Exploring alternatives to the 3-tiered immunogenicity testing paradigm for gene therapy programs

Robert Nelson PhD | 26 February 2025

BioAgilytix

Agenda

1. Bioanalysis of gene therapies
2. Alternatives to the 3-tier testing paradigm
3. Case 1: Anti-AAV capsid antibodies
4. Case 2: Anti-transgene protein antibodies
5. Concluding remarks

Bioanalysis of gene therapies

Bioanalysis for ‘traditional’ biotherapeutics is simple(r)

Non-clinical

- Pharmacokinetics (PK)
- Anti-drug antibodies (ADA)
- Biomarkers (PD, target engagement, safety, …)

Clinical Phase I/II

- Pharmacokinetics (PK)
- Anti-drug antibodies (ADA)
- Neutralising antibodies (NAb)
- Biomarkers (PD, target engagement, safety, …)

Clinical Phase II/III

- Pharmacokinetics (PK)
- Anti-drug antibodies (ADA)
- Neutralising antibodies (NAb)
- Biomarkers (PD, target engagement, safety, …)

Green – Assays typically used at this stage

Red – Assays sometimes used at this stage

Antibody graphic: Created with BioRender.com

Bioanalysis for gene therapies

Non-clinical

- Biodistribution & shedding (qPCR/ddPCR)
- Transgene product detection/quantitation
- TAb to vector (selection or stratification)
- Anti-transgene protein antibodies
- NAb to vector (selection or stratification)
- Biomarkers (PD, target engagement, safety, …)

Clinical Phase I & Beyond

- Shedding (qPCR/ddPCR)
- Transgene product detection/quantitation
- TAb to vector (inclusion/exclusion)
- NAb to vector (inclusion/exclusion)
- Anti-transgene protein antibodies
- NAb to transgene protein
- Cellular immunogenicity to vector (ELISpot/Flow cytometry)
- Cellular immunogenicity to transgene protein (ELISpot/Flow cytometry)
- Biomarkers (PD, target engagement, safety, …)

Green – Assays typically used at this stage

Red – Assays sometimes used at this stage

AAV graphic: Created with BioRender.com

- With so many bioanalytical endpoints possible, it is important to decide *what to measure and why*, and *what not to measure and why*

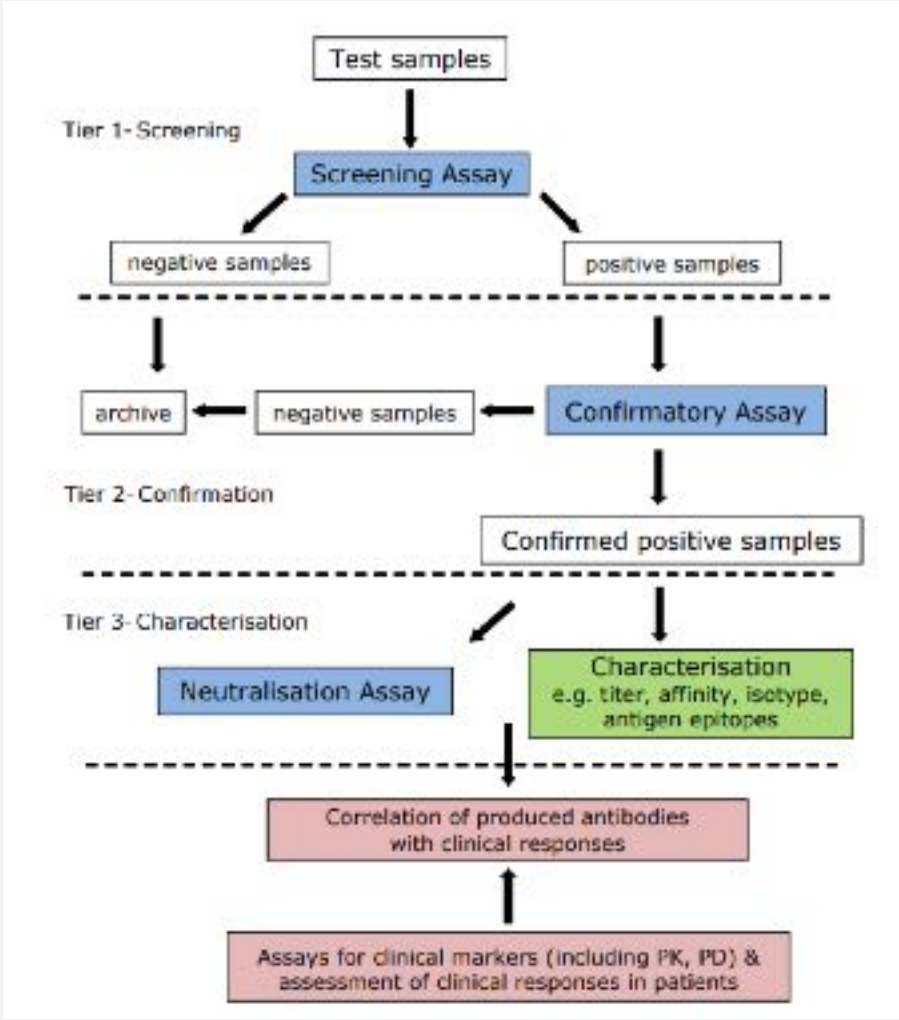
- Want to be as efficient as possible, so long as it does not increase risk to the patient

Alternatives to the 3-tier testing paradigm

Approach to clinical immunogenicity testing

- Clinical immunogenicity assessment typically follows a multi-tiered approach:
  - Screening
  - Confirmation
  - Characterisation (Titer, NAb, …)
- Strategy developed as industry consensus to harmonise the testing approach

Link: https://doi.org/10.1016/j.jpba.2008.09.020

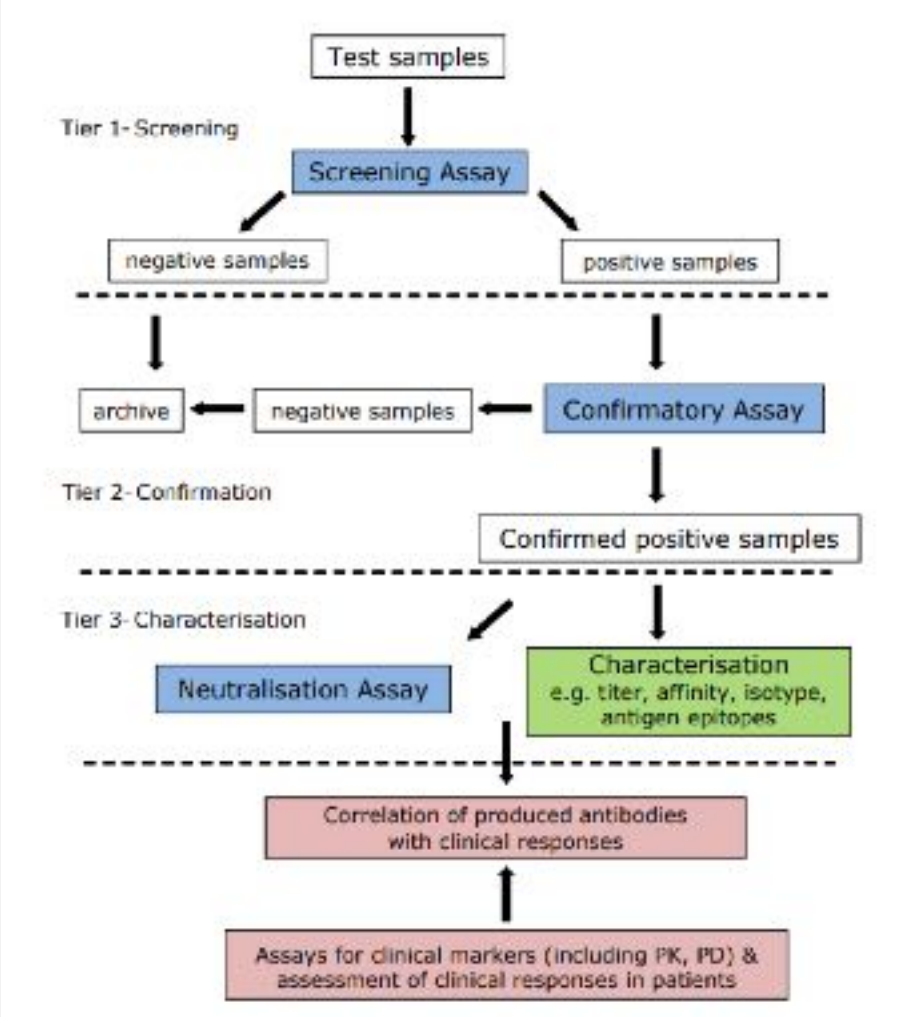


Approach to clinical immunogenicity testing

Limitations

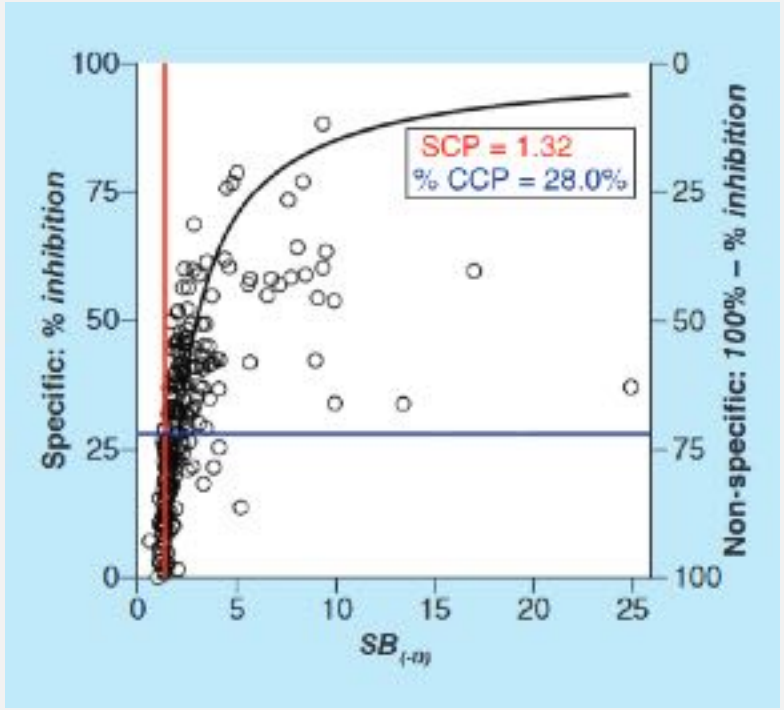
- Multiple assessments required for each sample – time & cost
- High sample consumption to cover all tiers
- Multiple freeze/thaw cycles

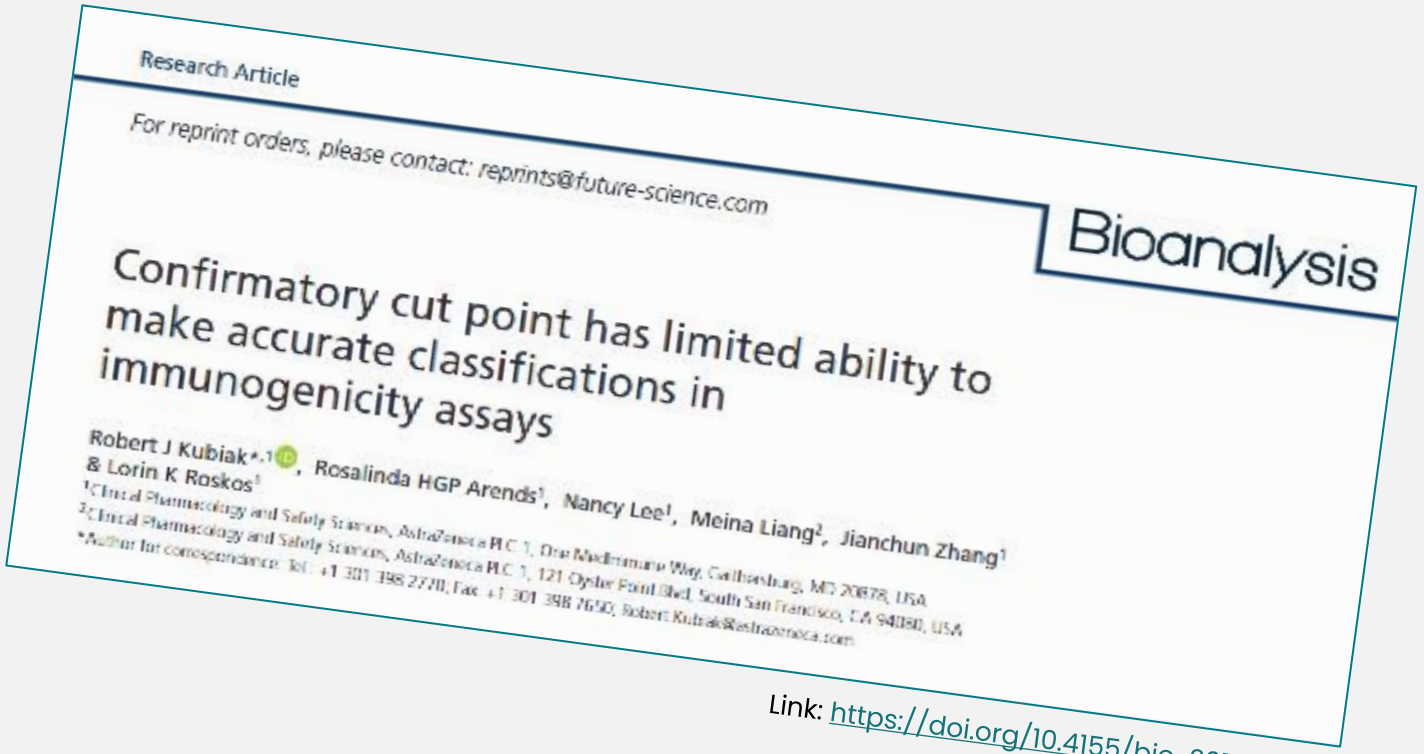
→ Can simpler approaches be applied without loss of information?



Alternatives testing apporaches

- Value of the confirmation tier has been questioned
  - Retrospectively, it is easy to see where confirmation did or did not add value
  - In our experience, bridging assay formats most often have strongest correlation of screen and confirmation tiers




Research Article

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Bioanalysis

Confirmatory cut point has limited ability to make accurate classifications in immunogenicity assays

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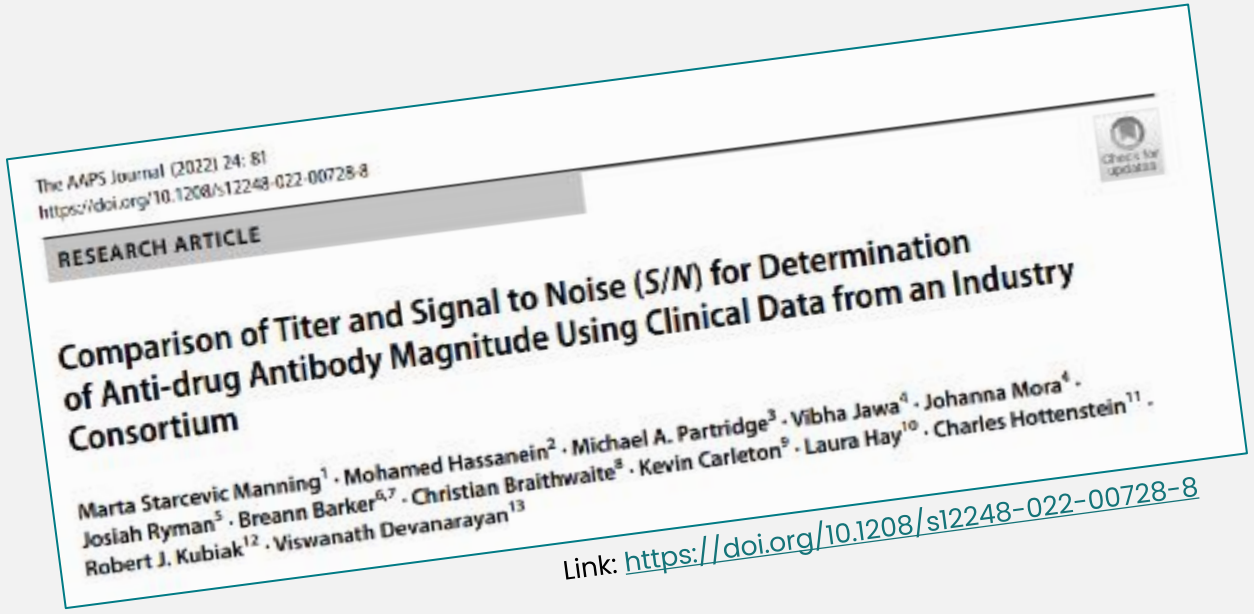
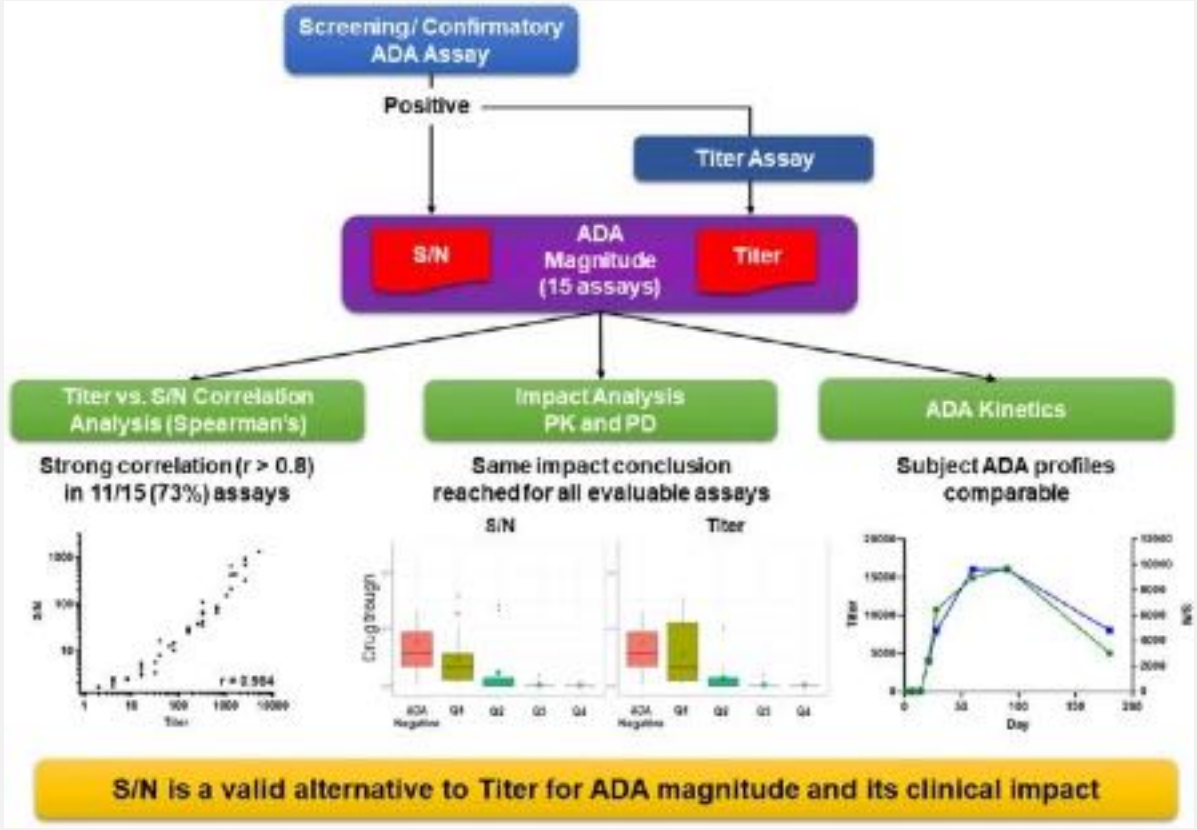
Link: https://doi.org/10.4155/bio-2019-0283

Alternatives testing approaches

- Signal-to-noise ratio (SNR) has been proposed as an alternative to titer

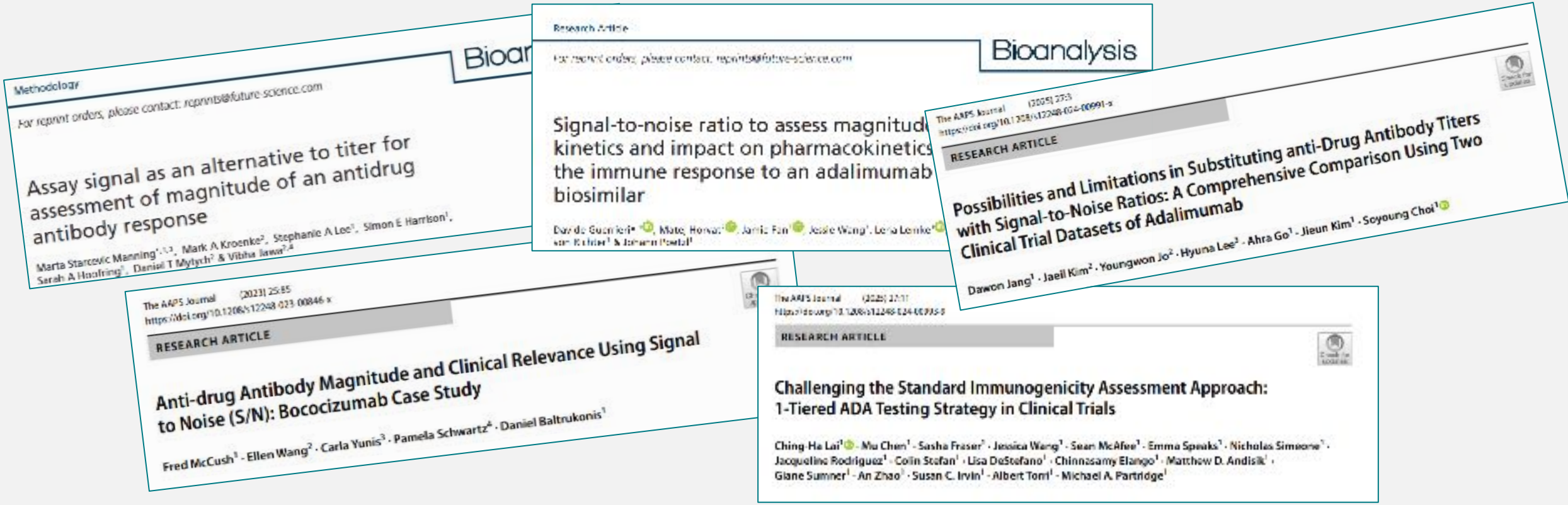


Link: https://doi.org/10.1208/s12248-022-00728-8



Alternatives testing approaches

- Increasing number of publications and presentations supporting the approach
  - Supporting numerous programs where correlation of SNR and titer is being evaluated – more data…!
  - Supporting several programs where 1-tier approach is being implement for clinical studies



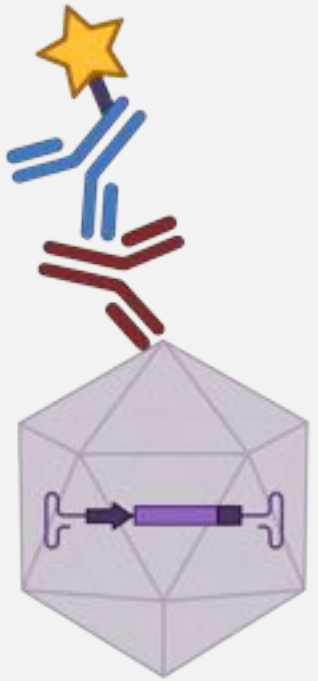
- Are there valid alternatives to the 3-tiered immunogenicity testing paradigm for gene therapy programs…?

Case 1: Anti-AAV capsid antibodies

Assay graphic: Created with BioRender.com

Assay summary

Anti-AAV capsid antibodies assay summary	
Drug Modality	AAVx vector expressing intracellular protein
Administration	Systemic
ADA assay type	Sequential sandwich assay using unlabelled capsid as capture and labelled anti-human IgG as detection
LPC, HPC	100, 5000 ng/mL
Platform	Colorimetric ELISA
Screening/Titer Cut Point	1.40
Drug tolerance	No AAV in circulation at sampling

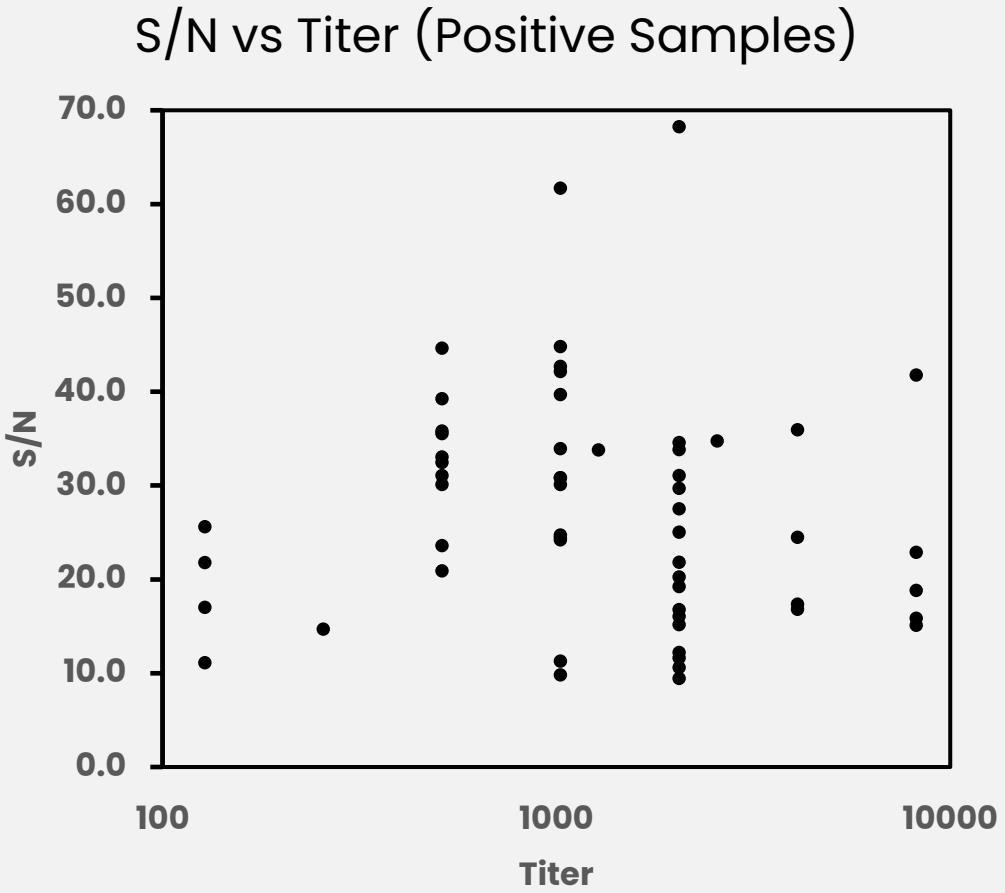


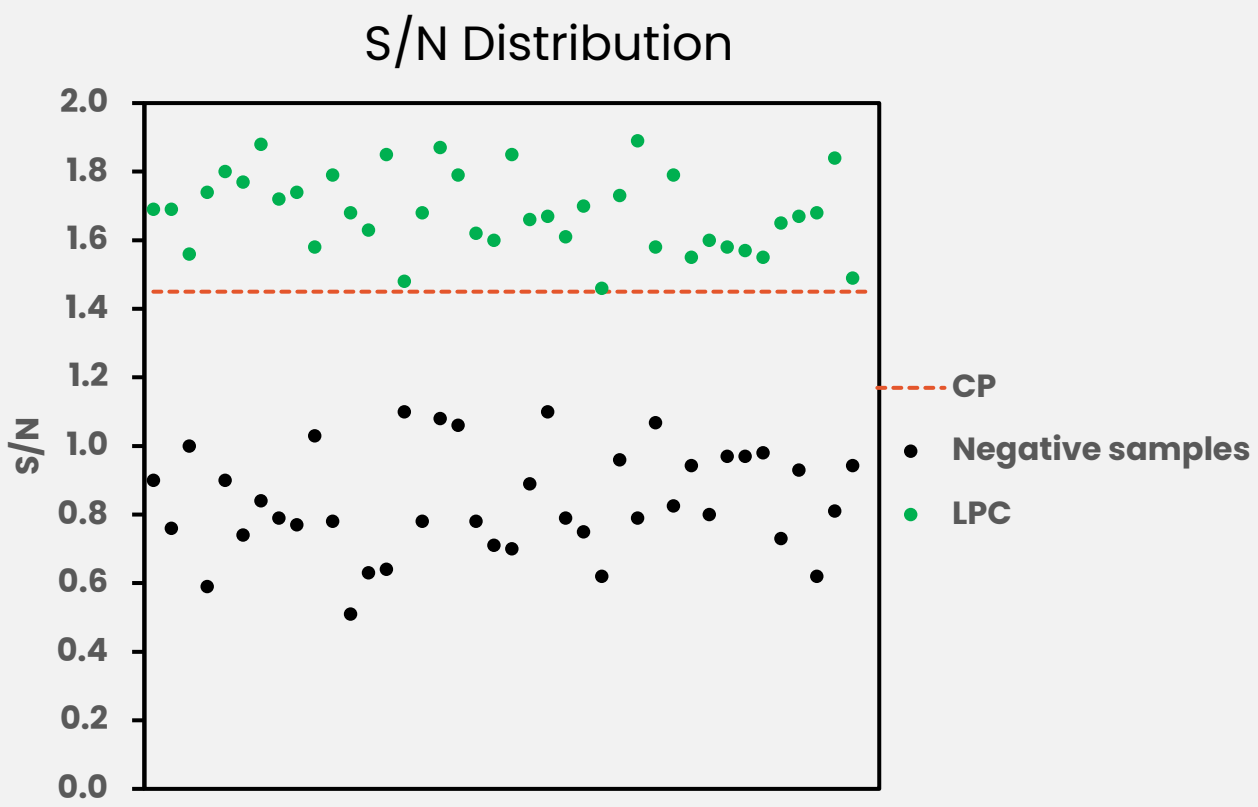
Jittered data based on real case study

Study sample analysis summary

Clinical study: Phase 1/2 Single Ascending Dose	
Total number of screened samples for pre-existing antibodies	52
Number of negative samples	4
Maximum titer observed	16000

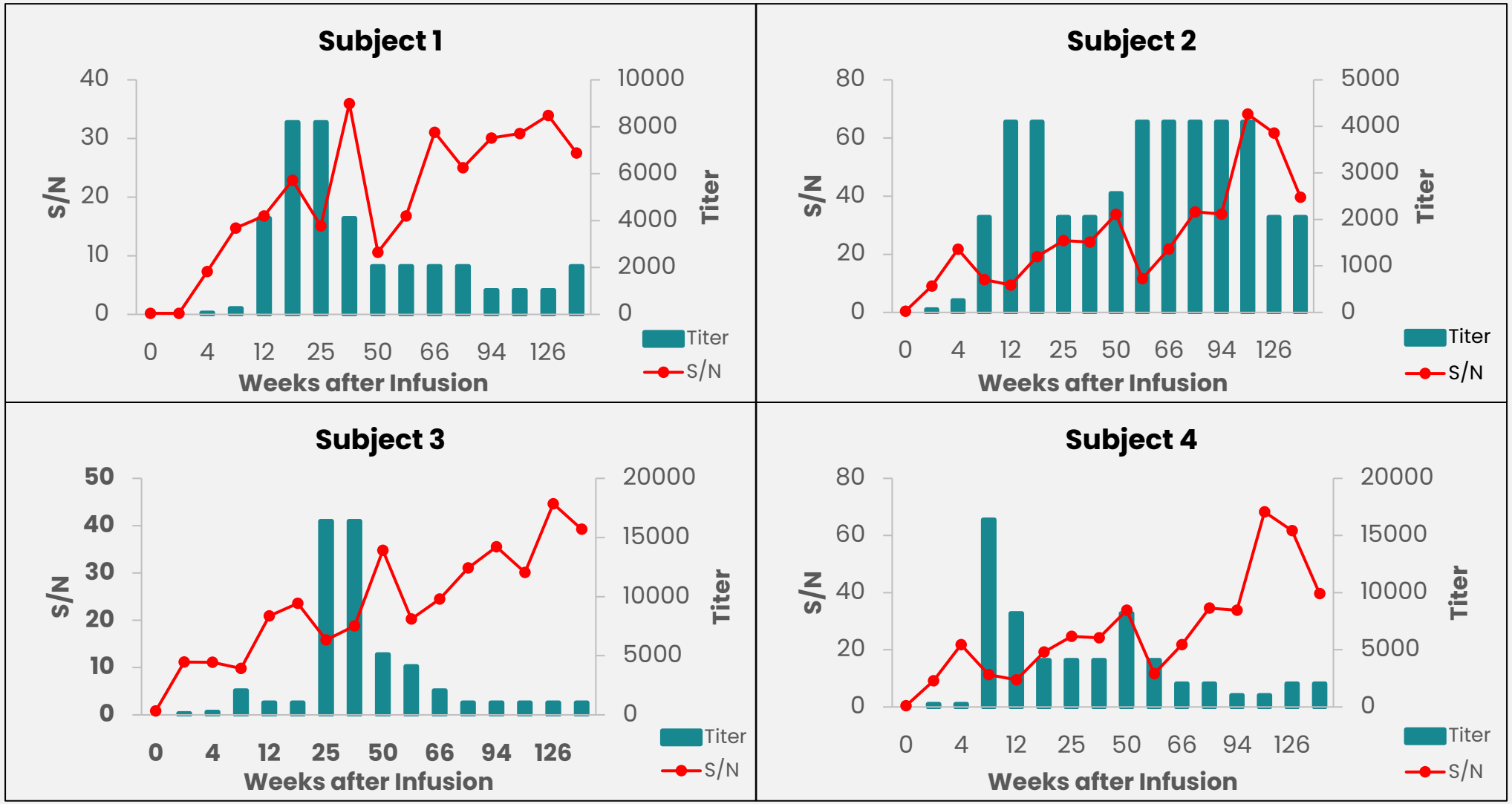
SNR vs Titer Correlation Plot



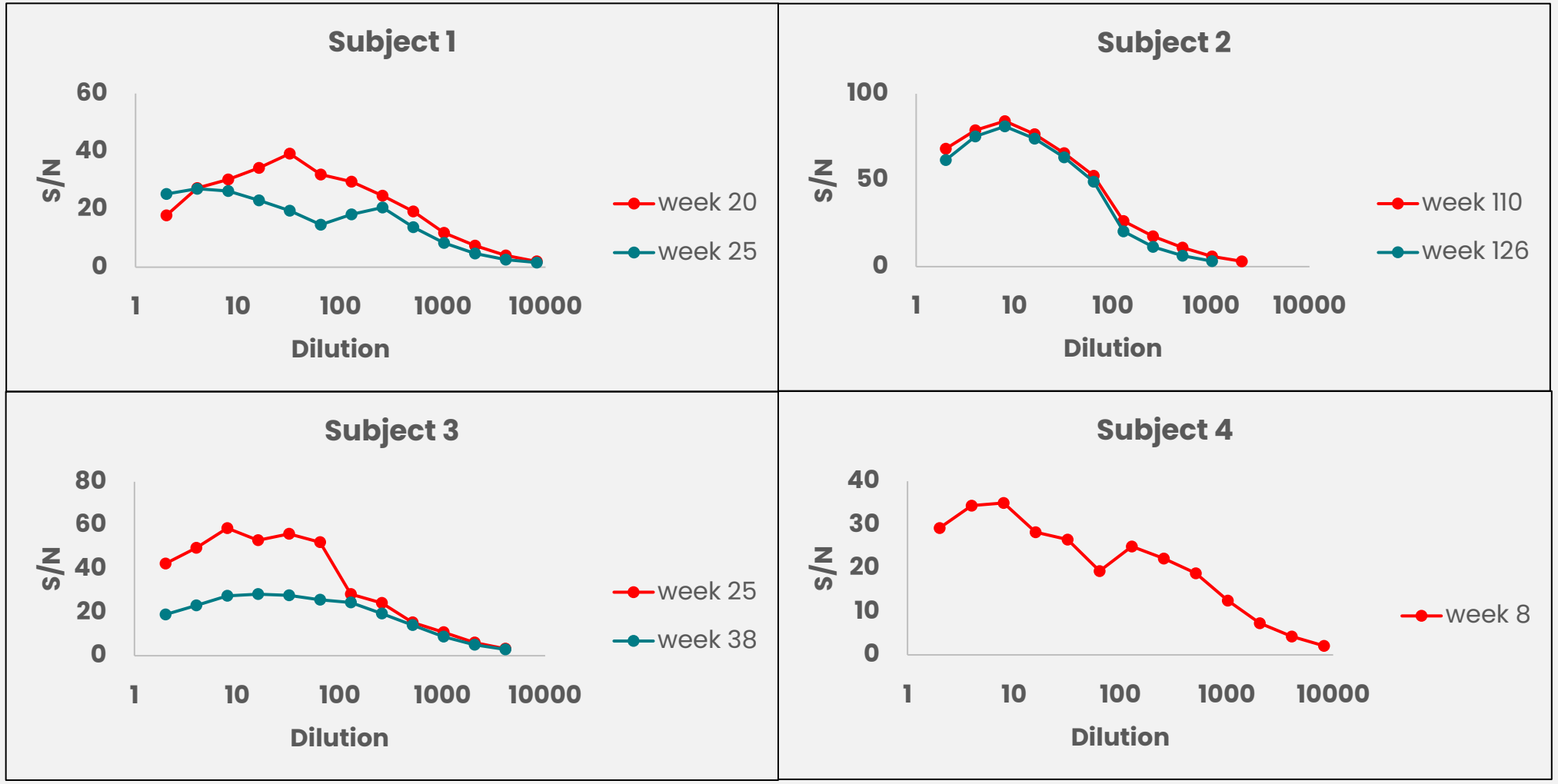


- Broad S/N distribution for same titers
- Low variability on negative samples and LPC

SNR vs titer individual profiles



SNR vs dilution in titer assessment



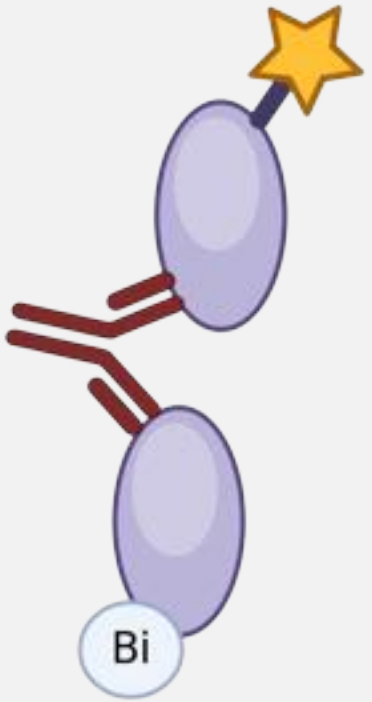
→ Hook effect observed, limits applicability

Case 2: Anti-transgene protein antibodies

Assay graphic: Created with BioRender.com

Assay summary

Anti-transgene protein antibodies assay summary	
Drug Modality	AAVx vector expressing secreted protein
Administration	Local
ADA assay type	Bridging assay using recombinant human protein as capture and detection
LPC, (MPC), HPC	10, (250), 5000 ng/mL
Platform	MesoScale Discovery (MSD)
Screening Cut Point	1.16
Titer Cut Point	1.31
Drug tolerance	Circulatory levels not detectable



Jittered data based on real case study

Anti-transgene protein antibodies assay data

Subject No.	Visit	ADA Result	Titer
001	D-1 Predose	Negative	N/A
	D14	Positive	<20.0
	D30	Positive	<20.0
	D60	Negative	N/A
	D120	Positive	<20.0
	D180	Negative	N/A
002	D-1 Predose	Negative	N/A
	D14	Negative	N/A
	D30	Positive	<20.0
	D60	Negative	N/A
	D120	Positive	20
	D180	Negative	N/A
003	D-1 Predose	Negative	N/A
	D14	Negative	N/A
	D30	Negative	N/A
	D60	Negative	N/A
	D120	Negative	N/A
	D180	Negative	N/A
004	D-1 Predose	Positive	20
	D14	Positive	20
	D30	Positive	20
	D60	Positive	20
	D120	Positive	40
	D180	Positive	20

- Higher than expected incidence of positive samples causes concern
  - Justified concerns…?
- Positive/negative and titer are not very informative to understand the data

Anti-transgene protein antibodies assay data

Subject No.	Visit	ADA Result	Titer	Screening Assay **SNR**
001	D-1 Predose	Negative	N/A	**1.09**
	D14	Positive	<20.0	**1.17**
	D30	Positive	<20.0	**1.24**
	D60	Negative	N/A	**1.14**
	D120	Positive	<20.0	**1.18**
	D180	Negative	N/A	**1.12**
002	D-1 Predose	Negative	N/A	**1.05**
	D14	Negative	N/A	**1.13**
	D30	Positive	<20.0	**1.24**
	D60	Negative	N/A	**1.14**
	D120	Positive	20	**1.32**
	D180	Negative	N/A	**1.06**
003	D-1 Predose	Negative	N/A	**1.14**
	D14	Negative	N/A	**1.07**
	D30	Negative	N/A	**1.08**
	D60	Negative	N/A	**1.04**
	D120	Negative	N/A	**1.10**
	D180	Negative	N/A	**1.04**
004	D-1 Predose	Positive	20	**1.34**
	D14	Positive	20	**1.28**
	D30	Positive	20	**1.31**
	D60	Positive	20	**1.37**
	D120	Positive	40	**1.45**
	D180	Positive	20	**1.30**

- Signal-to-noise ratio (SNR) brings more granularity to data interpretation
  - Visualisation brings additional understanding…

Anti-transgene protein antibodies assay data

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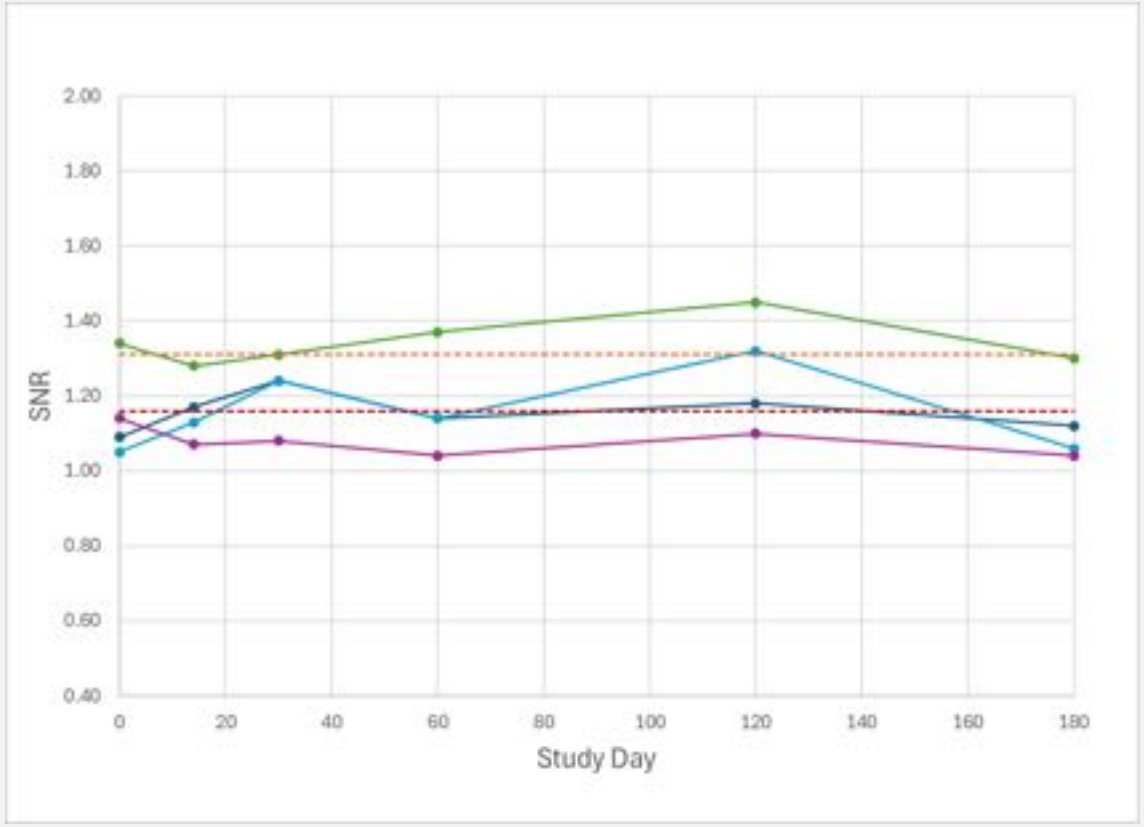

- A problem of scale…?
  - Getting lost in the weeds…?

Anti-transgene protein antibodies assay data

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001	D-1 Predose	Negative	N/A	**1.09**
	D14	Positive	<20.0	**1.17**
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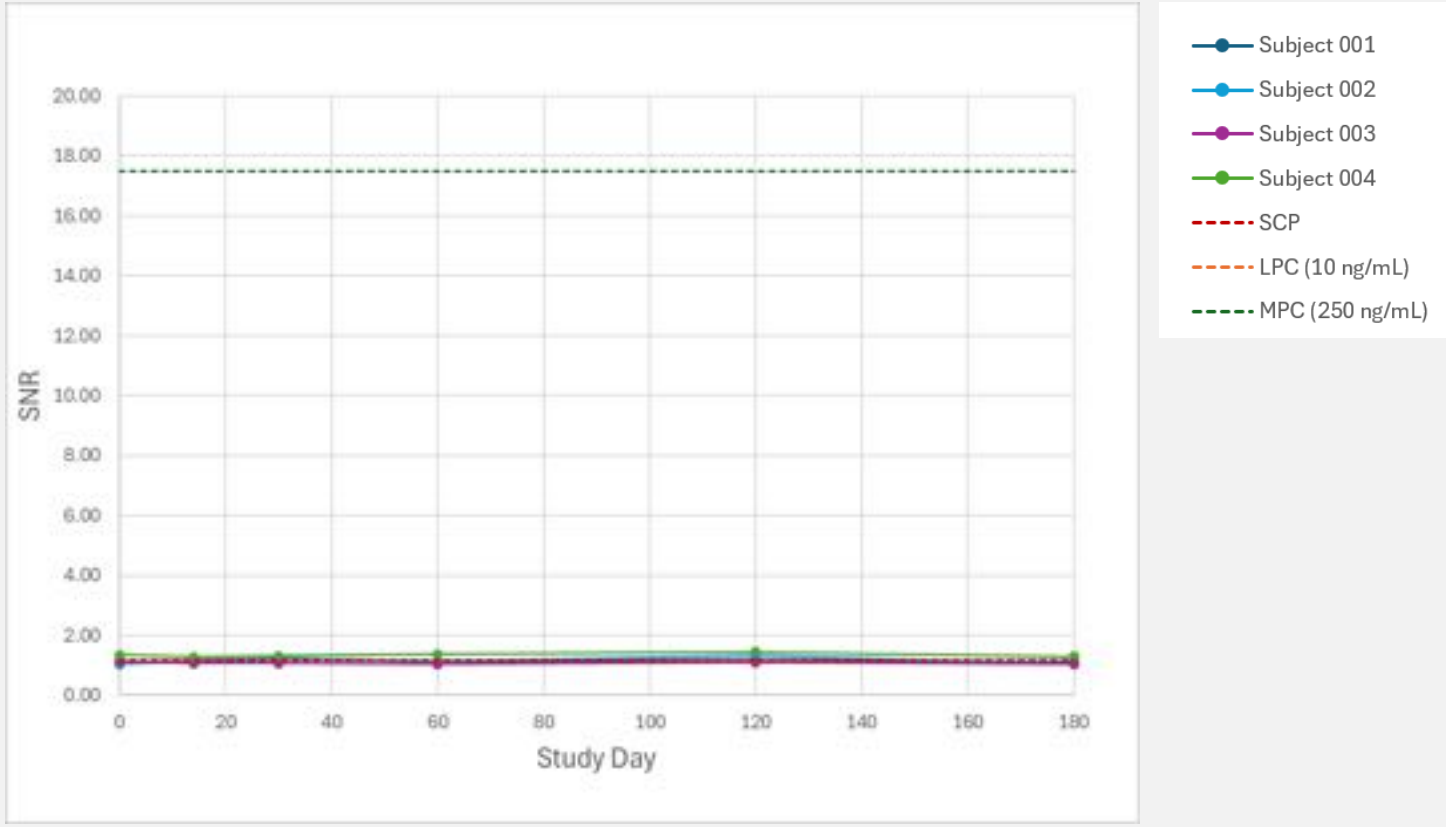

- Adding the SNR of 250 ng/mL PC for reference
- All sample responses are at the background in a very sensitive assay

Concluding remarks

Concluding remarks

- In studies with high incidence and/or high magnitude responses, SNR may be a suitable alternative
  - In the case study presented, titer provided more granularity for data interpretation
  - In other studies, SNR and titer provide very similar interpretation
  - Some liabilities may limit the approach, e.g., saturation point of SNR, drug interference
  - May depend on what is a clinically impactful response

- For many anti-capsid antibodies assays, we are running a *screen and titer* approach
  - Confirmation does not add value
  - Also consumes large quantities of (extremely) valuable reagent (Capsid DP)

Concluding remarks

- In studies with low incidence and/or low magnitude responses, SNR can provide a better understanding of the ADA data
  - SNR provides a longitudinal evaluation of response dynamics and relevance
  - Drug interference potentially a limiting factor
  - **Is a cut-point needed at all…?**

- Moving away from the 3-tier testing paradigm is possible
  - Many factors need to be considered:
    - the phase, drug modality, mode of action, etc.,
    - the performance of the assay and known liabilities
    - company risk tolerance

Acknowledgements

BioAgilytix colleagues	For case studies and scientific discussions
Sponsors	For exciting projects
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EIP	‘Obrigado’ for the opportunity to share our science

Thank You | Obrigado

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Abstract

- The 3-tiered paradigm for ADA testing (screening, confirmation, characterisation) evolved as the result of industry efforts to harmonise approaches and improve the reliability of immunogenicity testing data, at a time when biologics were a relatively new class of therapeutics. These industry best practices were soon absorbed into regulatory guidelines and have become the default approach to ADA testing.
- With more than two decades of experience, more sensitive assay platforms, and a plethora of biologic modalities, is the 3-tier testing approach still fit-for-purpose, or are there areas where we could be more time and resource efficient without compromising data quality or patient safety? The utility of the confirmatory tier to provide an orthogonal assessment of ADA positive/negative classification has been challenged (Kubiak et al, 2020), and signal-to-noise (SNR) has been proposed as an alternative to titer for reporting of ADA magnitude (Starcevic Manning et al, 2022).
- In gene therapy programs with viral vectors, the analytical assessment of humoral immunogenicity for anti-capsid antibodies and anti-transgene protein antibodies bear many similarities, but there are some clear distinctions in the data usage/interpretation and very different expectations with respect to prevalence of pre-existing reactivity and magnitude of response after dosing. Here we will present case studies from clinical gene therapy programs where we demonstrate that a screen and titer approach achieves study objectives in the assessment of anti-capsid antibodies, and that the evaluation of SNR can provide valuable insights when evaluating the relevance of anti-transgene protein antibodies.