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Taking the “false” out of ADA testing results

Towards better interpretation of clinical relevance

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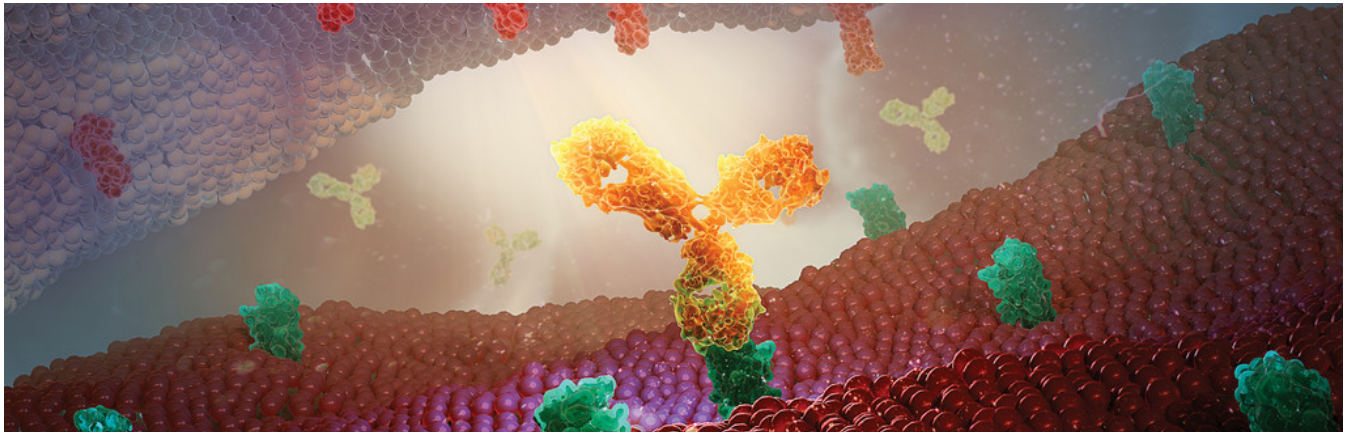
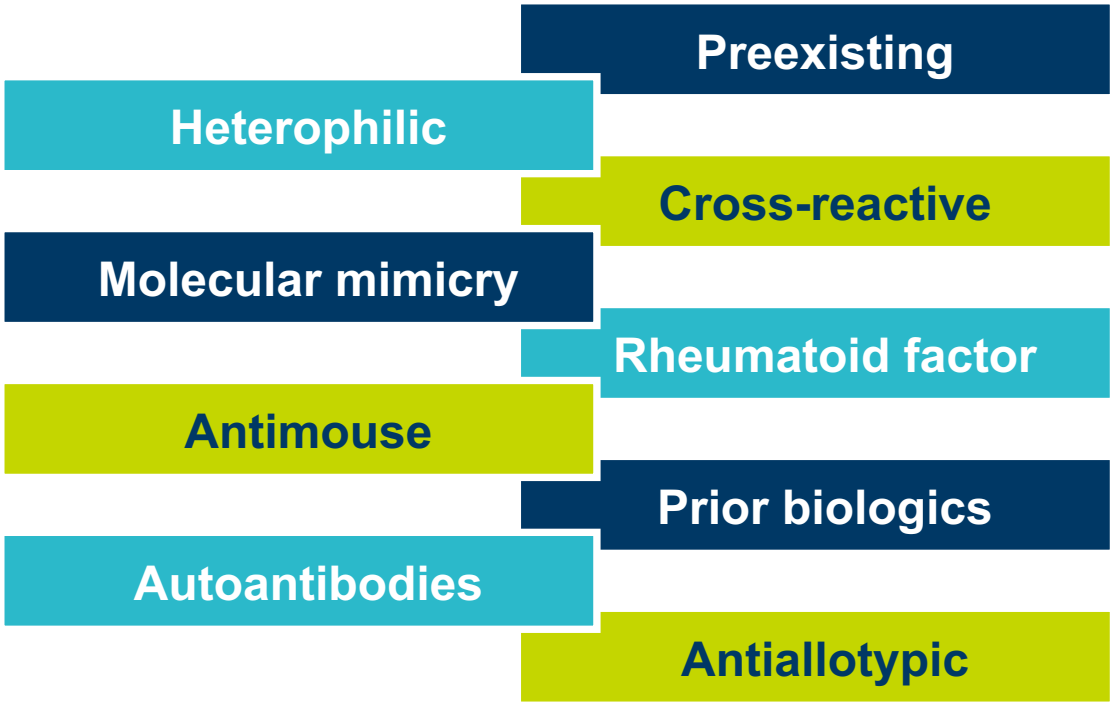


Similar prevalence and incidence of ADA positive patients commonly seen in placebo and treatment cohorts

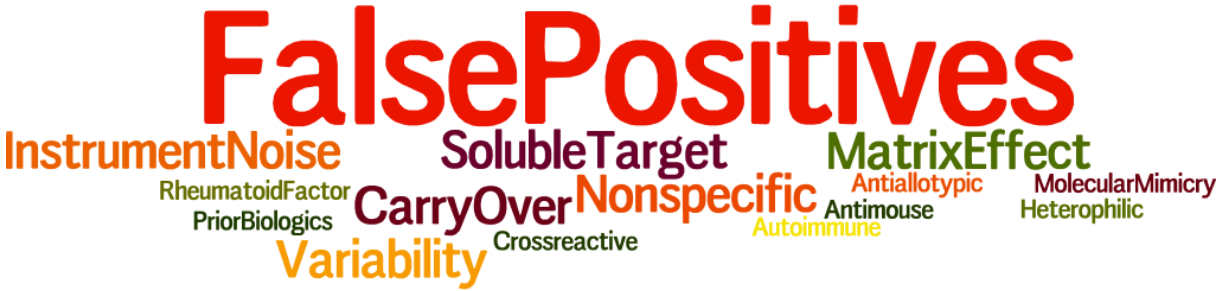
ADA results in AstraZeneca phase 3 trials

			Placebo		Treatment	
Drug	Construct	Disease	N	Prevalence Incidence	N	Prevalence Incidence
Durvalumab	Human mAb	Stage III NSCLC	200	5.0% 2.5%	401	4.5% 1.7%
Tralokinumab	Human mAb	Asthma	768	2.2% 0.8%	1160	1.8% 0.9%
Brodalumab	Human mAb	Psoriasis		Not reported	4369	2.3% 2.0%
Benralizumab	Humanized mAb	Asthma	847	4.0% 2.1%	820	14.9% 13.1%

Many immunological reasons proposed for placebo and baseline positives



In most cases the explanation is analytical



Source of expectation for fixed false positive rate (FPR):

2004 industry/FDA white paper

"A screening assay that picks up 5% positives that are subsequently shown to be due to NSB in a confirmatory (immunodepletion) assay provides assurance that true low positives can be detected"

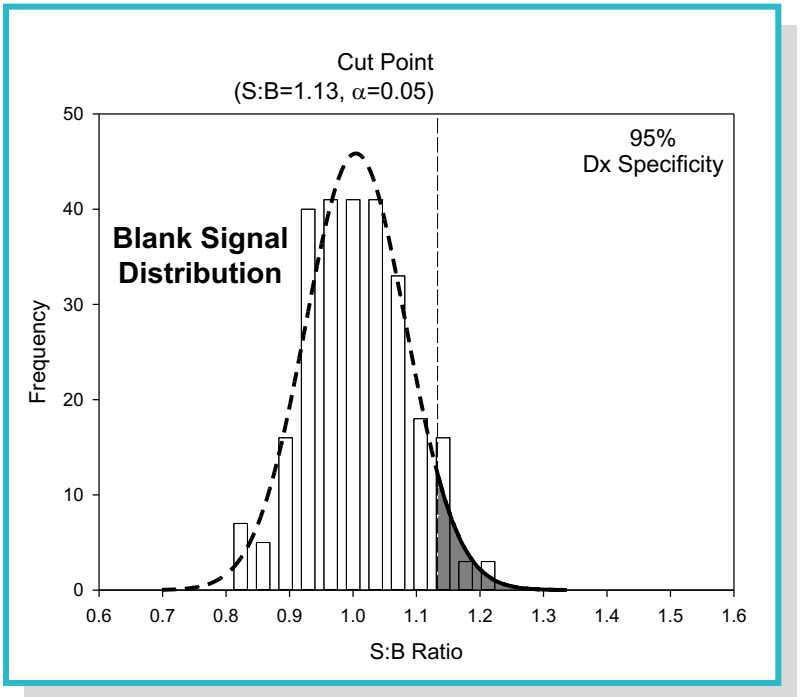
J Immunol Methods 2004 Jun;289(1-2):1-16.

Misclassifying ADA negative samples as positive has little relevance to detection of true positives

Definition:
specificity of ADA Assays

Dx specificity: probability that subjects who do not have ADA are classified correctly by the assay

Establishing Dx specificity for ADA assays



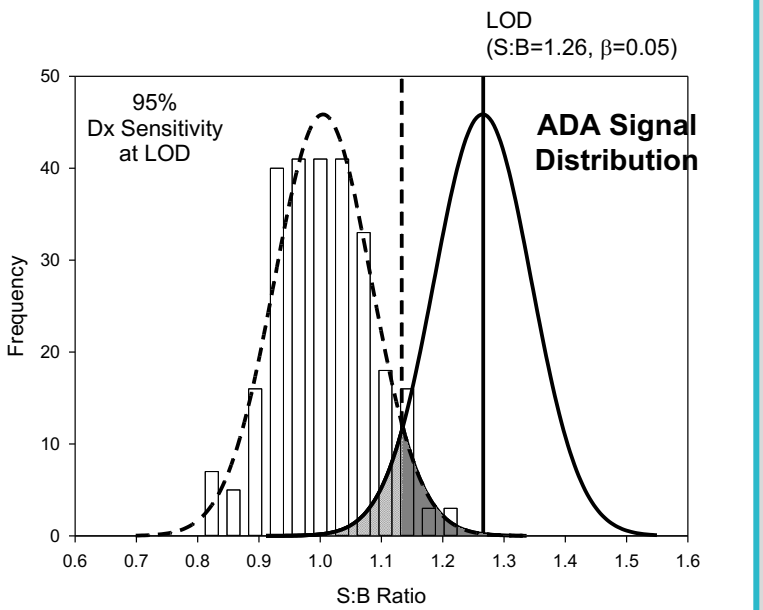
- Fixed 95% Dx specificity by FDA guidance
- Generally set after "outlier" exclusion which **increases** true FPR and **decreases** Dx specificity below 95%
- Can result very low CP

Definitions:
analytical and diagnostic sensitivity of ADA assays

Analytical sensitivity: lowest concentration of ADA that can be detected with a specified false negative error rate (Limit of Detection, LOD)

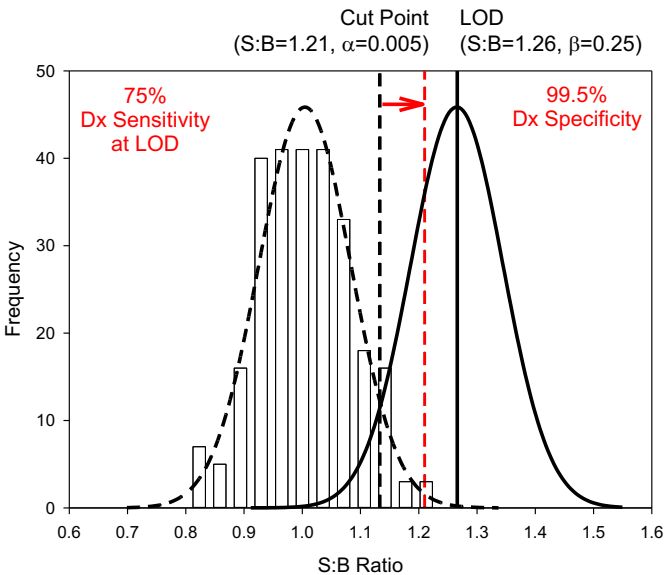
Diagnostic (Dx) sensitivity at LOD: probability that subjects who have ADA at LOD are identified by the assay

Establishing Dx and analytical sensitivity for ADA assays



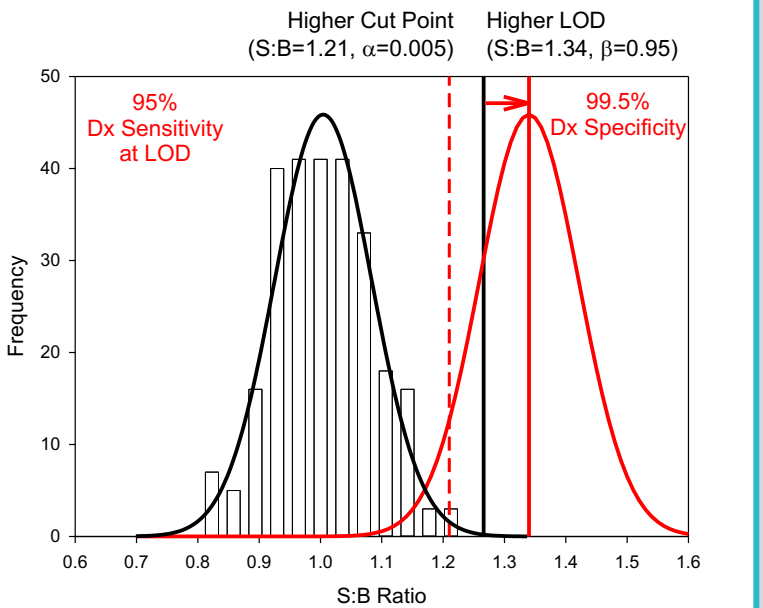
- Determine lowest ADA level associated with an acceptable false negative rate (FNR)
- This ADA concentration is the analytical sensitivity or LOD
- FNR defines Dx sensitivity at LOD (95% in this example)

Increasing Dx specificity decreases Dx sensitivity



- Dx specificity increased to 99.5% (FPR=0.5%)
- Small CP increase from 1.13 to 1.21
- Dx sensitivity decreased to 75%
- LOD unchanged! (provided FNR deemed acceptable)

Can maintain prior Dx sensitivity by increasing LOD



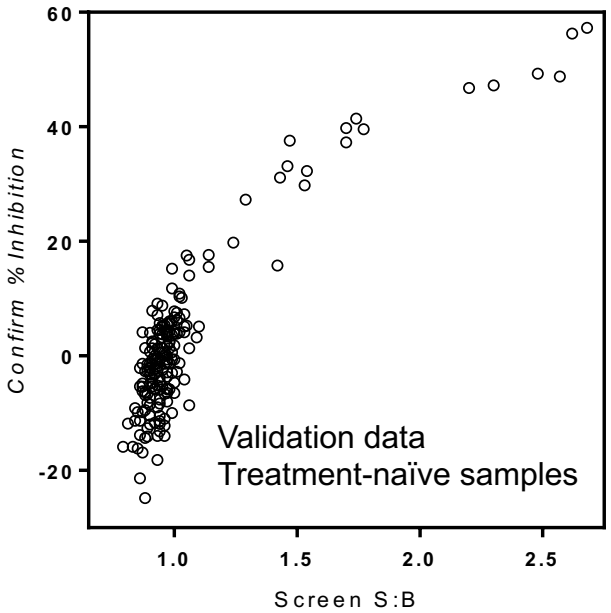
- 95% Dx sensitivity maintained, with 99.5% specificity
- Results in small increase in S:B for LOD (1.26 to 1.34)
- **Negligible loss of analytical sensitivity**

Decreasing specificity negligibly decreases LOD

Immunogenicity data from a clinical study of a therapeutic mAb were re-evaluated using cut point calculations recommended by the 2016 FDA draft guidance.

	Original validation	2016 draft guidance
Screening cut point	1.28	1.08
Confirmatory cut point	34.1%	29.7%
Limit of detection	8.65 ng/mL	5.27 ng/mL

Confirmatory assays do not adequately eliminate false positives



- Screening and confirmatory assays are usually correlated
- Correlation exists over entire range of S:B in treatment naïve samples
- False positive samples usually confirmed positive

Kubiak et al, J Pharm Biomed Anal. 2013 Feb 23;74:235-45.

The road to false positives is paved with good intent

1. Exclude “outliers” that are not pre-existing ADA
2. Set CP using unnecessarily high 5% FPR
3. Protect FPR by using lower confidence limit
   - But the nominal specificity is 95%!
4. Confirm positives in non-orthogonal assays
   - What is the problem?
5. Discount ADA results in placebo arms

Sensitivity and specificity are not good measures of assay performance in individual subjects

The overlooked importance of predictive value

Positive predictive value:

probability that subjects classified by the assay as ADA positive are true positive

Negative predictive value:

probability that subjects classified as ADA negative by the assay are true negative

- Positive and negative predictive values are the best measures of assay performance in individuals
- Clinical interpretation of ADA data usually is conducted at the individual subject level
- FDA/Industry recommendations for ADA testing result in low PPV for many products

Reduced specificity: low PPV for low incidence ADA

5% FPR heavily contaminates ADA data when incidence is low

Case #2b fixes problem by increasing specificity

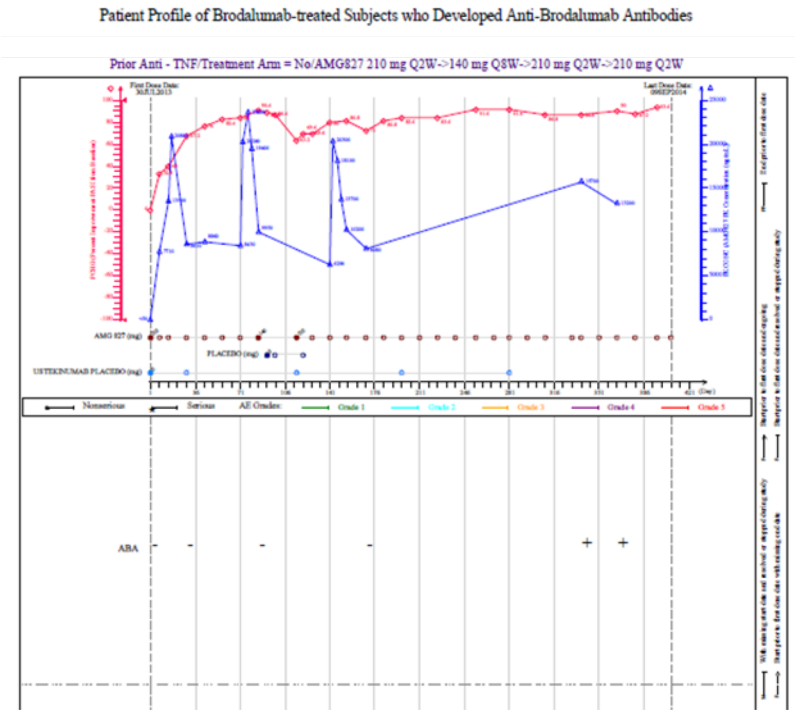
Case #1	Case #2a	Case #2b
Specificity = 95%	Specificity = 95%	Specificity = 99.5%
True ADA incidence = 50%	True ADA incidence = 2.5%	True ADA incidence = 2.5%
PPV = 50/(50+5) = 91%	PPV = 2.5/(2.5+5) = 33%	PPV = 2.5/(2.5+0.5) = 83%

EMA query: brodalumab

Individual patient data interpretation benefits from high assay PPV

> “With respect to antibody formation, the applicant is asked to make a comprehensive overview of the subjects tested positive in the complete program with any measure of PK, PD or efficacy tabulated (including sampling schedule for immunogenicity testing).”

Comprehensive overview showed no relationship between ADA status, PK, efficacy, or safety



- 107 individual ADA positive patients evaluated
- No clear evidence of true ADA positives
- Most profiles were single time point positive; all nAb neg
- No titer data available
- Assay likely has very low PPV

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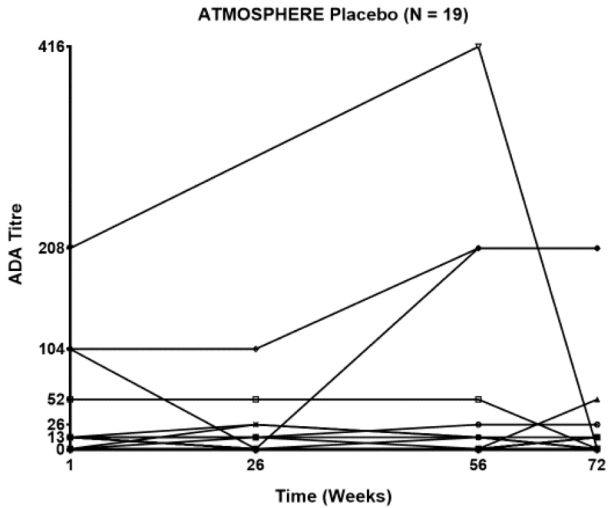
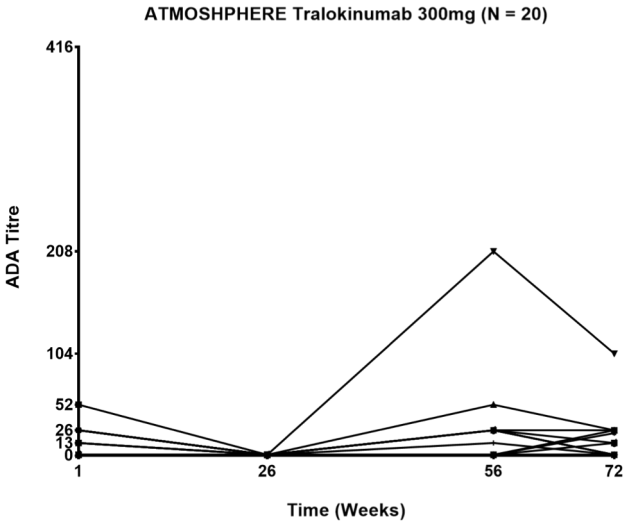
ADA titers for tralokinumab for placebo and treatment (pooled phase 3 trials)





- Longitudinal titer profiles similar for treatment and placebo
- No evidence of titer boosting relative to placebo

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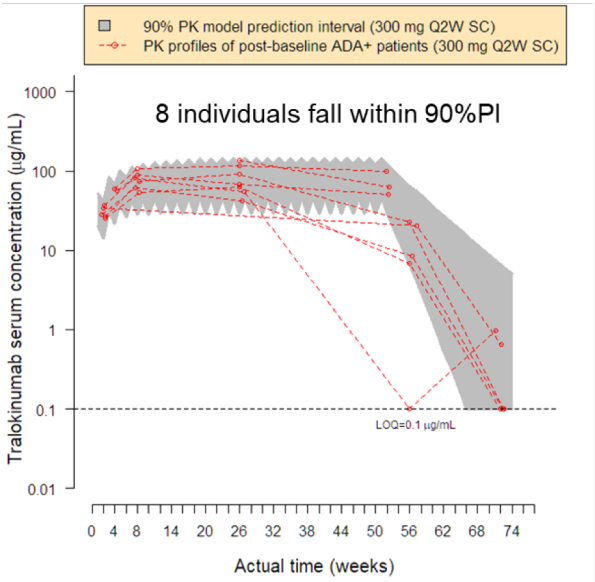
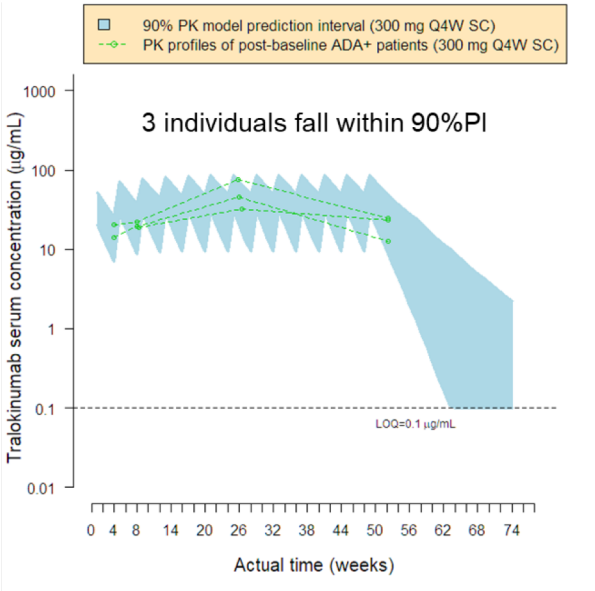
Population PK visual predictive check for tralokinumab: post-baseline ADA+ patients





- No clear effect of ADA status on tralokinumab PK
- One suspicious PK time point
- PPV ≤ 9%
- Low PPV is caused by strictly adhering to guidance

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Source: ECR 2014

An immunogenicity testing paradox

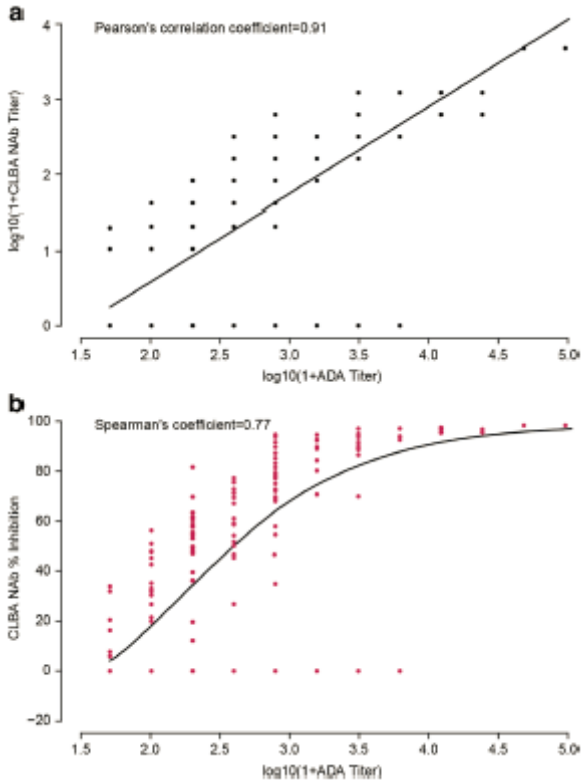
The drug-tolerant nAb assay

nAb data: the most misunderstood area of immunogenicity testing

Considerations for drug-tolerant NAb assay data

- NAb positive status indicates ADA can potentially neutralize drug
- Does not tell if ADA levels are sufficiently high to neutralize drug in vivo
- Does not identify neutralization by other means (increased clearance or impaired biodistribution)
- A robust pharmacodynamic endpoint is better than any nAb assay

Most ADA to human mAbs are to neutralizing epitopes

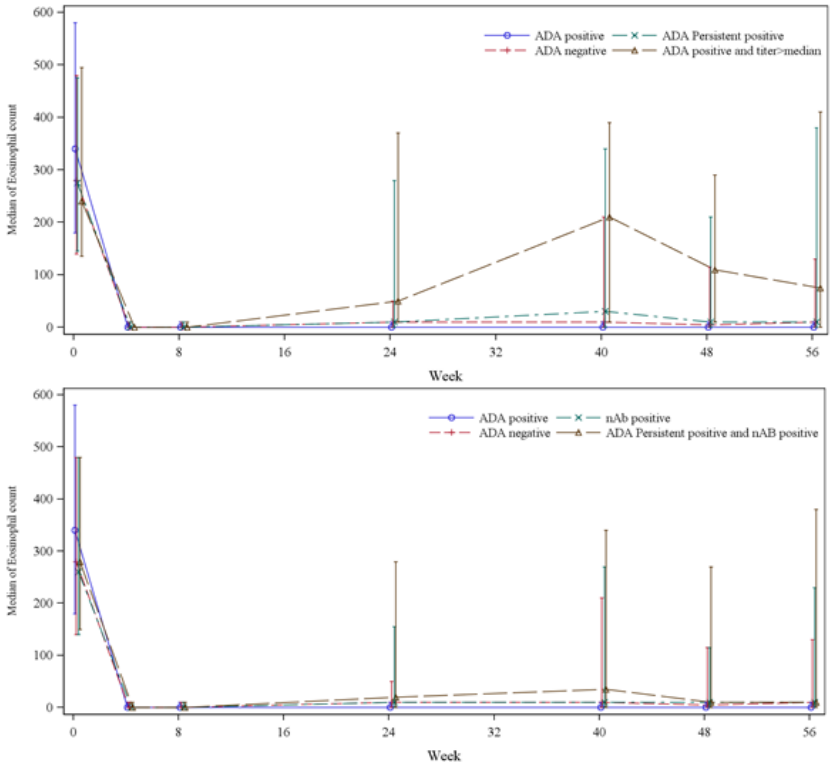


- For benralizumab, strong correlation between ADA titer and nAb titer
- ADA positive samples with no measurable nAb were from placebo cohorts or patients with drug levels higher than drug tolerance
- NAb status tells little about ability to neutralize in vivo

Wu et al,
AAPS J. 2018 Mar 14;20(3):49.

Evaluation of ADA effects by titer is usually best approach

Eosinophil depletion by benralizumab (30 mg Q8W) pooled CALIMA and SIROCCO phase 3 studies



- For benralizumab, ADA effect on median pharmacodynamic profile at trough seen when ADA titer > median
- ADA effect on PD not seen in overall ADA positive or nAb positive subsets

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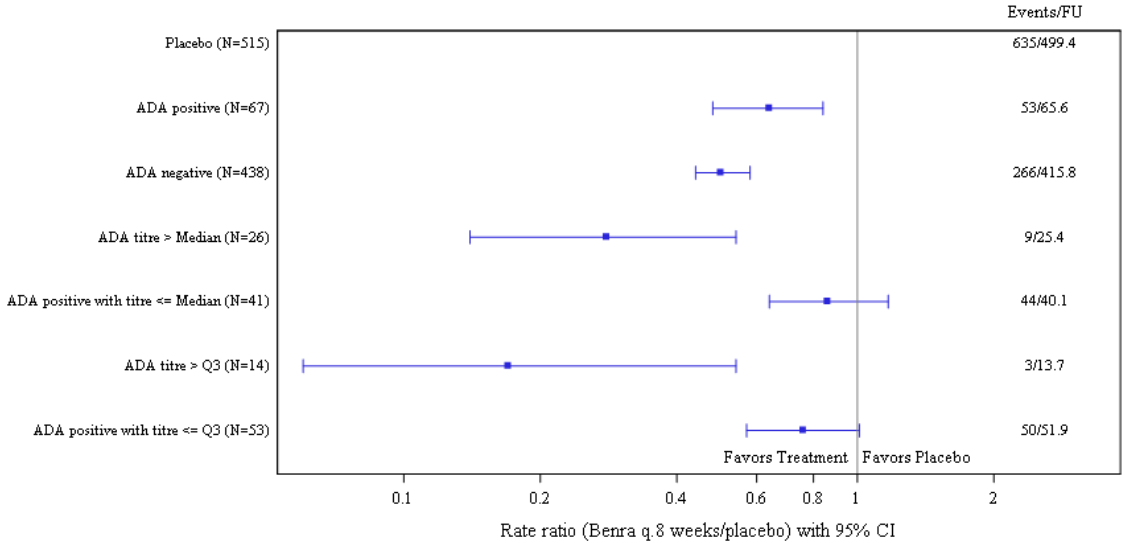
Primary efficacy endpoint evaluated by ADA titer

Annual asthma exacerbation rate (full analysis set) pooled CALIMA and SIROCCO phase 3 studies



No effect of ADA on primary and secondary efficacy endpoints for benralizumab, even in patients with high titers (ADA titer > Q3)

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Despite limited value of nAb assays for mAbs, health authorities still query drug tolerance

EMA query for brodalumab

"Due to the low drug tolerance of the Nab assay (1.25 µg/mL) which is much lower than that of the screening assay … the conclusion that no subject developed neutralizing antibodies cannot be endorsed."

PMC from FDA for durvalumab

"Provide a comprehensive report that supports the approach AstraZeneca proposes to use in classifying the majority of ADA binding responses as neutralizing responses."

Conclusions

- ADA testing that strictly follows guidance can heavily contaminate ADA results with false positives
- The resulting low PPV for many ADA assays is counterproductive when associating ADA status with clinical outcome
- A low PPV ultimately will lead to false or missed associations
- Increase specificity by modifying outlier exclusion practice and decreasing false positive rate if the desired LOD and drug tolerance can be maintained
- Regulatory requirements for nAb testing should be eliminated for most mAbs; monitor ADA titers and PK-PD instead

Acknowledgements

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