NIBR
Translational Medicine

Assessment of lean approach for ADA determinations

EBF Autumn Meeting, Sep 21, 2023

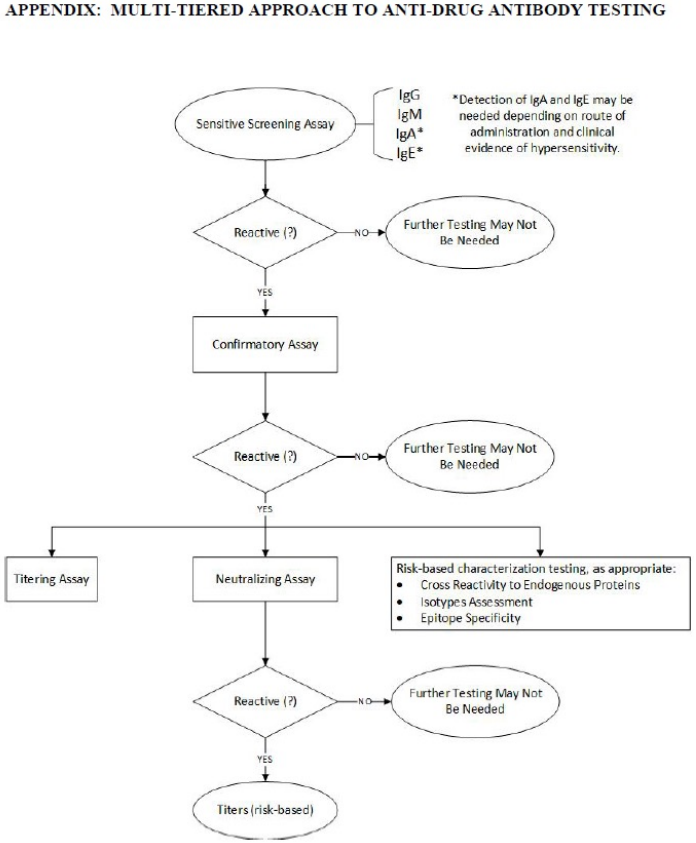
Carsten Krantz

NOVARTIS | Reimagining Medicine

Changing the current practice of ADA testing

- ADA analysis is performed in a three-tiered assay (screening, confirmatory, titration)
- Can ADA analysis be more “lean” i.e. can we omit the titration step or use solely the screening assay
- In this presentation focus on whether S/N is a viable alternative of replacing titration
    - ❖ What are the benefits/ shortcomings of S/N vs titer?
    - ❖ How do we gain regulatory acceptance?

Current practice of ADA determinations



adopted from FDA guidance 2019

- 3- tiered approach for ADA testing generally accepted
- Screening: Detection of anti-drug antibodies that bind to the therapeutic in a specific matrix
- Confirmation: Determines specificity of the assay by adding drug to the assay that suppresses the signal
- Titration: Semiquantitative measurement of anti-drug antibodies by dilution
- Neutralizing: determines the portion of ADA that compete with target binding
- Further investigations: Isotyping , epitope specificity assessment

Regulatory landscape

FDA

A. Assays for ADA Detection

Screening assays, also known as binding antibody assays, are used to detect antibodies that bind to the therapeutic protein product. The specificity of ADA for the therapeutic protein product is usually established by competition with a therapeutic protein in a confirmatory assay. ADAs are characterized further using titration and neutralization assays. Titration assays characterize the magnitude of the ADA response. It is important to characterize this magnitude with titration assays because the impact of ADA on pharmacokinetics, pharmacodynamics, safety, and efficacy may correlate with ADA titer and persistence rather than incidence (Cohen and Rivera

K. Reporting Results for Qualitative and Quasi-Quantitative Assays

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. The most common approach is qualitative, with subjects reported as having a positive or negative antibody response.

For subjects who are confirmed to be ADA positive, determining antibody levels can be informative because it allows for stratified assessment of ADAs and their impact on safety and efficacy. Positive antibody levels may be evaluated using a titer. Reporting levels of antibodies in terms of titers is appropriate and generally understood by the medical community. Most frequently titer is determined from the reciprocal of the highest dilution that gives a value at or just above the cut-point of the assay. Alternatively, titer may be determined by extrapolating the dilution to the assay cut-point using the linear portion of the dose response curve. All sample dilutions, such as the MRD and acid dissociations, should be factored into the calculations of titers and provided when reporting titers.

- Preference to quantify the ADA response using titration
- Alternative approaches may be possible but will need discussion with the agency

EMA

antibody response is important as it may correlate with clinical consequences.

If antibodies are induced in patients, serum or plasma samples need to be characterised in terms of antibody level (titre), neutralizing capacity and possibly other criteria determined on a case-by-case basis according to the biological product, the type of patients treated, the aim of the study, clinical symptoms and possibly other factors. These may include antibody class and subclass (isotype), affinity and specificity. The degree of characterization required will differ depending on the study purpose and

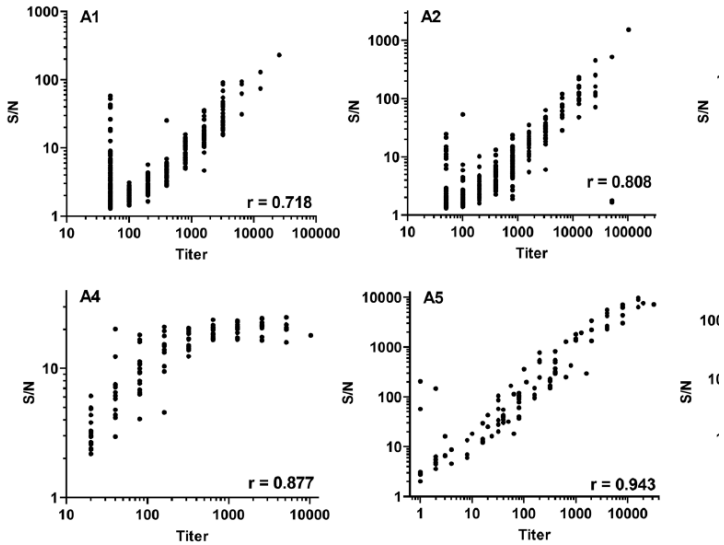
- Characterization of antibody level by titer

Titration to determine ADA levels

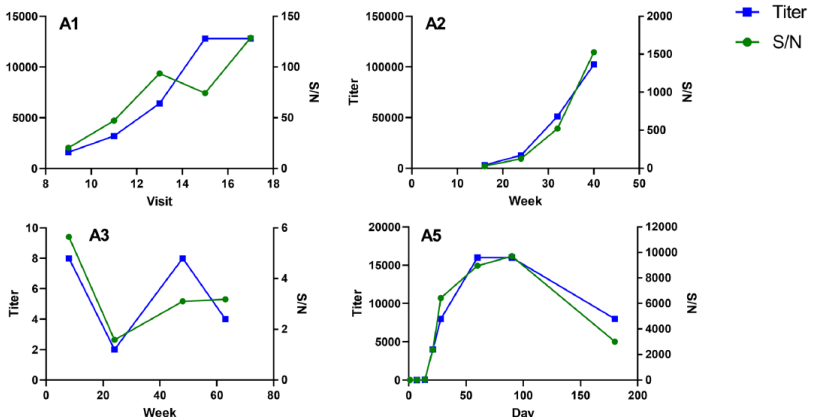
Pro	Contra
Titer used classically to determine the magnitude of ADAs	Titration often applied for ELISA assays, today's ECLIA have higher dynamic range and improved DT and sensitivity
Titration used for patient stratification	May be biased against low affinity antibodies and poor precision in the lower range of the assay range
Mitigate assay saturation, or hook effect	Titration as the 3rd step (FT cycles, sample handling) may compromise sample integrity
	Volume limitations (especially for pre-clinical and pediatric studies
	Critical reagent, time and money consuming

Signal to noise to assess ADA levels

S/N correlates well with Titer in majority of ADA assay formats across modality, IG risk level, study population and IG incidence



- Good correlation in 73% (Spearman's >0.8)
- Strong correlation in most cases also of S/N with PK and PD
- S/N follows ADA kinetics in most individuals

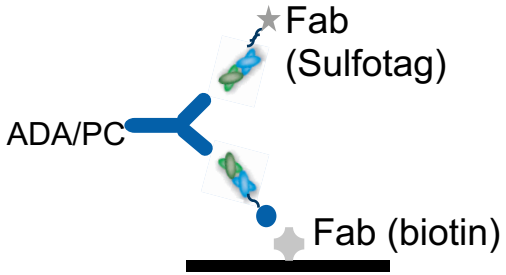


adopted from Starcevic et al, 2022

Case studies

- Case study 1 Fab in immunology, preclinical
- Case study 2 Fab in immunology clinical
- Case study 3 Recombinant protein in oncology, clinical

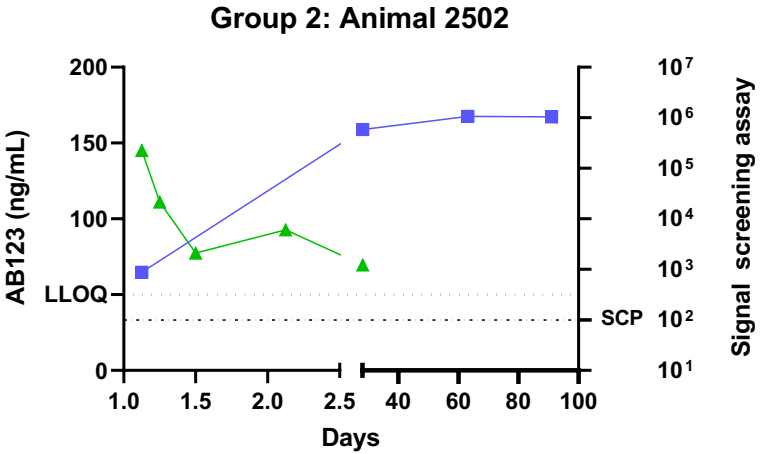
Case Study 1-Preclinical Cyno Tox

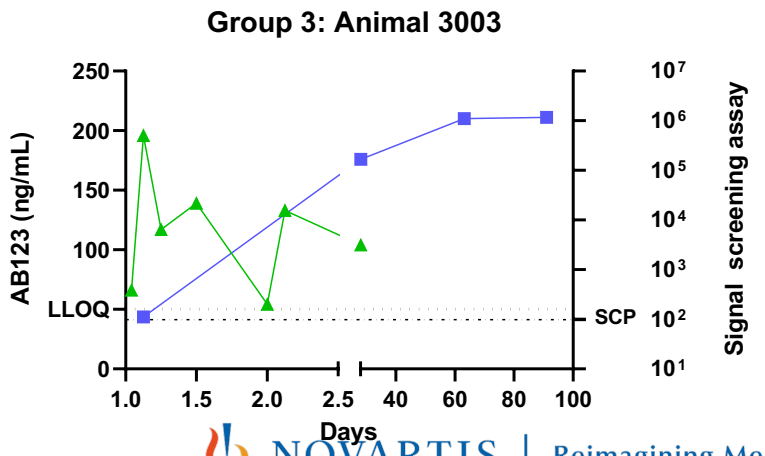


- Fab against soluble target in immunology
- Daily dosing
- Homogenous bridging MSD assay
- Sensitivity 20 ng/mL of PC in 100% cyno serum
- CPF: 1.33 (99.9th percentile)
- Screening assay only

Case Study 1-Preclinical Cyno Tox

- 100 % IG incidence in preclinical 13 wk Cyno Tox study manifesting from study day 28 onwards
- Signals of ADA screening assay correlated well with loss of exposure in some animals
- S/N good marker to express the ADA magnitude preclinically

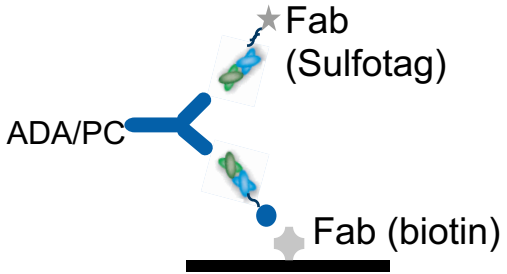




NOVARTIS | Reimagining Medicine

Case Study 2-Clinical PHI and PHII

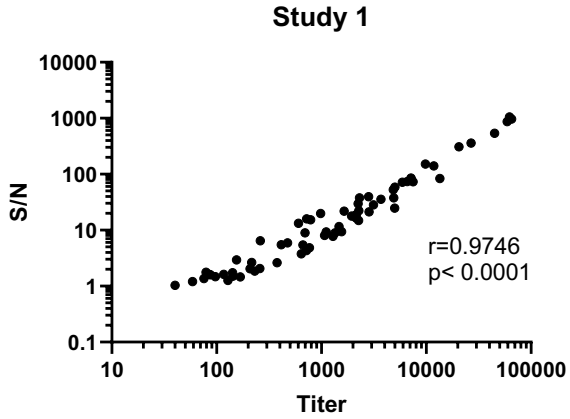
- Fab against soluble target in immunology
- Homogenous bridging MSD assay
- Sensitivity 20 ng/mL of PC in 100% human serum
- SCP: 1.7018, CCP:74%

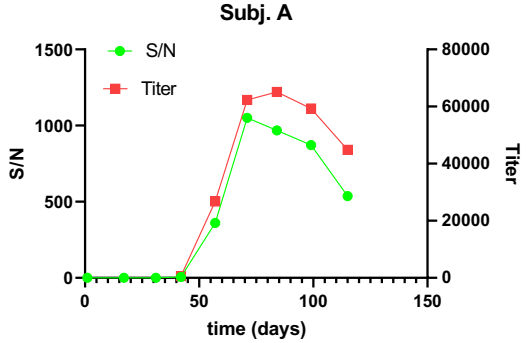


	Study 2	Study 1
Total samples	250	909
% screening positive	38 (96)	75 (702)
% confirmed positive	27 (67)	65 (605)
% false positive	12 (29)	11 (103)

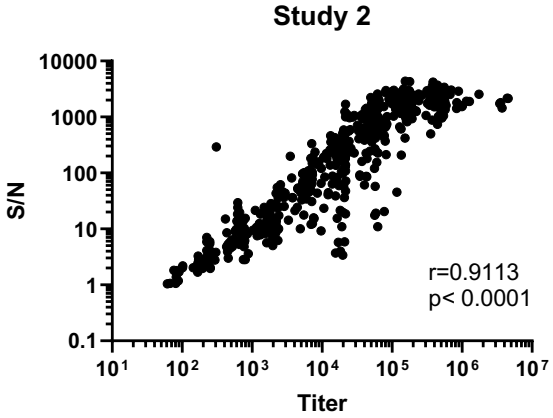
Case study 2- Fab against soluble target in immunology-Clinical

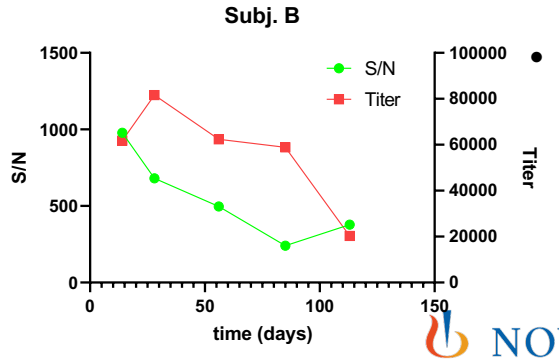
PHI





PHII

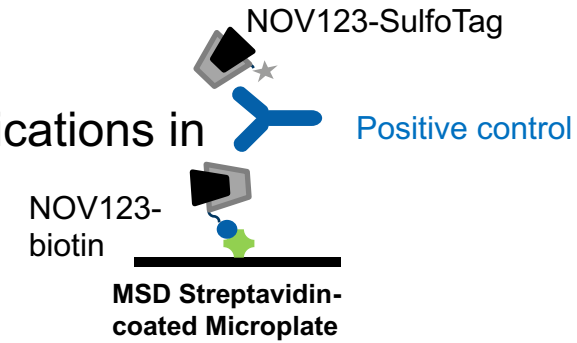




- Highly significant correlations observed in both studies
- In PHII study S/N reached a plateau where the correlation was not linear
- Individual time courses demonstrated good overlap between S/N and ADA titer except at S/N> 1000

Case Study 3

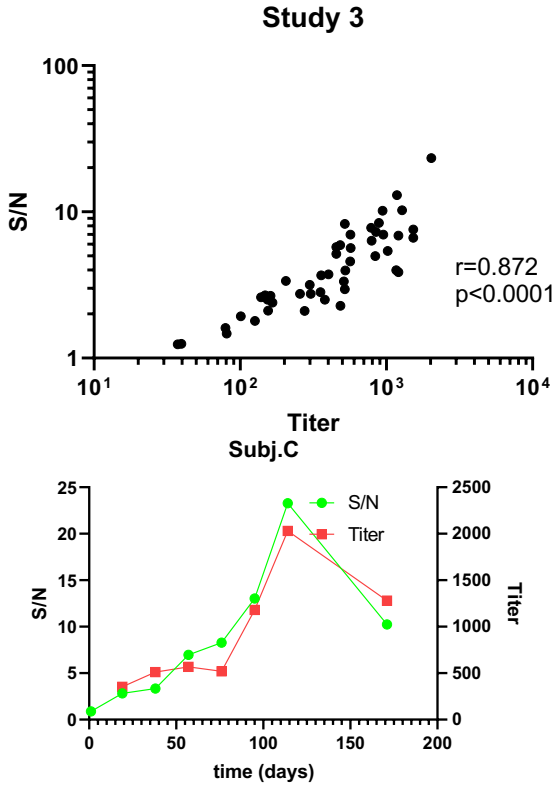
- Recombinant protein against soluble target in different indications in oncology in PH1/1b
- Homogenous bridging MSD assay
- Sensitivity 100 ng/mL of PC in 100% human serum
- SCP: 1.06 (CPF)*Mean of NC, CCP:35%



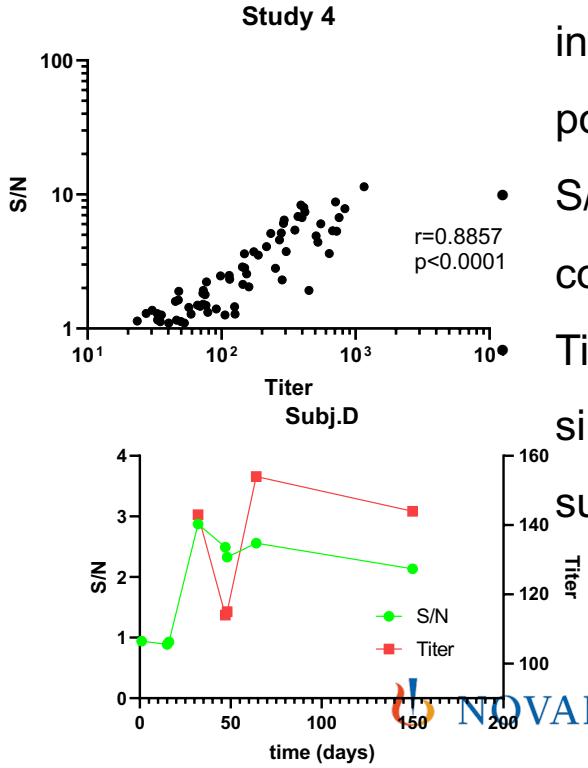
	Study 1	Study 2
Total samples	224	382
% screening positive	24 (54)	20 (78)
% confirmed positive	23 (53)	18 (69)
% false positive	2 (0.9)	2 (9)

Case study 3- Recombinant protein against soluble target in oncology

PHI /Ib
Indication A



PHI /Ib
Indication B



- Both studies correlated well independent if the patient population changed
- S/N vs titer comparison was consistent in both studies
- Titer and S/N followed similar trend in most of the subjects

Summary

- In preclinical studies S/N can help to understand the magnitude of the ADA response
- Statistical relevant correlation of S/N to titer was demonstrated in two different modalities, within different clinical phases and indications using ECLIA assays
- At high S/N plateaus were observed that led non- linearity between titer and S/N

Conclusion and recommendation

- S/N approach can explain ADA magnitudes in pre-clinical studies (for molecules where an ADA assay is deemed necessary e.g. risk classification, dosage, route of administration ...)
- Titration should be considered in preclinical species only if S/N does not work on a case-by case base
- Using standard bridging assays using the ECLIA system high correlation between S/N vs titer can be observed in clinical studies and S/N could replace titration, however non-linearities should be accounted for
- It is recommended to validate the S/N-titer dependencies in non-pivotal studies taking into account assay parameters, ADA status and patient centric parameters (e.g. matrix composition, co-medication, dose...) and approach HA for discussion before usage of S/N

Acknowledgements

- Florent Bender
- Maria Jadhav
- Hisanori Hara

and the whole BAMSO team

Thank you