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# Comparing Signal-to-Noise with titer results

## A retrospective analysis

*Karin Benstein*
*Translational Medicine Unit, Sanofi*

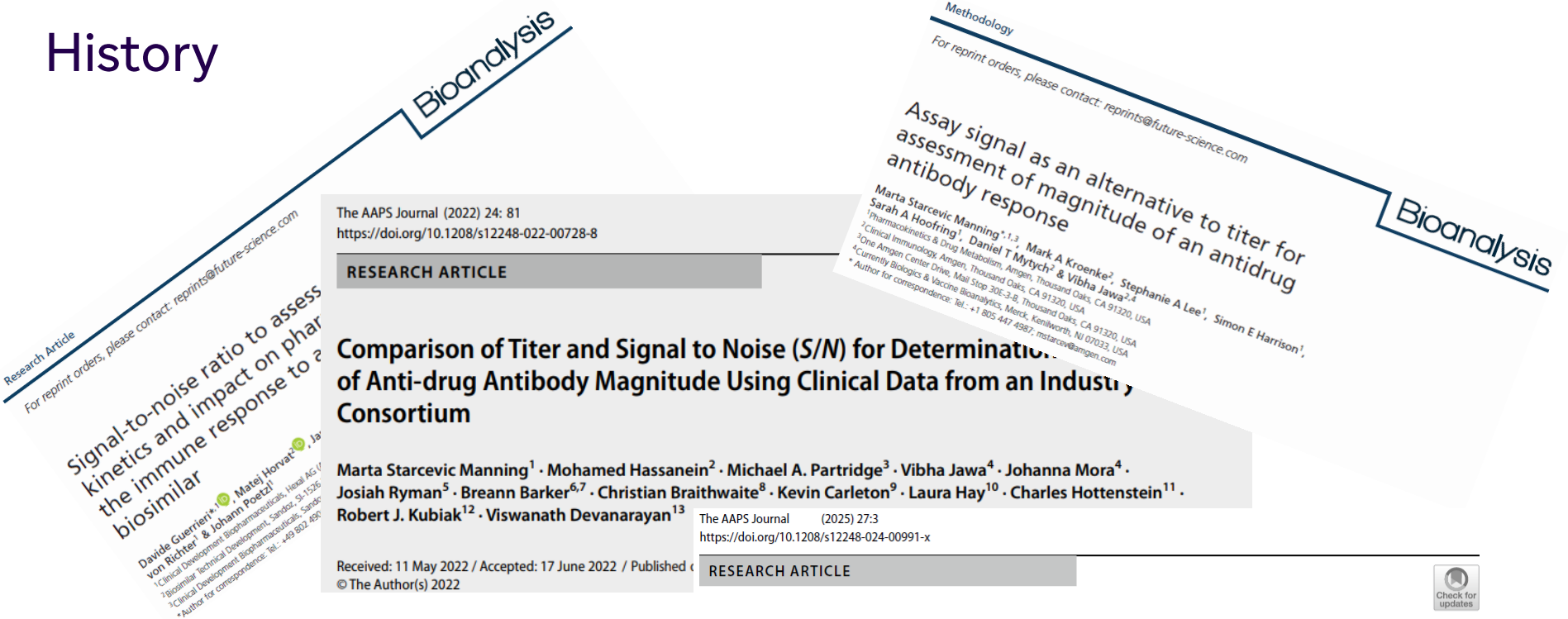
*16th Open Scientific EIP Symposium 2025*

# Contents



# 01 Background

# History


Bioanalysis
Research Article
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Signal-to-noise ratio to assess kinetics and impact on pharmacokinetics of the immune response to a biosimilar
Davide Guerrieri*[1], Matej Horvat[2], Jan von Richter[1] & Johann Poetzl[1]
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[3]Clinical Development Biopharmaceuticals, Sandoz
*Author for correspondence: Tel.: +49 802 490
The AAPS Journal (2022) 24: 81
https://doi.org/10.1208/s12248-022-00728-8
RESEARCH ARTICLE
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
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Methodology
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Bioanalysis
Assay signal as an alternative to titer for assessment of magnitude of an antidrug antibody response
Marta Starcevic Manning*[1,3], Mark A Kroenke[2], Stephanie A Lee[1], Simon E Harrison[1], Sarah A Hoofring[1], Daniel T Mytych[2] & Vibha Jawa[2,4]
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The AAPS Journal (2025) 27:3
https://doi.org/10.1208/s12248-024-00991-x
RESEARCH ARTICLE
Check for updates
Possibilities and Limitations in Substituting anti-Drug Antibody Titers with Signal-to-Noise Ratios: A Comprehensive Comparison Using Two Clinical Trial Datasets of Adalimumab
Dawon Jang[1] · Jaeil Kim[2] · Youngwon Jo[2] · Hyuna Lee[3] · Ahra Go[1] · Jieun Kim[1] · Soyoung Choi[1]
Received: 29 June 2024 / Accepted: 3 November 2024
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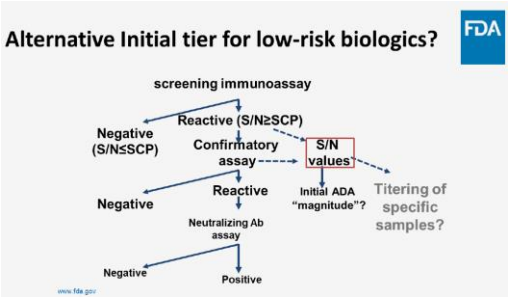
# Current Understanding

***Regulatory Perspective (João Pedras-Vasconcelos, FDA)***

Alternative methods of ADA quantitation besides titer may be used:

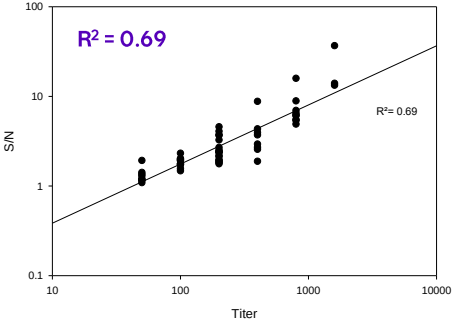
- 2019 Immunogenicity Guidance:
  - "Several approaches may be used to report positive antibody responses, and the appropriateness of the approach used should be evaluated on a **case-by-case basis**."
- Useful for sample semi-quantitation **in early-stage development**, prior to development of titer assays
  - "fit for purpose" (i.e. qualified) assay(s)
- Alternative **initial** tier approach for **low immunogenicity risk products**
- For late-stage Development: Sponsors should provide a justification for choice of S/N for ADA quantitation in eCTD 5.3.1.4 (Reports of Bioanalytical and Analytical Methods for Human Studies), 2.7.1 (Summary of Bioanalytical Methods) and 5.3.5.3 (Integrated Summary of Immunogenicity)
  - S/N and titer development data
  - Early clinical study data correlating the effect of ADA on PK using both S/N and titer
  - Establish S/N criteria for assigning study samples as treatment-boosted ADAs

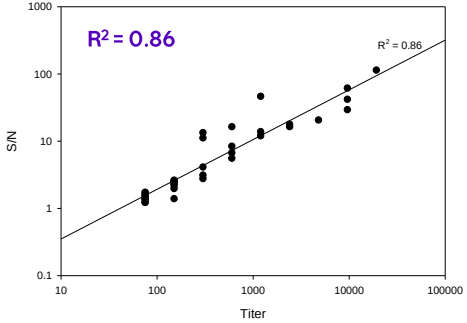



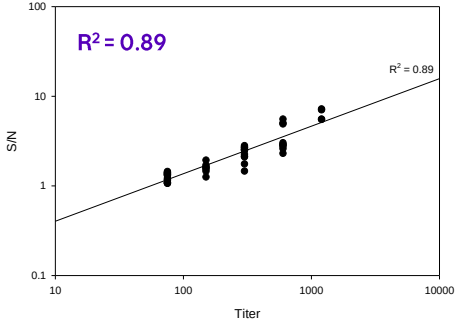
# 02 S/N versus Titer correlation

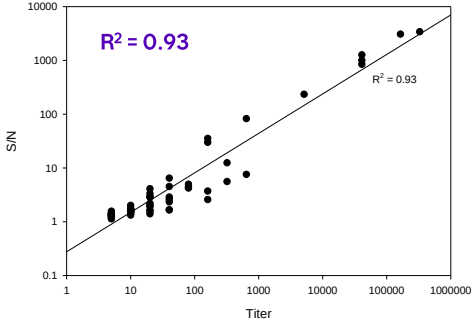
# S/N vs Titer

## *Overall Correlation*



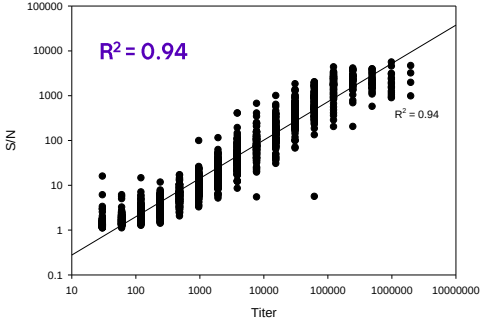



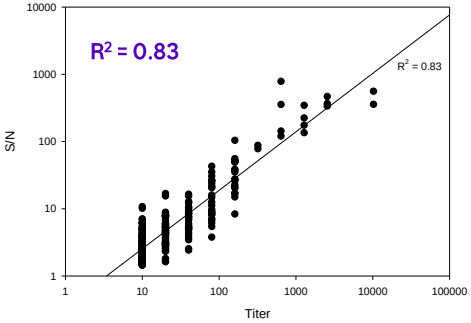



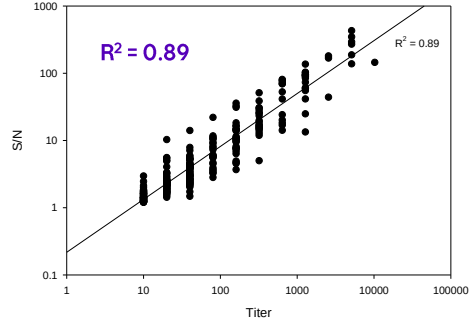




**Modalities:**

- Peptide
- Monoclonal Antibodies
- Multi-specific Nanobodies®
- NK-cell engager
- Fusion protein



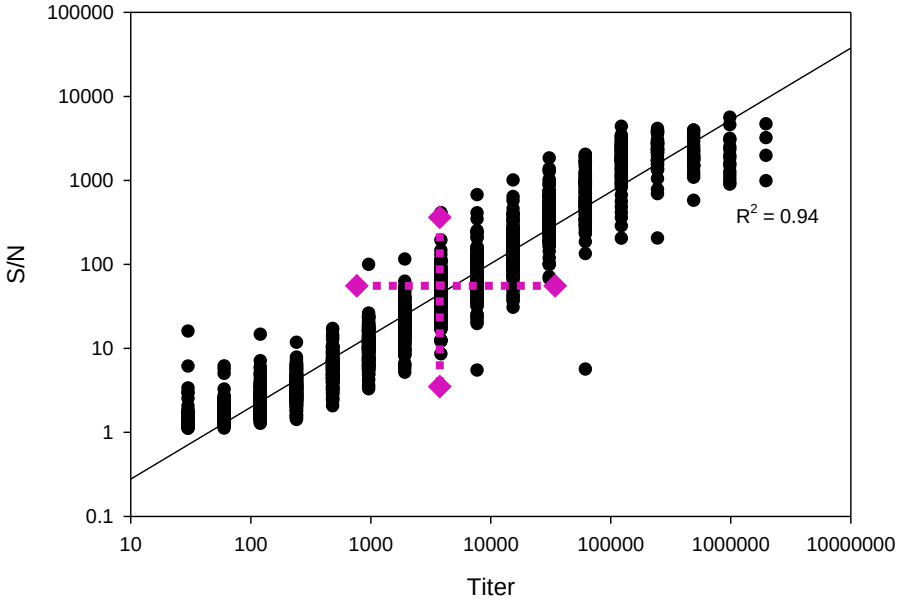



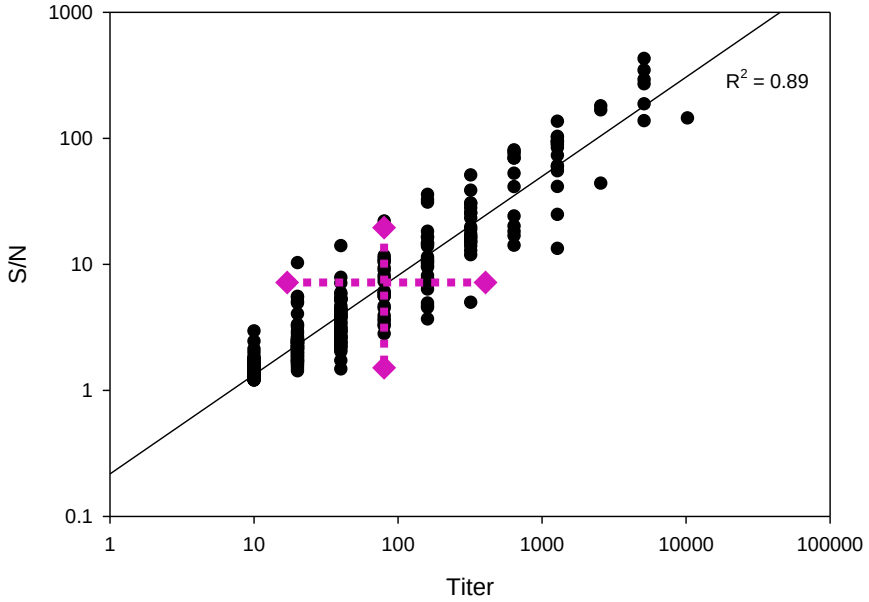




➢ Good overall correlation of titer and S/N

# S/N vs Titer

***Overall Correlation***

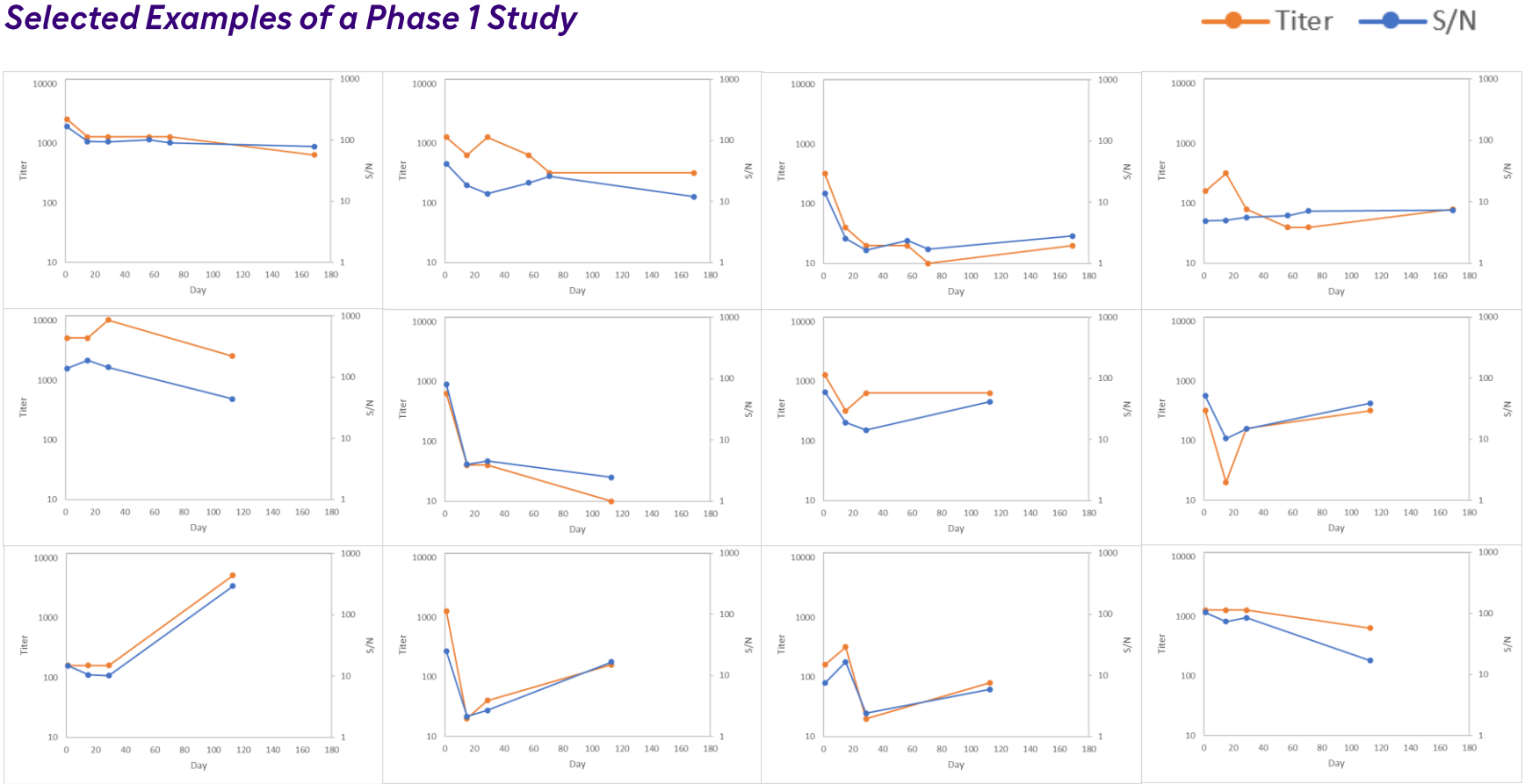



- **Due to the discrete values of titer, a titer correlates with a range of S/N values**
- **But also S/N correlates with approx 4-5 titer steps**

# S/N vs Titer – Individual profiles

***Selected Examples of a Phase 1 Study***



# S/N vs Titer – Individual profiles

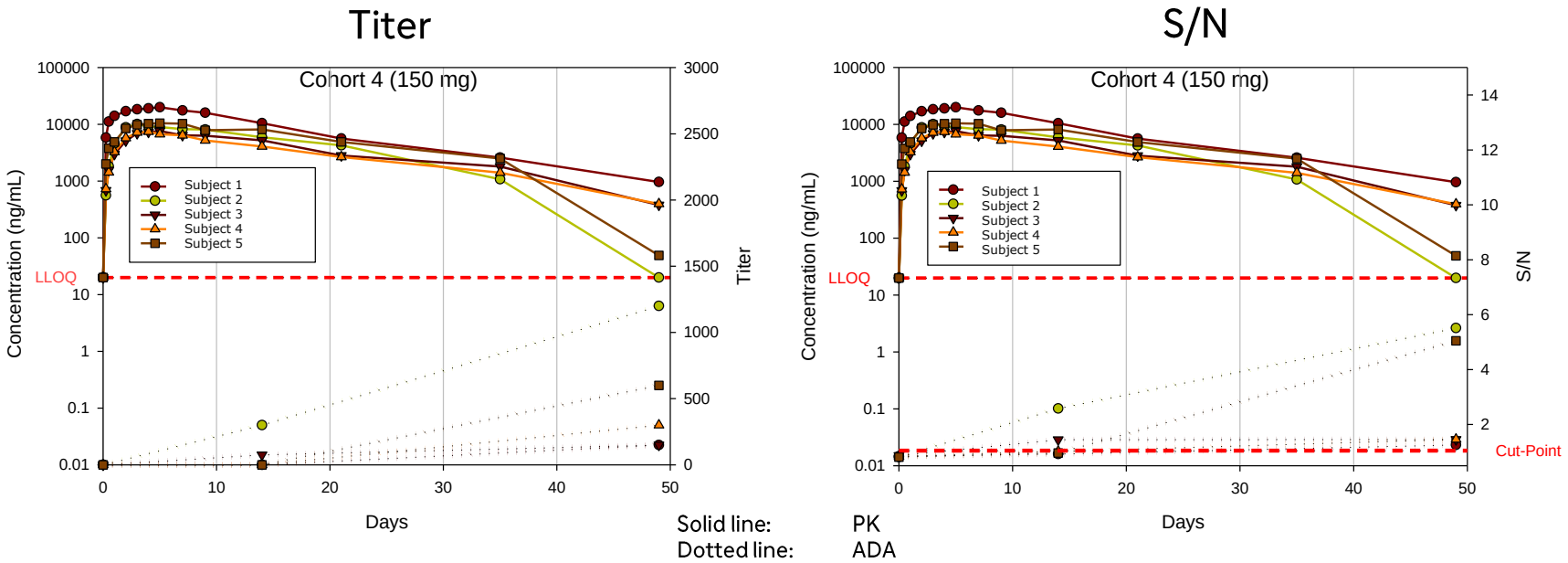
***Selected Examples of a Phase 3 Study***


➢ Similar profiles of titer and S/N over time

# 03 Impact of ADAs on PK

# S/N vs Titer – Impact of ADAs on PK

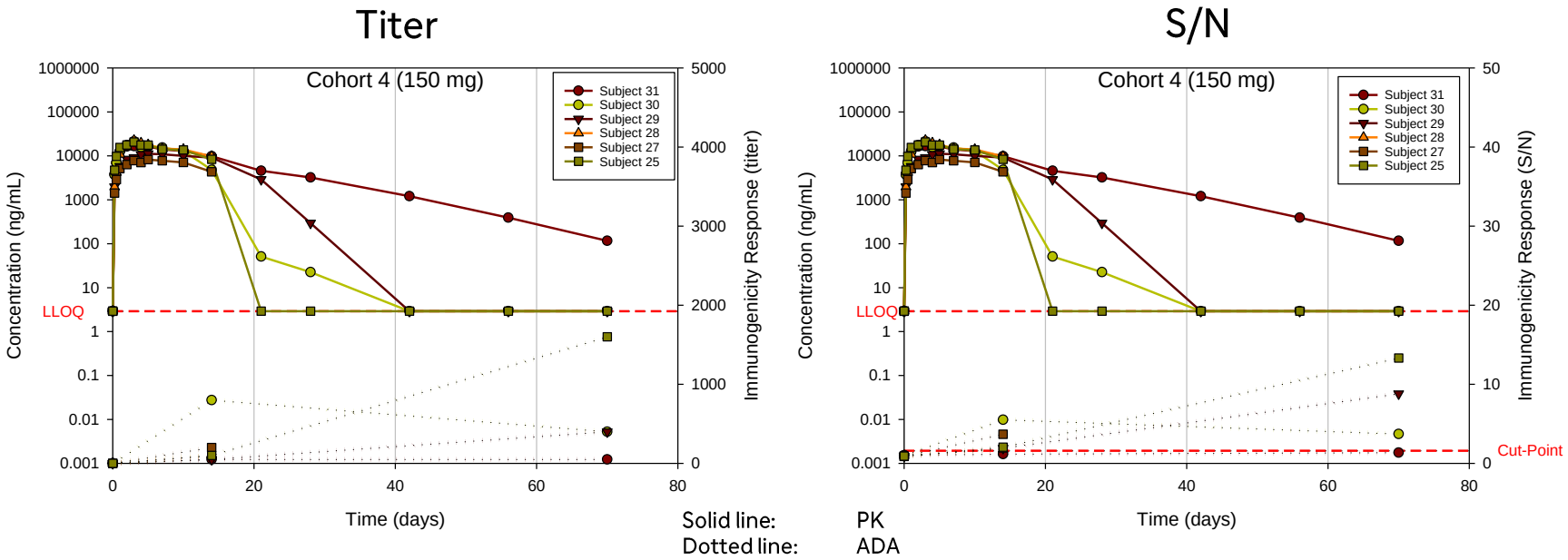
***Selected Example of a Phase 1 Study***




- S/N correlates better with PK profile than titer

# S/N vs Titer – Impact of ADAs on PK

***Selected Example of a Phase 1 Study***

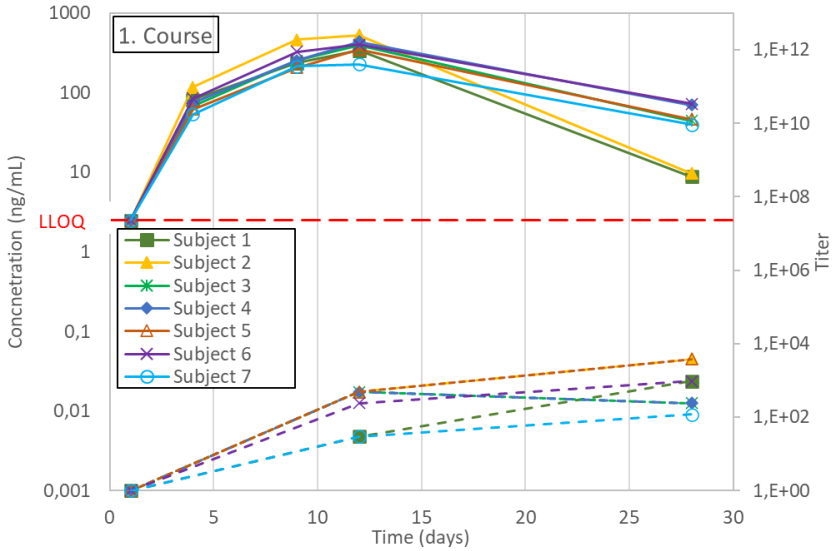



➢ Similar correlation of titer and S/N with PK profile

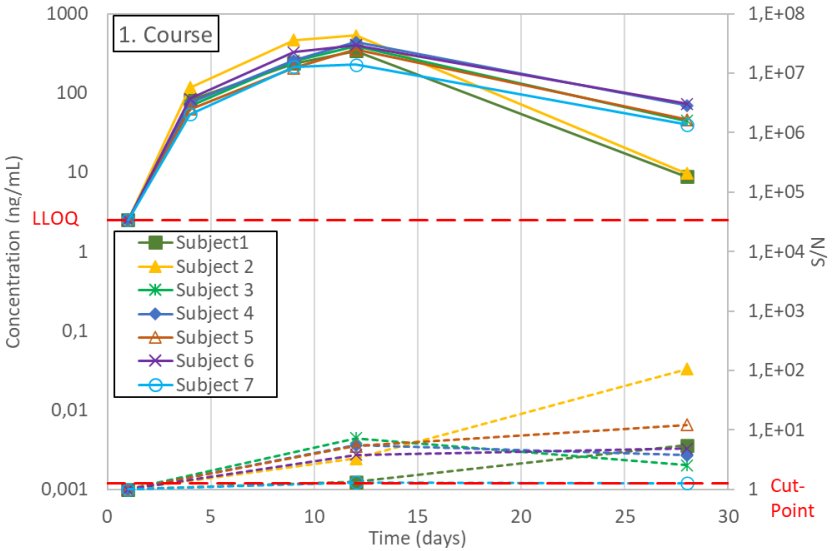
# S/N vs Titer – Impact of ADAs on PK

***Selected Example of a Phase 3 Study***

Titer




S/N

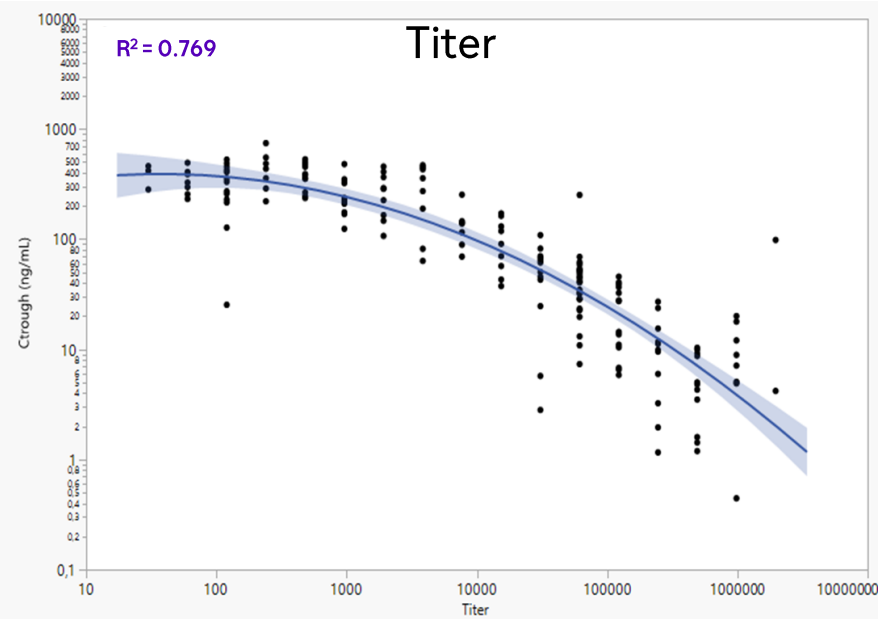


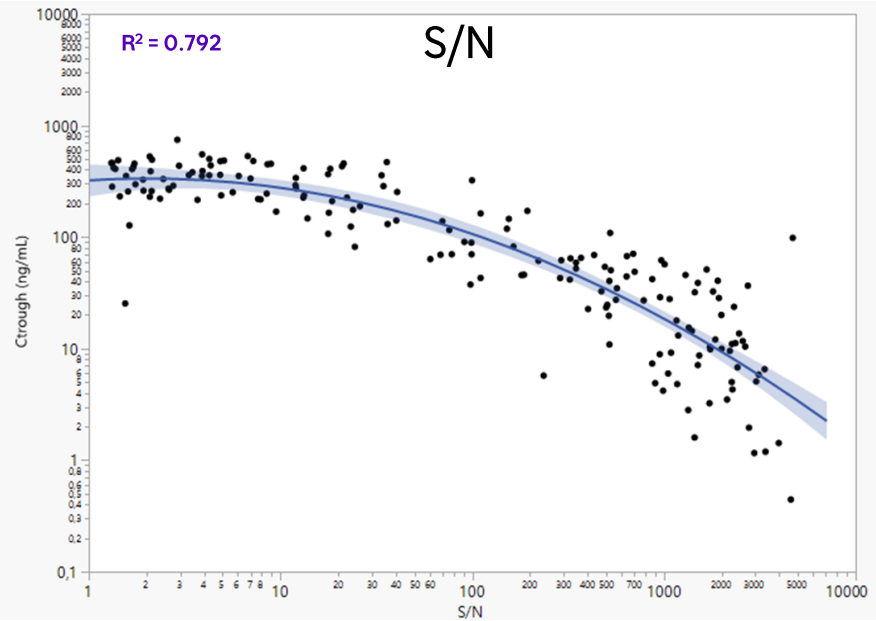

Solid line: PK
Dotted line: ADA

➢ Similar correlation of titer and S/N with PK profile

# S/N vs Titer – Correlation with PK ($C_{trough}$)

***Selected Example of a Phase 3 Study***




➢ Similar correlation of titer and S/N with $C_{trough}$

# 04 Criterion for treatment-boosted ADAs

# S/N vs Titer – Criterion for Treatment-Boosted ADAs

- Switching to S/N would require to define a criterion for treatment-boosted ADAs
  - Minimum significant ratio (MSR) is useful for defining Titer or S/N Precision
  - MSR can be used to define a criterion for treatment-boosted ADAs

$$\mathbf{MSR} = \mathbf{10}^{\mathbf{t(0.05,n-1)}*\sqrt{\mathbf{2}}*\mathbf{SD}}$$

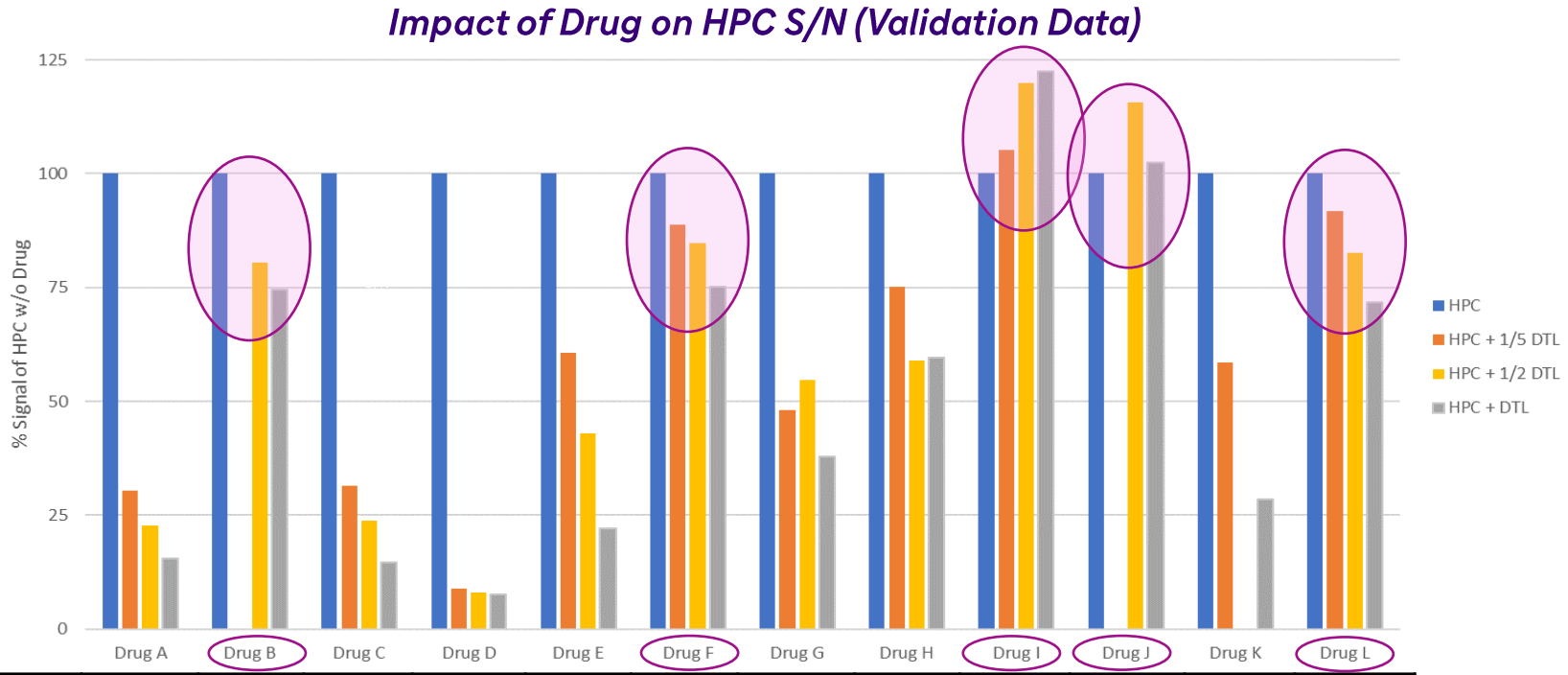
| | LPC | | MPC | | HPC | |
|---|---|---|---|---|---|---|
| Run | S/N | Log10(S/N) | S/N | Log10(S/N) | S/N | Log10(S/N) |
| 1 | 1,73 | 0,238 | 29,7 | 1,473 | 1420 | 3,152 |
| 2 | 1,64 | 0,215 | 29,0 | 1,462 | 1400 | 3,146 |
| 3 | 1,58 | 0,199 | 27,3 | 1,436 | 1240 | 3,093 |
| 4 | 1,80 | 0,255 | 33,5 | 1,525 | 1580 | 3,199 |
| 5 | 1,77 | 0,248 | 32,2 | 1,508 | 1640 | 3,215 |
| 6 | 1,67 | 0,223 | 29,7 | 1,473 | 1470 | 3,167 |
| 7 | 1,67 | 0,223 | 28,8 | 1,459 | 1300 | 3,114 |
| 8 | 1,76 | 0,246 | 31,7 | 1,501 | 1510 | 3,179 |
| 9 | 1,63 | 0,212 | 30,1 | 1,479 | 1500 | 3,176 |
| 10 | 1,87 | 0,272 | 37,3 | 1,572 | 1860 | 3,270 |
| 11 | 1,67 | 0,223 | 26,6 | 1,425 | 1280 | 3,107 |
| 12 | 1,61 | 0,207 | 26,4 | 1,422 | 1230 | 3,090 |
| mean | | 0,230 | | 1,478 | | 3,159 |
| SD | | 0,0218 | | 0,0435 | | 0,0537 |
| t(0.05;n-1) | | 2,201 | | 2,201 | | 2,201 |
| MSR | | **1,17** | | **1,37** | | **1,47** |
| **Median MSR** | | | | **1,37** | | |

- MSR can be retrieved from inter-assay precision data of the positive controls (i.e. no additional wet work necessary) during validation
- Final MSR = Median of MSR from different control levels

**Any on-treatment sample having a at least 1.37-fold higher S/N value than its corresponding pre-treatment sample will be called “treatment-boosted”**

# 05 Possible Limitations

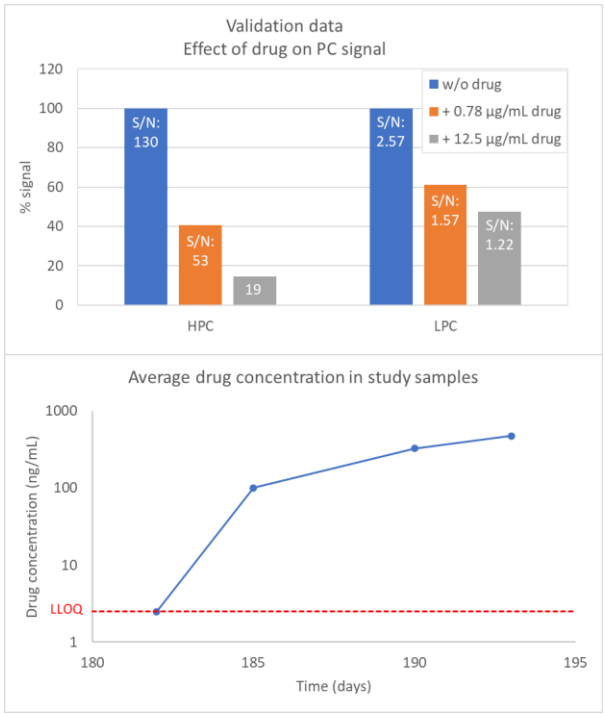
# S/N vs Titer Limitation – Impact of Drug

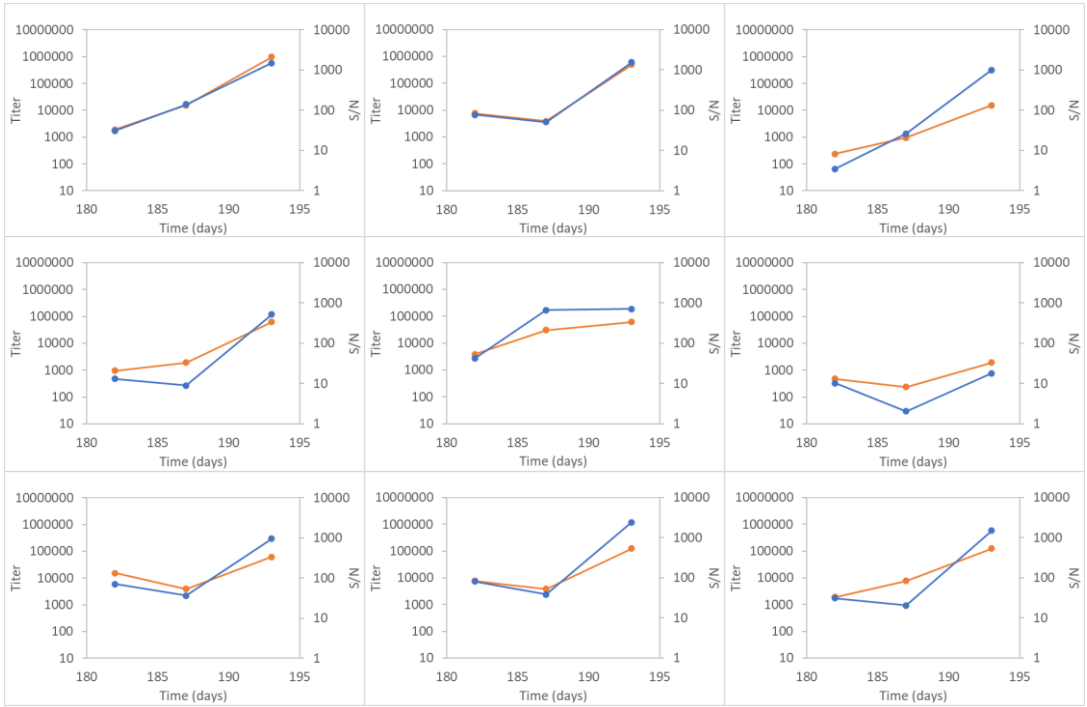



| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MSR | 1.4 | 1.3 | 1.31 | 1.44 | 1.22 | 1.52 | 1.37 | 1.39 | 1.64 | 1.39 | 1.42 | 1.5 |
| DTL (µg/mL) | 500 | 100 | 12.5 | 1 | 12.5 | 0.8 | 1000 | 1000 | 200 | 1000 | 50000 | 5000 |

# S/N vs Titer Limitation – Impact of Drug

***Selected Examples of Phase 3 Study Data***



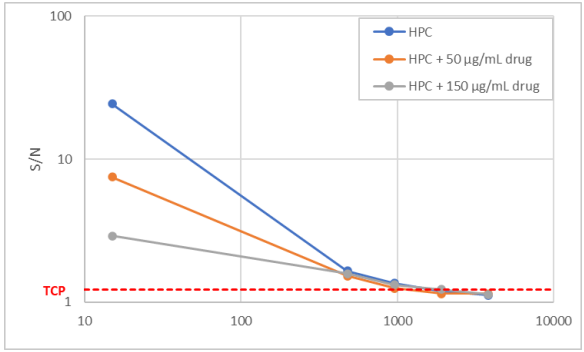

➢ No clear impact of drug on titer and S/N

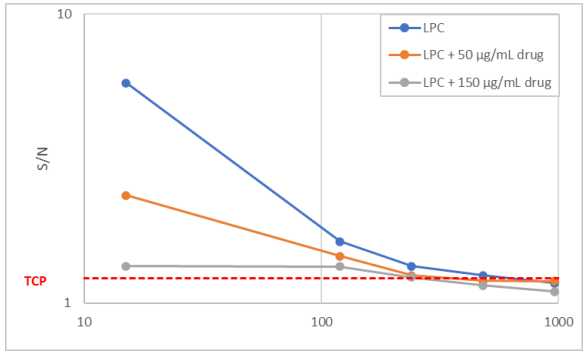
# S/N vs Titer Limitation – Impact of Drug

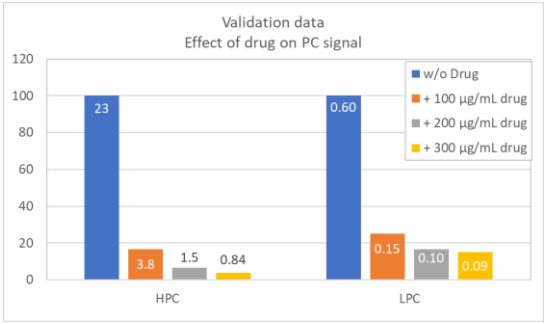
***Titration of PCs with or without Drug***

| HPC | Titer | S/N |
|---|---|---|
| w/o drug | 960 | 20.1 |
| + 50 µg/mL drug | 960 | 6.2 |
| + 150 µg/mL drug | 1920 | 2.4 |

| LPC | Titer | S/N |
|---|---|---|
| w/o drug | 480 | 4.8 |
| + 50 µg/mL drug | 240 | 1.9 |
| + 150 µg/mL drug | 240 | 1.1 |



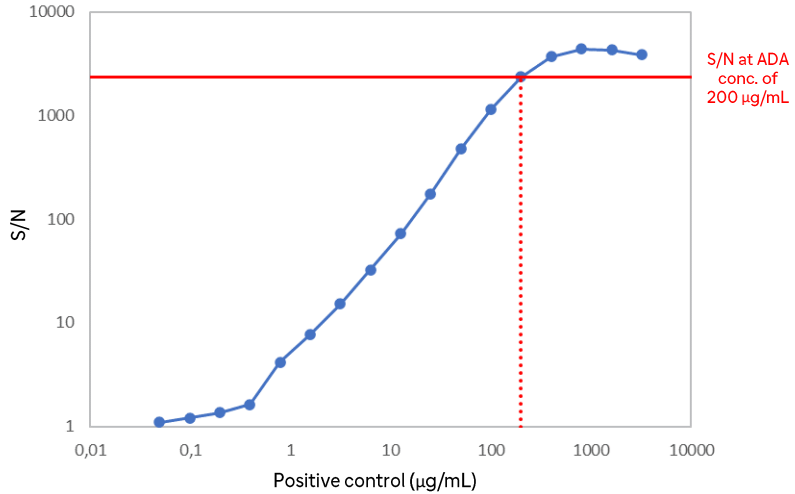

- S/N is impacted with increasing drug concentrations

# S/N vs Titer Limitation – Assay Dynamic Range

***Saturation Range of the ADA Assay***

S/N data (in contrast to titer values) have the intrinsic disadvantage to plateau at high signals

- This is frequently observed with colorimetric read-outs (such as ELISAs) due to their limited dynamic range, but could also be seen with ECL assays (if reagents become limited)
- This can be evaluated during assay validation using high concentrations of the positive control
  - Based on internal and literature data, it is extremely unlikely that ADA responses will show concentrations > 200 µg/mL
  - S/N is deemed feasible if the ADA assay is still in its dynamic range (not plateauing) at an antibody concentration of 200 µg/mL



# 06 Conclusion & Outlook

# Workload Savings

| Project | Total Number of Plates (Accepted Plates) | Titer Plates (% of total) |
|---|---|---|
| Drug A (Phase 1) | 79 | 50 (63.3%) |
| Drug B (Phase 1) | 32 | 19 (59.3%) |
| Drug C (Phase 2) | 119 | 21 (17.6%) |
| Drug D (Phase 3) | 179 | 65 (36.3%) |
| Drug E (Phase 3) | 644 | 441 (68.4%) |

# Summary & Outlook

- Good overall correlation (but debatable if this is the appropriate parameter)
- Similar individual ADA profiles between titer and S/N
- Impact of ADAs on PK can be retrieved with both S/N and titer
- Treatment boosted ADAs can be defined using the MSR for S/N values
- The assay needs to have a sufficient dynamic range to define an upper acceptance limit
- The influence of an excess of drug needs to be evaluated during validation
- Significant saving of time and money using S/N instead of titer

Proposal:

- Use S/N in early (non pivotal) clinical trials for non-high risk immunogenicity programs (if dynamic range of the assay allows and impact of drug is within MSR)
  - Will allow higher throughput and permit earlier ADA result availability
- Continue to use titer for pivotal- and potential post-marketing trials (until final consensus regarding the use of S/N with authorities is achieved)

# Special THANKS to:

Daniel Kramer

Nathalie Mace
Eric Gouillaud

All bioanalytical teams involved

# Questions?

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