Sandoz Biopharmaceuticals

Signal-to-Noise for a more accurate determination of immunogenicity response

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Disclaimer

I work for Sandoz (a Novartis division). Views expressed are my own.

The regulatory requirements for the approval of a biosimilar are markedly different from novel biologics

Define strategy which allows a sensitive comparison

- In Biosimilar development it is critical to ensure the most accurate evaluation of immunogenicity is applied ensuring meaningful comparison of the Biosimilar's immunogenicity profile to its Reference
- Titer is a widely used and understood method for determination of magnitude of ADA but:
  - The Accuracy/Precision of titer results is impacted by the use of an individually set dilution scheme (1:2, 1:3, etc.)
  - Titer is therefore a categorical variable
  - Obtained titer values have a low capacity for discrimination at low ADA levels
- S/N ratio has become an alternative using techniques comprising high dynamic range:
  - S/N is a direct measure of ADA magnitude integrating measurement of ADA amount and avidity of a polyclonal ADA response
  - S/N is therefore a continuous variable
  - Applying S/N enabling new (*and more sensitive*) ways to assess immunogenicity of Biosimilars to Reference Product

S/N ratio replacing Titer analysis
Current understanding: Regulators perspective (1/2)

- **Lin Zhou: Assay Signal-to-Noise Ratio (S/N) as A Potential Alternative to Titer for An ADA Response: Regulatory Perspectives from An FDA Office of Clinical Pharmacology Assessor (WRIB 2022)**
  - Demonstrate that using either S/N or titer leads to a similar conclusion about evaluating the impact of ADA on PK using data from clinical development program (incl. phase 3)
  - Choose a sufficiently sensitive dataset and conduct correlation analyses for PK vs. S/N, PK vs titer, and S/N vs titer
  - Data from ADA negative patients should be included (anchor to demonstrate clinical impact of ADA)
  - Immunogenicity assay should have adequate drug tolerance for the range of observed PK data
  - Define criteria for treatment-boosted ADA+ using S/N
  - S/N may be acceptable on a case-by-case basis and depend on factors like immunogenicity risk of the product as well as the stage of development and assay performance

S/N ratio replacing Titer analysis
Current understanding: Regulators perspective (2/2)

- **João Pedras-Vasconcelos : Using Signal-to-Noise (S/N) as an Alternative to Titer Assessment: Regulatory Perspectives from an FDA Office of Biotechnology Products Immunogenicity Assessor (WRIB 2022)**
  - Alternative methods of ADA quantitation besides titer may be used according to FDA Immunogenicity Guideline (2019): *several approaches may be used to report positive antibody responses*
  - Justification for choice of S/N to determine magnitude of ADAs to be provided in eCTD 5.3.1.4 (BA Reports), 2.7.1 (Summary of BA Methods) and 5.3.5.3 (ISI)
  - Discuss S/N with Agency during program development including S/N development data
  - Agency may still request titer analysis (keep and store samples adequately)
  - Still to be addressed:
    - Reporting of S/N values on product label
    - Historical use of titers may make if difficult for Health Care Practitioners to accept S/N concept

Exploring a novel immunogenicity endpoint: *anti-drug-antibody (ADA) signal-to-noise (S/N) ratio in a PK bridging study* (article submitted)

ADA Results (titer)	Study Day											Total
	1[1)]	3	7	10	16	23	30	44	58	72	E.T.	
Negative titer	329	329	322	320	219	193	231	233	176	129	6	2487
Positive titer	1	0	6	7	108	129	90	85	140	184	1	751

- Healthy population
- Dense sampling study
- Single-dose study
- Highly immunogenic molecule (Adalimumab)

Setup most suited for immunogenicity analysis of ADA S/N ratio versus ADA titer parameters, due to the highly immunogenic nature of the drug tested in immunocompetent healthy volunteers

Assay newly validated *for use of the new continuous variable S/N ratio as readout*

- Fully validated state-of-the-art ECL immunoassay (more sensitive, drug-tolerant performance, with lower matrix interference, lower sample volume requirement and much wider dynamic range compared to ELISA)

Assay re-validation as per Starcevic Manning et al 2017, 2022

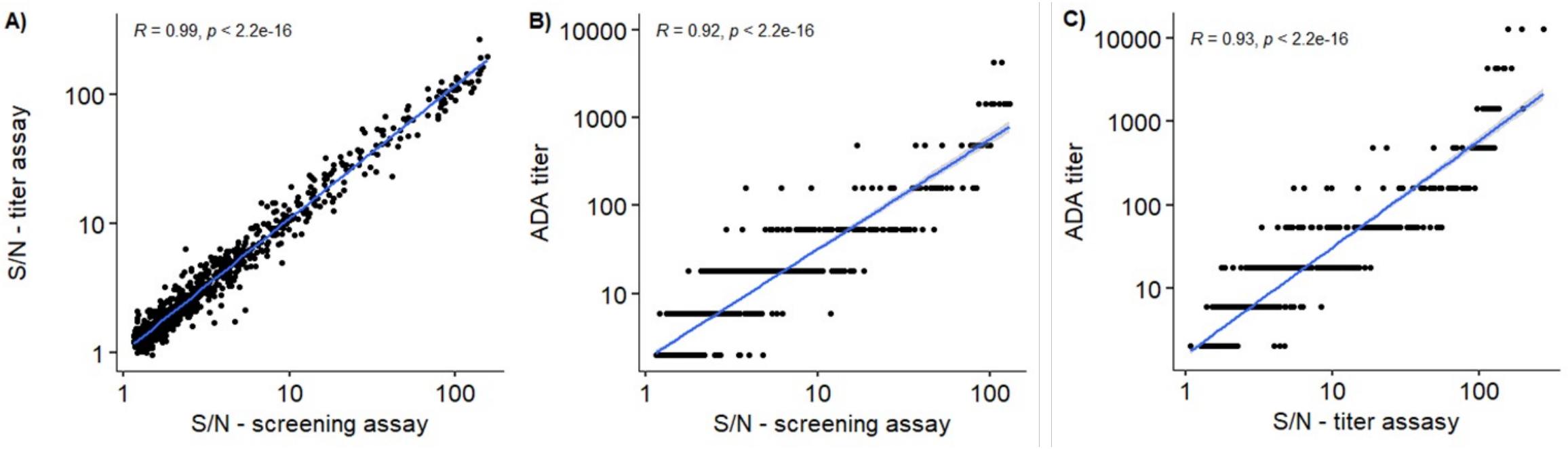
- Continuous variable S/N ratio: average of the 2 ADA ECL signals on MSD, divided by the average of 2 blank values of the corresponding plate
- New validation **with S/N ratio as readout**

Parameter	Specification	Testing
Linearity (incl. *Hook Effect*, if required)	(S/N ratio −1) ±50% of the value expected at next dilution step[1)]	Performance evaluated within the validated assay range at each concentration level
Precision	CV (%) ≤ 20 for S/N ratio at each concentration level	Using all QC sets of all runs from inter-assay, precision analysis
Drug Tolerance	Acceptable drug impact on S/N ratio	Performed considering range of PK data

- Reevaluation of the **in-study precision with S/N ratio as readout** (all CV% QC <20%)

Demonstrate correlation of S/N and Titer in ADA assay

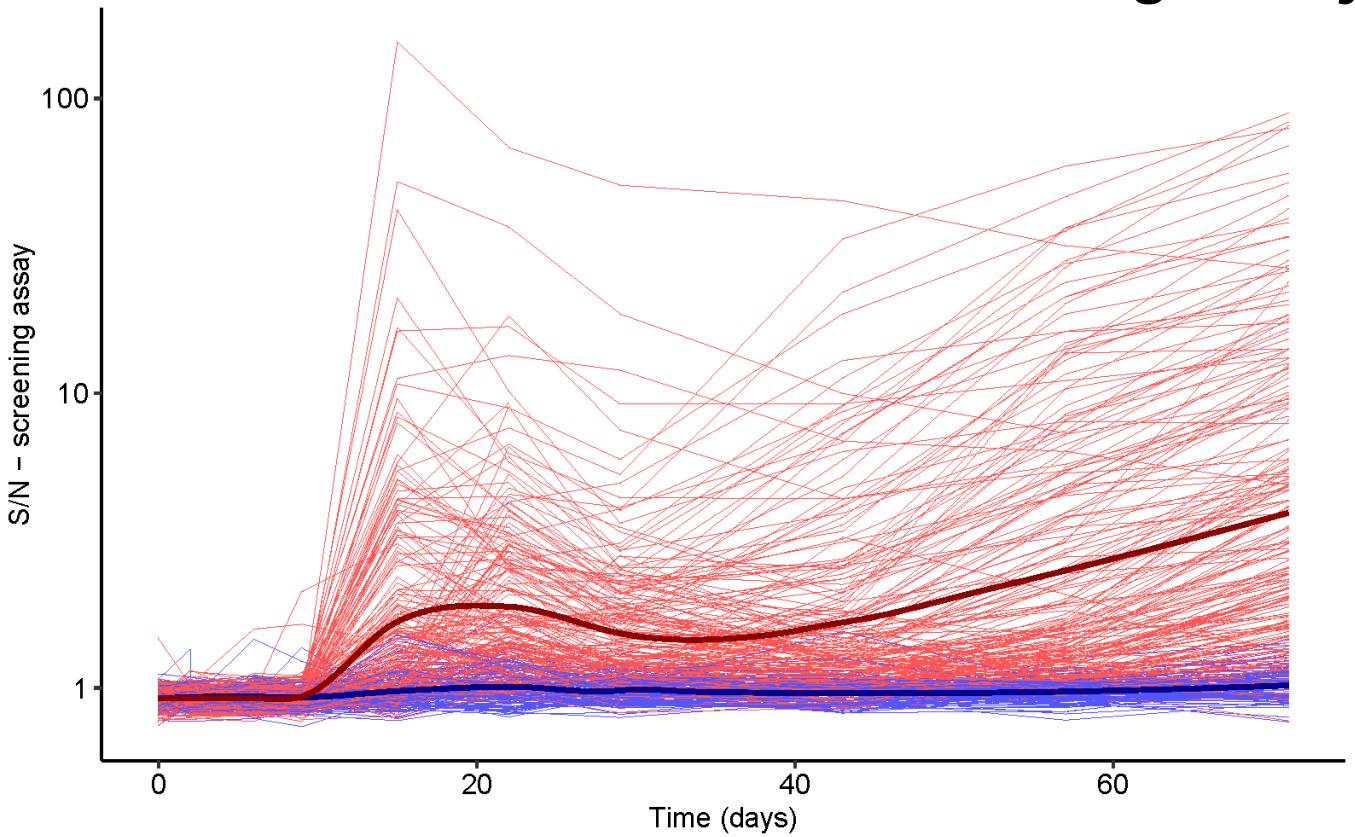
Strong correlation of S/N and Titer



Correlation between ADA S/N ratio and ADA titers. (A) Blank normalized S/N ratio at screening assay vs. titer assay, (B) S/N ratio at screening vs. ADA titer, and (C) S /N ratio at titer assay vs. ADA titer

ADA over time using continuous variable S/N ratio

Immunogenicity profiles



Individual representation of the ADA profile of a healthy volunteer over time;

- red line: ADA positive;
- blue line: ADA negative;
- Black lines: median values for ADA positive and ADA negative

The continuous nature of the ADA S/N ratio allows for the integration over time of the ADA magnitude

New ADA parameters similar to drug concentrations assessment in PK calculations:

- **S/N_{max},**
- **ADA S/N AUC,**
- **T_{max} (to S/N_{max}),**

to assess immunogenicity profiles of immune responses at individual and population level

ADA parameters analyzed at population level

Group	n	ADA AUC S/N ratio					S/N_{max}					ADA T_{max}				
		H-L est	C.I. low	C.I. high	Range Min	Range Max	H-L est	C.I. low	C.I. high	Range Min	Range Max	H-L est	C.I. low	C.I. high	Range Min	Range Max
All	322	2392	2227	2660	133	191915	3.005	2.511	3.442	0.94	154.95	48	44	48	1	72
ADA+	210	3621	3212	4256	807	191915	5.777	4.606	7.367	1.16	154.95	48	48	51	10	72
ADA-	112	1655	1640	1670	133	1967	1.119	1.098	1.144	0.94	1.79	34	27	38	1	72

Early termination subjects not included. ADA, anti-drug antibody; C.I., confidence interval; H-L est, Hodges-Lehmann estimate.

The ADA-positive and the ADA-negative subgroups are clearly distinct,

- non-overlapping confidence intervals of the Hodges-Lehmann estimators in both **ADA AUC S/N ratio** and **S/N_{max}**,
- max range difference of ~100-fold for **ADA AUC S/N ratio** and of ~86-fold for **S/N_{max}** between the positive and negative group

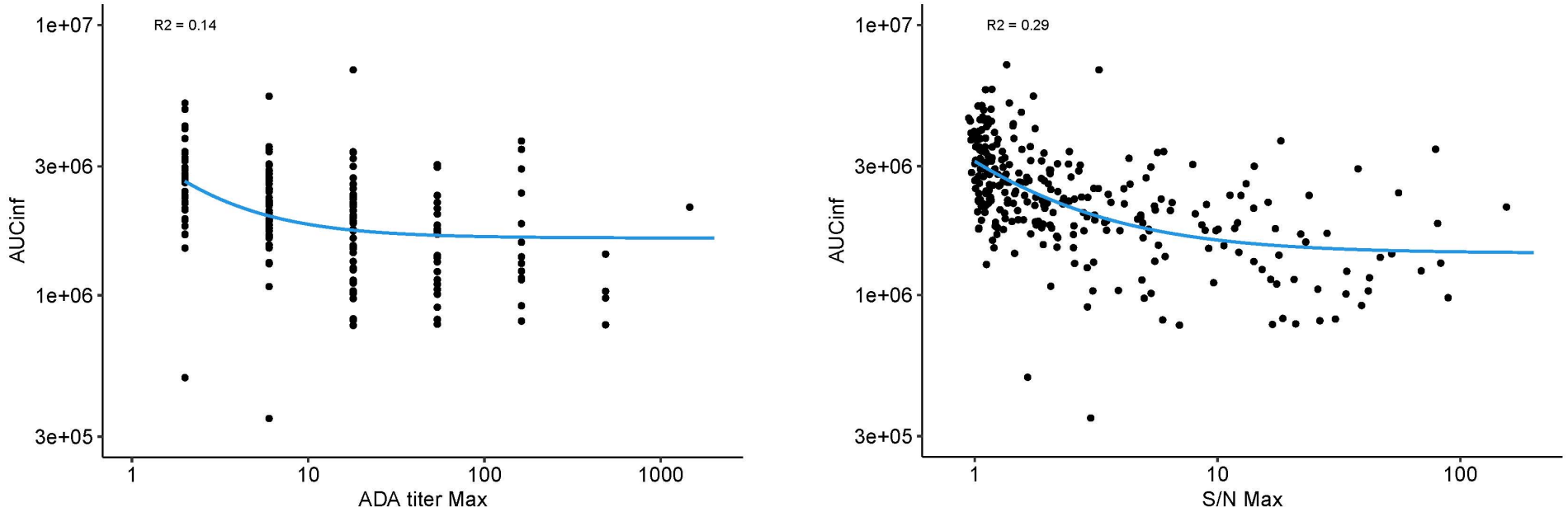
Very limited range overlap of **ADA AUC S/N ratio** and **S/N_{max}** between positive and negative subpopulations: possible potential for future refinement of the threshold of meaningful ADA positivity?

Broad range of T_{max} (occurring all throughout the duration of the study, from day 1 to day 72) suggests a notable complexity of the dynamic of the immune response at individual level, making prediction of the maximum immune response almost impossible.

Correlation between ADA response and PK at individual level

S/N_{max} versus Titer max to assess impact of ADA on PK AUC

Correlation analysis between ADA titer max value (positive ADA) and S/N_{max} (positive and negative ADA) on PK parameters AUC_{inf}

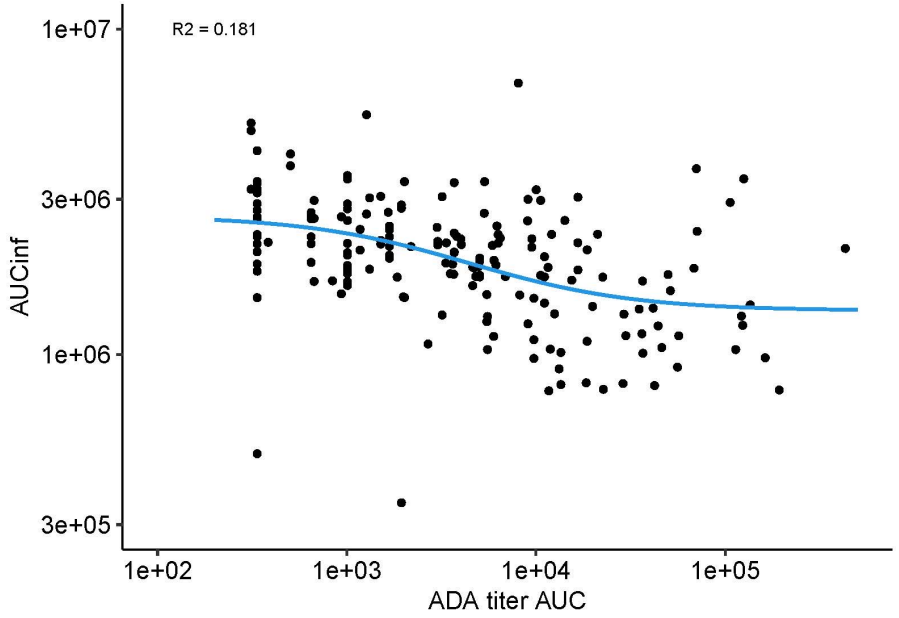


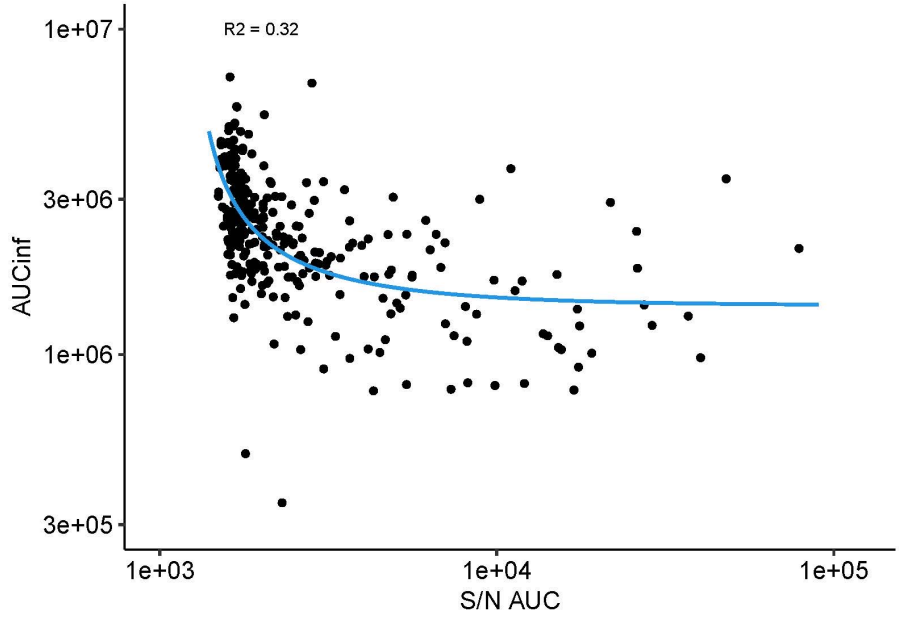
When assessing impact of ADA on PK, ADA parameters calculated using S/N ratio exhibit a higher correlation to PK parameters than using ADA titers

Correlation between ADA response and PK at individual level

S/N AUC versus Titer AUC to assess impact of ADA on PK AUC

Correlation analysis between ADA titer AUC (positive ADA) and S/N AUC (positive and negative ADA) on PK parameters AUC_{inf}

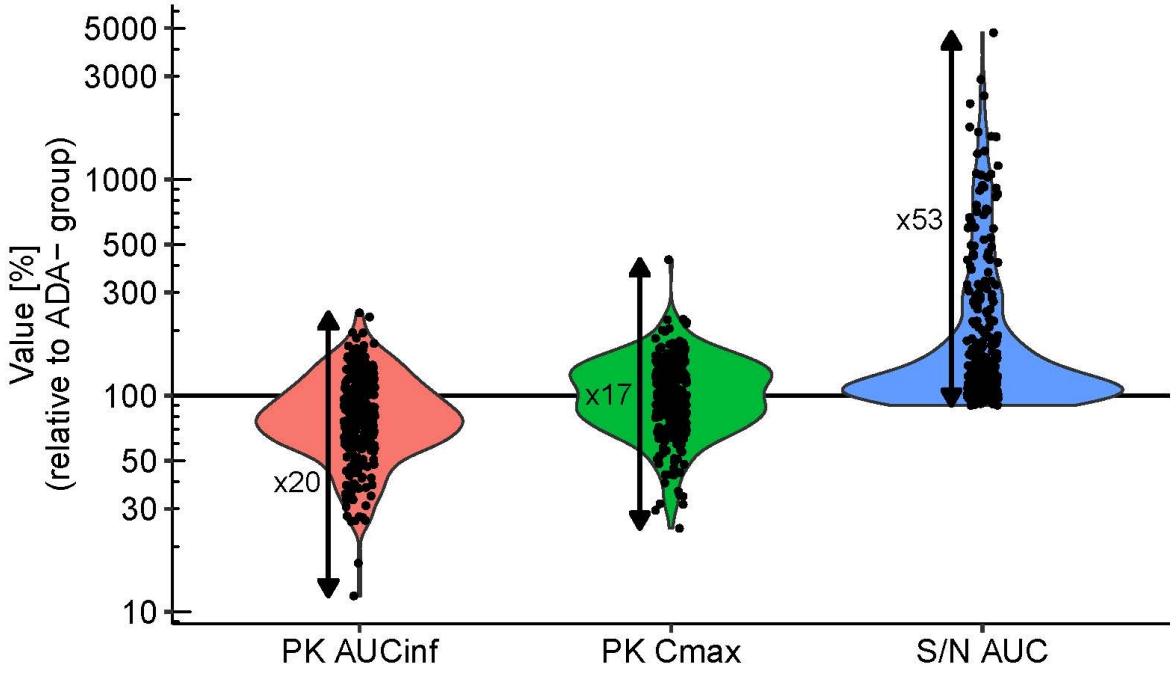




When assessing impact of ADA on PK, ADA parameters calculated using S/N ratio exhibit a higher correlation to PK parameters than using ADA titers

Extent of impact of immunogenicity on ADA S/N compared with PK parameters C_{max} and AUC_{inf}

Ranges of PK parameters AUC_{inf} and C_{max} compared with AUC of ADA S/N ratio following administration of single doses to healthy subjects



The plotted values are normalized relative to the respective geometric mean of the sub-group of subjects that did not exceed the ADA screening assay threshold at any sampling time point

ADA S/N varied within a wider dynamic range than PK AUCinf and Cmax after a single dose, which indicates a richer information content of this measure

Summary & Outlook

- **S/N ratio can be an alternative to titer for the determination of the magnitude of ADAs also from a Biosimilar perspective**:
  - ➢ It is important to define a strategy which allows a sensitive comparison of the Biosimilar to its Reference
  - ➢ S/N ratios enable the application of new ways to look at immunogenicity which increase the information derived from analysis per sample to support a tailored Biosimilar development program

- **Several aspects still need to be discussed and aligned on as**:
  - ➢ Harmonization of validation parameters and acceptance criteria for applying S/N
  - ➢ Definition of treatment boosted ADAs when using S/N
  - ➢ Communication of S/N to Health Care Practitioners and patients: Immunogenicity of Biosimilars is compared to historical data which used titers to determine magnitude of ADAs
  - ➢ Testing of S/N and correlation analysis to titers in “early” clinical studies in Biosimilar development may not be possible in case of having only one integrated PK(PD), efficacy and safety study

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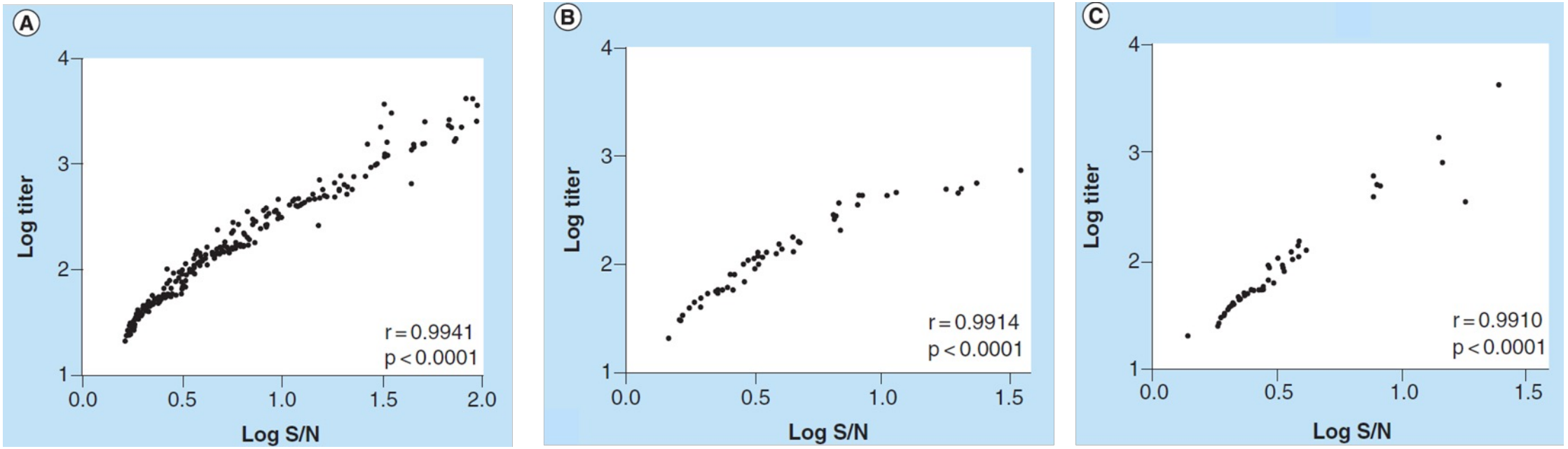
Supplemental slides

S/N ratio replacing Titer analysis
Current understanding: Industry perspective (1/2)

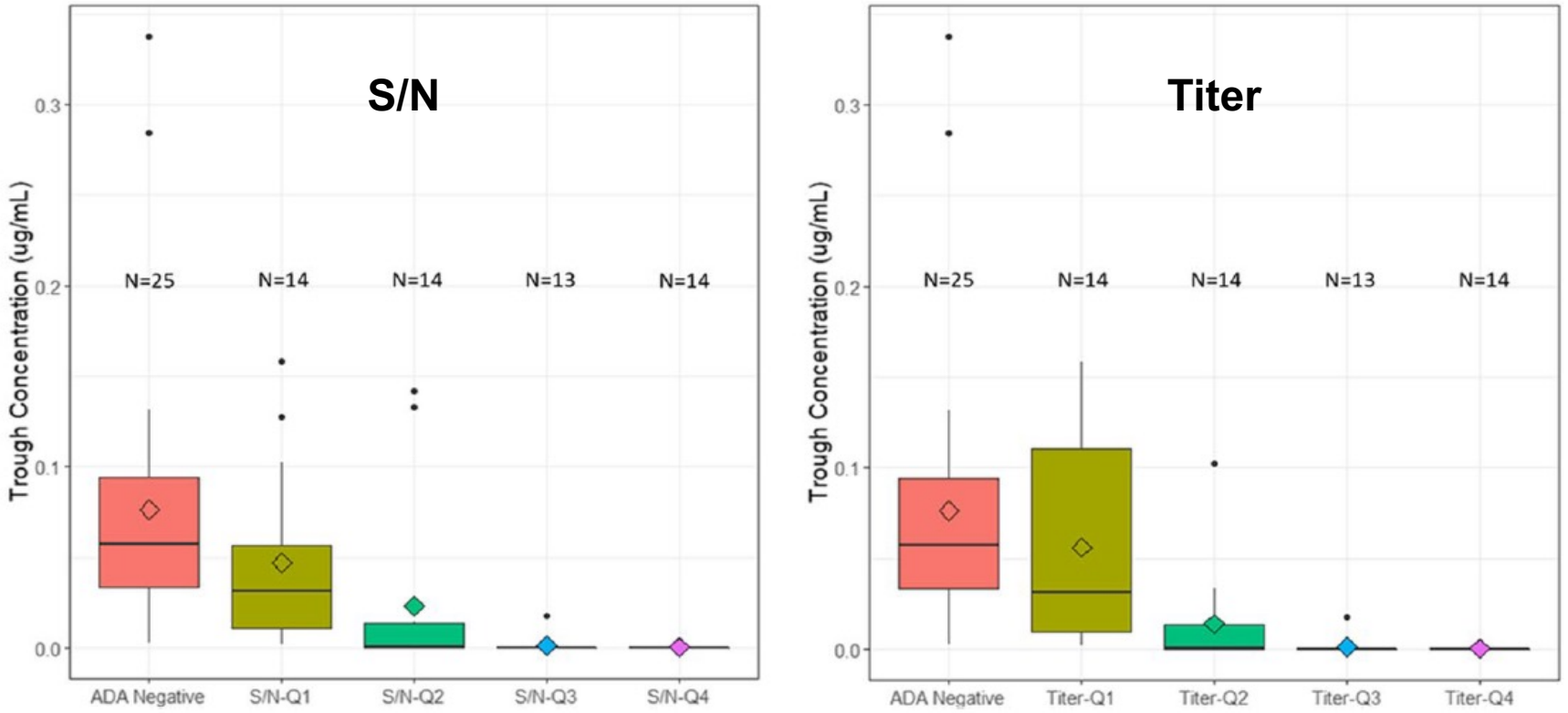
- **Manning et al., Bioanalysis (2017): Assay signal as an alternative to titer for assessment of magnitude of an antidrug antibody response**
  - S/N correlated strongly with titer



Correlation of S/N and titer in samples from Phase II clinical studies. S/N strongly correlates with titer for the three **(A)** mAb1, **(B)** mAb2, and **(C)** mAb3 clinical studies evaluated. S/N: Signal-to-noise.

S/N ratio replacing Titer analysis
Current understanding: Industry perspective (2/2)

- **Manning et al., AAPS Journal (2022): Comparison of Titer and Signal to Noise (S/N) for Determination of Anti-drug Antibody Magnitude Using Clinical Data from an Industry Consortium**
  - Conclusions regarding ADA impact on PK were similar using S/N or titer



Impact of ADA magnitude on dose-normalized drug mean trough concentrations (example from strongest correlation)