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Challenges with pre-existing anti-drug antibodies

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EBF - Focus Workshop
Current analysis of immunogenicity – Best Practices and Regulatory Hurdles
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Agenda

1. Introduction to pre-existing anti-drug antibodies (pre-ADA)
2. Challenges with pre-ADA
3. Examples for assay cut point evaluation / assay strategy in case of the presence of pre-ADA
4. Summary/Considerations

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1. Introduction

Pre-existing anti-drug antibodies are:

- Present before a subject is treated with a biotherapeutic
- Specific (e.g. anti-PEG-ABs, ABs to antibody fragments) or cross-reactive (e.g. Rheumatoid factors, heterophilic ABs)
- Either part of the natural antibody population or antibodies of an adaptive immune response to similar biotherapeutics or environmental antigens
- Potentially affecting PK, efficacy, safety, occasionally without clinical impact

Gorovits *et al*, *AAPS J*, 18(2), 2016

Found for biotherapeutics which are potentially foreign to a patient

→ e.g. new constructs

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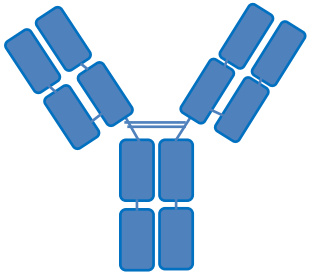
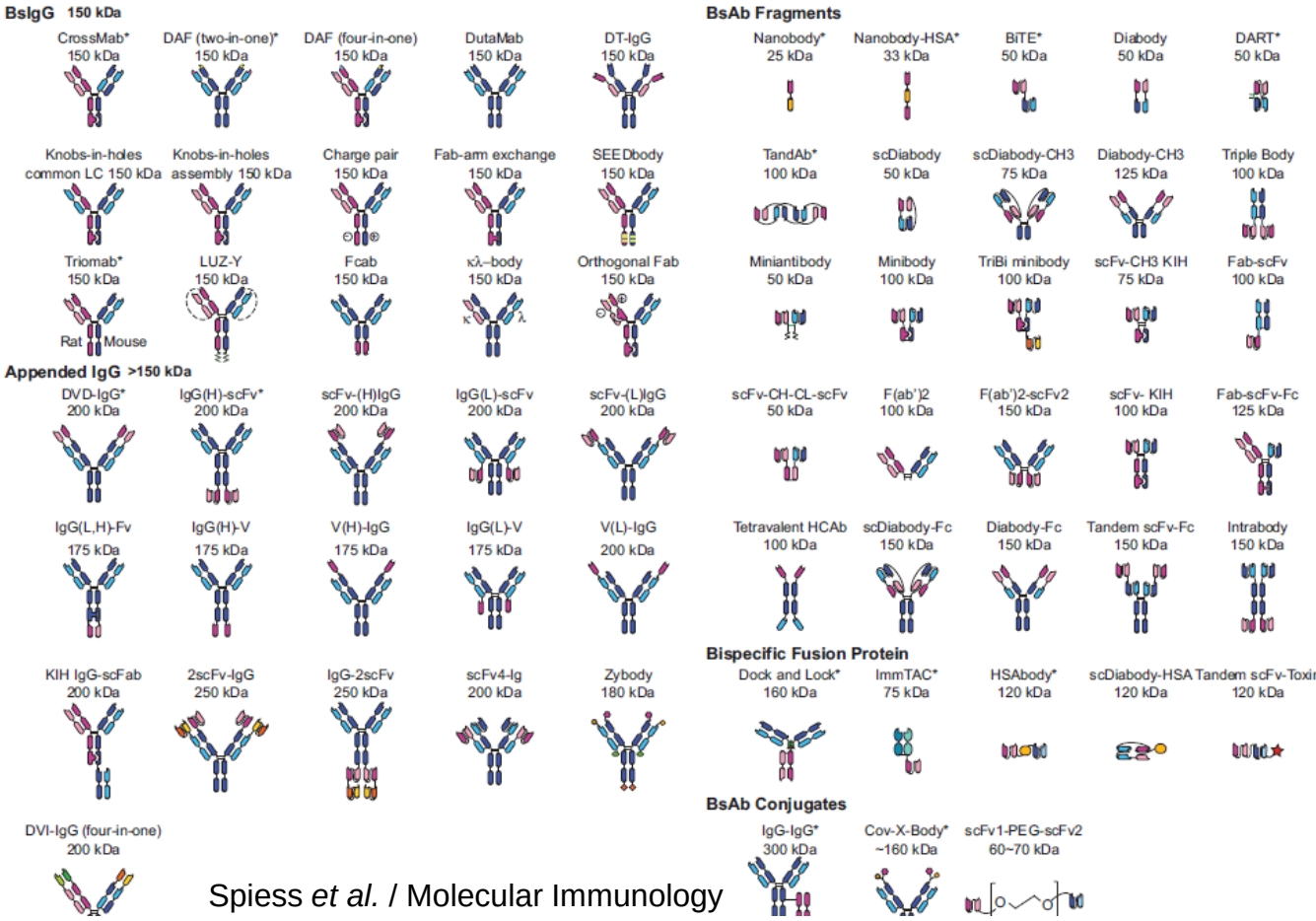
1. Introduction

Examples for new constructs:



Humanized AB
-umab



Spiess *et al.* / Molecular Immunology

-> growing evidence, also in literature, about existence of pre-ADA

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1. Introduction

- *Literature example: TAS266* - agonistic tetravalent Nanobody ® targeting the DR5 receptor

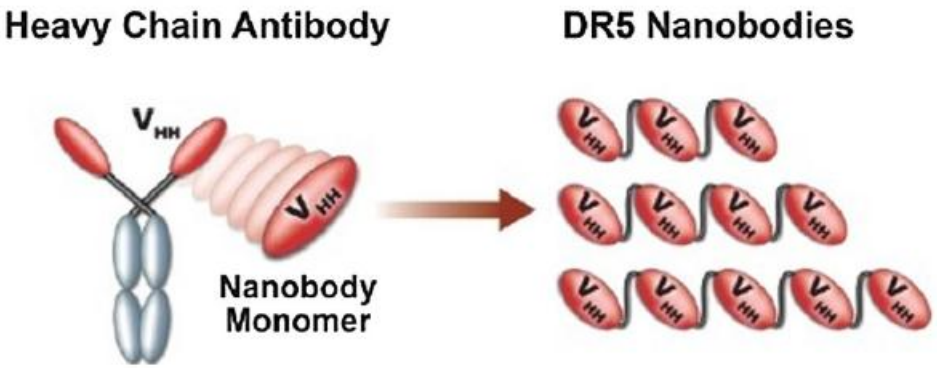

Table 3 TAS266 Immunogenicity

Patient	Study day	Anti-TAS266 antibody presence	Optical density (OD)
1	1	Yes	0.68
	10	Yes	1.94
	15	Yes	2.03
2	1	No	
	8	No	
	14	No	
	21	No	
	29	Yes	0.41
3	1	Yes	0.24
	9	Yes	1.05
	15	Yes	1.64
4	1	Yes	1.00
	5	Yes	0.81
	8	Yes	1.610
	15	Yes	2.06

Positive: OD >0.16. Patients 1, 3, and 4 experienced liver enzyme adverse events

Papadopoulos KP, *et al*. Unexpected hepatotoxicity in a phase I study of TAS266, a novel tetravalent agonistic Nanobody® targeting the DR5 receptor. Cancer Chemother Pharmacol. 2015;75(5):887-95

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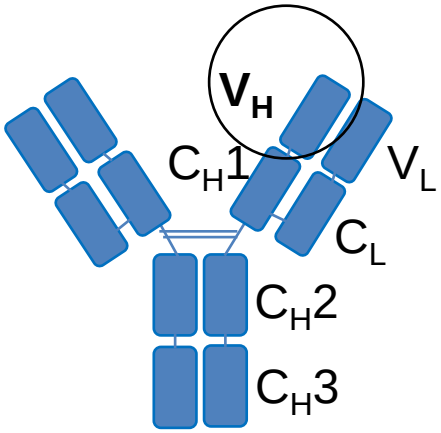
1. Introduction

- *Literature example: GSK1995057* - fully human, single heavy chain variable domain (V_H) antibody directed against the TNFR1 receptor



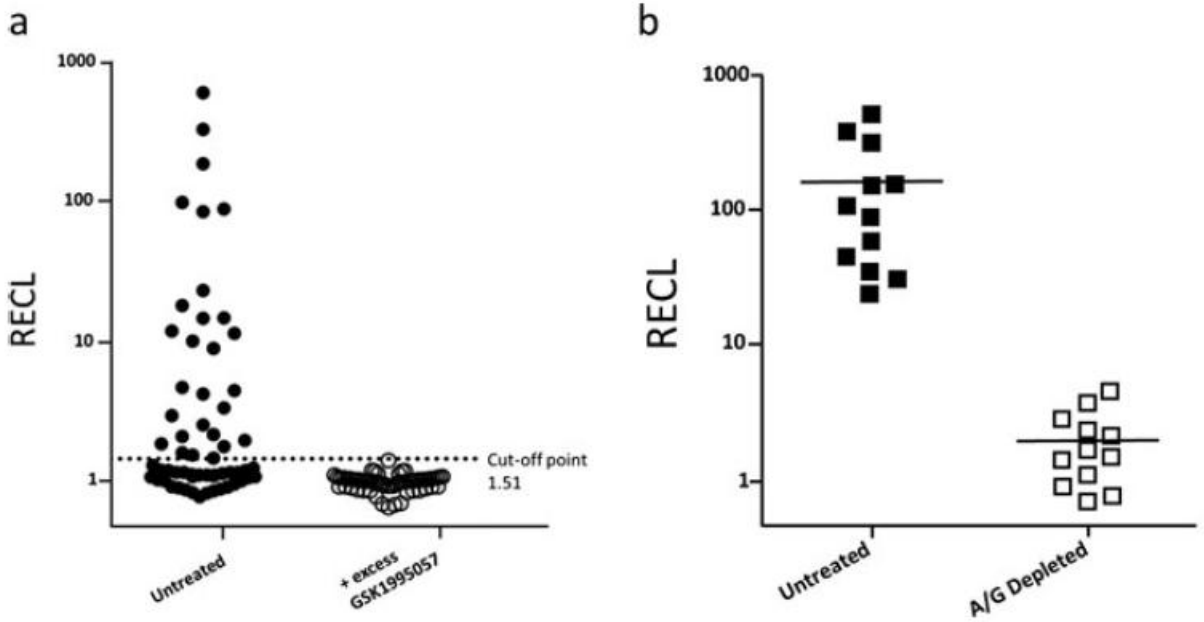


Fig. 2 **a** Screening of a serum panel from normal subjects (n=60) in the antibody detection assay. The screening cut-off point was a relative electrochemiluminescent (RECL) level of 1.51. Pre-incubation with excess unlabelled GSK1995057 (immuno-competition) reduced the signal below this cut-off point in all subjects (signal inhibition >40.5 %; data not shown). **b** Samples identified as autoantibody-positive (n=13) were depleted of immunoglobulin (Ig) using Protein A/G. Depleted samples were tested in the antibody detection assay and compared to non-depleted control samples.

Holland MC, *et al.* Autoantibodies to variable heavy (VH) chain Ig sequences in humans impact the safety and clinical pharmacology of a VH domain antibody antagonist of TNF-α receptor 1. J Cli Immunol.. 2013 Oct; 33(7):1192-203

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1. Introduction

- *FDA Draft Guidance for Industry - Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products; April 2016*

  – "The sponsor should identify those samples with pre-existing antibodies, ... and remove them from the cut point analysis."

  – "If the presence of pre-existing antibodies is a confounding factor, it may be necessary to assign positive responses or a cut point based on the difference between individual patient results before and after exposure."

  – "An alternative to the qualitative screening assay approach may be needed to assess the quantity and quality of ADA when pre-existing antibodies are present. For example, testing samples for an increase in ADA"

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1. Introduction

- *EMA Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins; Sept 2015*
  - "Pre-existing antibodies against a variety of protein therapeutics are frequently encountered."
  - "While the impact of pre-existing antibodies on safety and/or efficacy of biologics is poorly understood, consequences could be severe. Therefore, potential cross-reactivity with pre-existing antibodies should be considered."
  - "Some individual's/patient's samples may contain pre-existing antibodies ... for this is necessary to ensure that post-treatment data can be interpreted correctly in terms of treatment emergent antibodies."
  - "Evaluate impact of pre-existing Abs on pharmakokinetics, safety and efficacy"

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2. Challenge

Setting up an assay strategy which:

- Distinguishes pre-ADA from false-positive results due to other interfering factors, e.g. multimeric drug target
- Avoids generation of an inappropriately high cut point
- Reduces risk of false-negative results
- Measures treatment-boosted ADA

→ No standard procedure exist to determine an immunogenicity assay cut point when a high prevalence of pre-ADA is observed

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3. Examples for assay cut point evaluation / assay strategy in case of presence of pre-ADA

- Example 1: Usage of Immunoglobulin (Ig) depleted naive human sera for assay cut point evaluation (cell-based therapy)
- Example 2: Measuring samples without assay cut point (gene therapy)
- Example 3: Usage of drug inhibited naive human sera for assay cut point evaluation (nanobody therapy)

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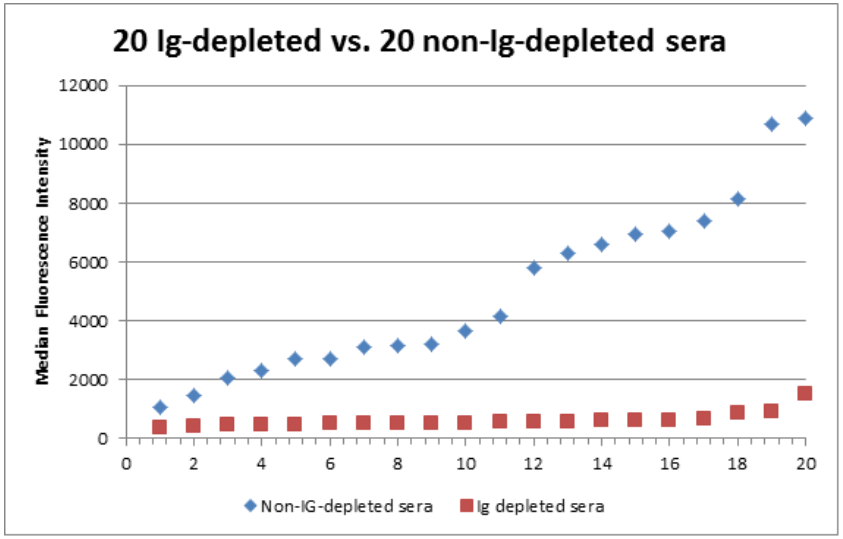
Example 1: Usage of Ig depleted naive human sera for assay cut point evaluation

- *Challenge:*

  Pre-ADA against drug were detected in majority of evaluated normal human sera.



- *Possible solution:*

  IgG/IgM depleted sera were used to establish the cut point factor (CPF)

Using Ig-depleted individual sera	Using naive individual sera
Low CP: majority of individual samples which showed high signal were reported as IG positive – safe approach	High CP: >50% of the samples which showed high signal would have been reported as IG negative – risky approach

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Example 1: Usage of Ig depleted naive human sera for assay cut point evaluation

- *Cut point evaluation:*
  - Naive human Ig-depleted sera (62) were 3x analyzed
  - CPF was defined with a non-parametric approach as the 99.9th percentile
- *Result evaluation:*

  1) Screening assay:
  - 1st: all samples were evaluated with the assay specific cut point
  - 2nd: pre-dose sample was used to calculate a subject-specific cut point

  2) Titration assay:
  - For ADA+ subjects the pre-dose and a post-dose sample with the highest signal was titrated

  3) Conclusion:
  - Evaluation of potentially boosted ADA response, Evaluation for sustained or transient ADA, Conclusion on clinical significance

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Example 2: Measuring samples without assay cut point

- *Challenge:*

  Literature reports pre-ADA in up to 100% of monkeys

- *Possible solution:*

Strategy:

1. No screening assay cut point factor determination
2. ADA assessment directly in confirmatory assay
3. Confirmed positive sample titration

Source: Lydia Michaut/ Kerstin Kentsch (Novartis) - Based on Nov-2015 presentation at EBF meeting

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Example 2: Measuring samples without assay cut point

- *Cut point evaluation:*
  - No CPF was evaluated
  - Negative control pool based on sera with very low signal un-specific for the drug
  - Global confirmatory assay precision was determined (n=108): 24%
- *Result evaluation:*

  1) Confirmatory assay:
  - Samples were analyzed: drug-spiked and naive
  - Drug-inhibited samples compared to the same naive sample ≥ 24% -> ADA+

  2) Titration assay:
  - ADA+ samples titrated in 4 dilution steps
  - Titer Cut Point = 2 x plate mean negative control

  3) Result:
  - 89% of the samples were and analyzed in the titer assay

Source: Lydia Michaut/Kerstin Kentsch (Novartis) - Based on Nov-2015 presentation at EBF meeting

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Example 3: Usage of drug inhibited naive human sera for assay cut point evaluation

- *Challenge:*

  Naive samples contained both ADA+ and ADA-

- *Possible solution:*

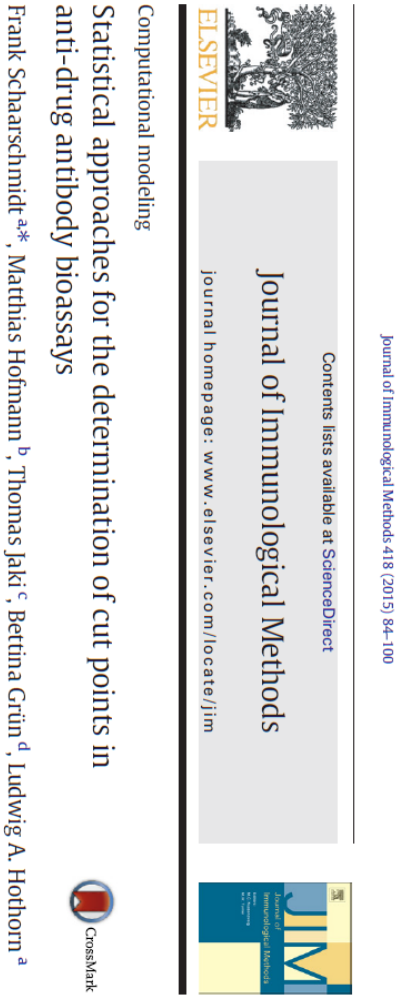
Strategy:

1. Selecting ADA- sera assuming a mixed distribution model for cut point determination

Examples published →

2. ADA assessment directly in confirmatory assay based on a subject specific cut point

3. Confirmed positive sample quasi-quantified



Source: Kerstin Kentsch, Matthias Hofmann (Novartis)

Example 3: Usage of drug inhibited naive human sera for assay cut point evaluation

- *Cut point evaluation:*
  - Sera were classified into ADA+ and ADA-

Example: F. Schaarschmidt *et. al*.

  - Sera with the lowest signal were used for determination of CPF and confirmatory CP

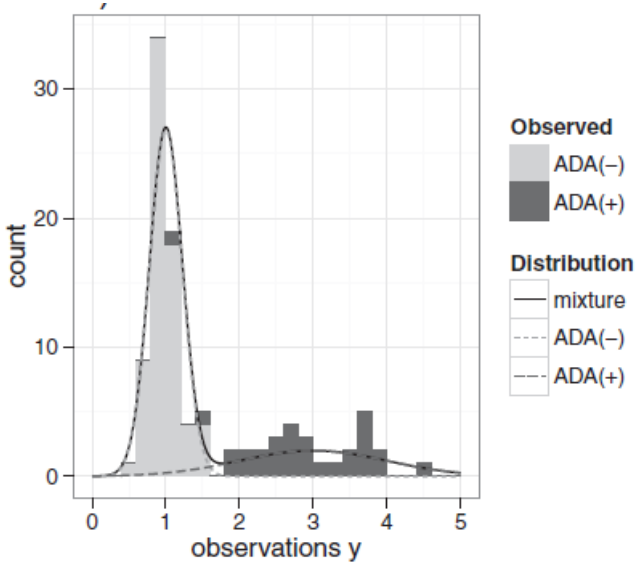


Fig. 2. Histograms of 100 observations with two subgroups. a) 100 observations drawn at random from a mixture model with 2 normally distributed subgroups, (75% truly ADA^-, mean = 1, variance = 0.05; 25% truly ADA^+ (mean = 3, variance = 1). Dotted and dashed lines depict the underlying normal densities of the subgroups.

- *Result evaluation:*

1) Confirmatory assay:
- Result of the drug-spiked sample was disregarded in case the signal of the drug-unspiked sample was below the assay cut point

2) Titration assay:
- In ADA+ the immune response was quasi-quantified to assess the intensity of the ADA response.

Source: Kerstin Kentsch, Matthias Hofmann (Novartis)

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Summary/Considerations

- Different ways possible to set up an IG assay for biotherapeutics with high prevalence of pre-ADA
- Immunogenicity can be related to safety, therefore avoid false negative results -> lower cut point should be preferred
- Report prevalence of pre-ADA along with treatment-boosted and treatment-induced ADA response
- Evaluate impact of pre-ADA on Immunogenicity, e.g. higher IG in subjects with *vs.* without pre-ADA
- Evaluate clinical impact of pre-ADA on PK, PD, safety, efficacy
- Pre-ADA should be included in the immunogenicity risk assessment

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Thank you

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