
https://doi.org/10.1208/s12248-022-00728-8

RESEARCH ARTICLE

Check for updates

Comparison of Titer and Signal to Noise (*S/N*) for Determination of Anti-drug Antibody Magnitude Using Clinical Data from an Industry Consortium

Marta Starcevic Manning[1] · Mohamed Hassanein[2] · Michael A. Partridge[3] · Vibha Jawa[4] · Johanna Mora[4] · Josiah Ryman[5] · Breann Barker[6,7] · Christian Braithwaite[8] · Kevin Carleton[9] · Laura Hay[10] · Charles Hottenstein[11] · Robert J. Kubiak[12] · Viswanath Devanarayan[13]

Received: 11 May 2022 / Accepted: 17 June 2022 / Published online: 12 July 2022

Abstract
During biotherapeutic drug development, immunogenicity is evaluated by measuring anti-drug antibodies (ADAs). The presence and magnitude of ADA responses is assessed using a multi-tier workflow where samples are screened, confirmed, and titered. Recent reports suggest that the assay signal to noise ratio (*S/N*) obtained during the screening tier correlates well with titer. To determine whether *S/N* could more broadly replace titer, anonymized ADA data from a consortium of sponsors was collected and analyzed. Datasets from clinical programs with therapeutics of varying immunogenicity risk levels (low to high), common ADA assay platforms (ELISA and MSD) and formats (bridging, direct, solid-phase extraction with acid dissociation), and titration approaches (endpoint and interpolated) were included in the analysis. A statistically significant correlation between *S/N* and titer was observed in all datasets, with a strong correlation (Spearman's $r > 0.8$) in 11 out of 15 assays (73%). For assays with available data, conclusions regarding ADA impact on pharmacokinetics and pharmacodynamics were similar using *S/N* or titer. Subject ADA kinetic profiles were also comparable using the two measurements. Determination of antibody boosting in patients with pre-existing responses could be accomplished using similar approaches for titer and *S/N*. Investigation of factors that impacted the accuracy of ADA magnitude measurements revealed advantages and disadvantages to both approaches. In general, *S/N* had superior precision and ability to detect potentially low affinity/avidity responses compared to titer. This analysis indicates that *S/N* could serve as an equivalent and in some cases preferable alternative to titer for assessing ADA magnitude and evaluation of impact on clinical responses.

Keywords Immunogenicity · Anti-drug antibodies · Biotherapeutic · Magnitude · Titer

✉ Marta Starcevic Manning
mstarcev@amgen.com

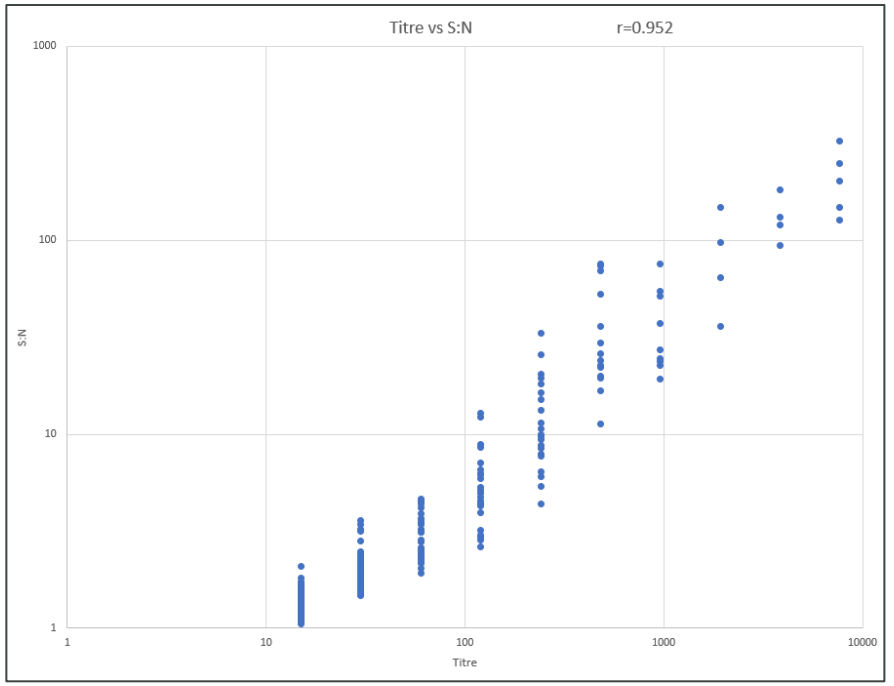
[1] Translational Safety and Bioanalytical Sciences, Amgen Research, One Amgen Center Drive, MS 30E-3B, Thousand Oaks, California 91320, USA
[2] Early Clinical Development, Precision Medicine, Pfizer Inc., Cambridge, Massachusetts, USA
[3] Bioanalytical Sciences, Regeneron, Tarrytown, New York, USA
[4] Non-clinical Disposition and Bioanalysis, Bristol Myers Squibb, Princeton, New Jersey, USA
[5] Quantitative Pharmacology and Pharmacometrics, Merck, Kenilworth, New Jersey, USA
[6] Specialty Bioanalytics, Teva Pharmaceuticals, West Chester, Pennsylvania, USA
[7] Present Address: Incyte Corporation, Wilmington, Delaware, USA
[8] BioAgilytix, Boston, Massachusetts, USA
[9] Janssen Preclinical Safety and Translational Sciences, Spring House, Pennsylvania, USA
[10] Bioanalytical Lab, PPD Clinical Research Services, Thermo Fisher Scientific, Richmond, Virginia, USA
[11] Bioanalysis, Immunogenicity and Biomarkers, GlaxoSmithKline, Collegeville, Pennsylvania, USA
[12] Clinical Pharmacology and Safety Sciences, AstraZeneca, Gaithersburg, Maryland, USA
[13] Data Science, Eisai Inc., Nutley, New Jersey, USA

aaps

Springer

- Titre assays represent a significant component of bioanalytical activity
  - Associated blood volume required
  - Finance and time considerations are large
  - Arguable how well titration data are understood
- Correlation between titre and S:N discussed in publications

- Further discussion needed with HA to gain acceptance



Conclusions

- Incretin mimetics are well established as peptide therapeutics for metabolic disease
- Immunogenicity risk assessment will typically class as higher risk molecules owing to sequence homology
- Review of approved molecules highlights differing incidence of ADA but with little clinical consequence
- Significant burden of bioanalytical testing in the clinic
- ADA analyses performed should add value to the programme
  - Challenge futility of nAbs assessments given drug tolerance
  - Challenge follow-up of ADA+ subjects at end of treatment in absence of safety signals
  - Consider S:N as alternative to titres

Acknowledgements

- Jo Goodman
- Robert Kubiak
- Jenny Cheng
- Hongtao Yu

Thank you

Chris Jones

Integrated Bioanalysis,
AstraZeneca