Recent Developments in the ***PK, PD, ADA Integrated Approach*** versus in vitro ***NAb Assay - New case studies and evolving trends***

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Outline

Clinical Relevance of anti-drug antibodies

Importance of the integrated data approach – high level

Case study

Regulatory feedback

Future perspectives

Relevant ADA information for doctors and patients in the label?

Clinically relevant ADA to be reported in product labels

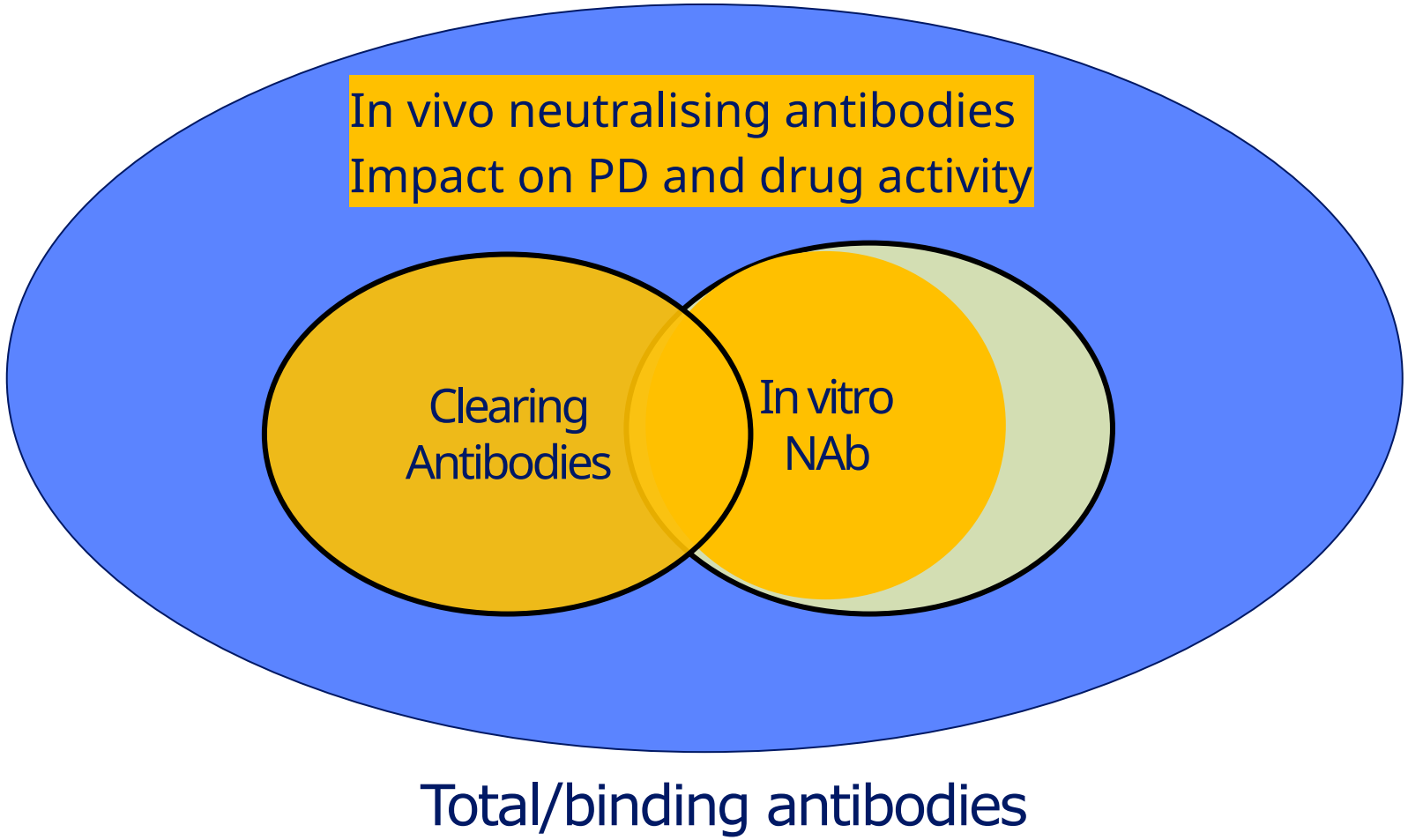
Standalone NAb assay:

- Translation to *in vivo* can be complex
- POS/NEG for NAb not always conclusive
- Lacking knowledge about the clinically relevant levels of NAbs

Integrated data approach (holistic view):

- Evaluate impact on **exposure**, **efficacy** and **safety** by correlating **levels and persistence of binding ADA** to PK, PD, biomarkers and safety events
- Can also include relevant *in vitro* NAb assay

Clinical relevance of antibodies



Clearing antibody *versus* neutralizing antibody

Binding antibodies (ADA)

ADA with clinical impact
(*In vivo* neutralising antibody positive)

ADA without clinical impact
(*In vivo* neutralising antibody negative)

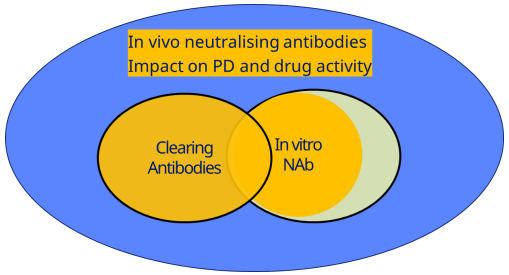
Affected

- Clinical PD marker
  - bleeding, hyperglycemia or growth
- In vitro PD marker
  - aPTT, HbA1c or IGF1
- Loss of drug activity
- Reduced drug due to clearing

Not Affected

- Clinical PD marker
  - bleeding, hyperglycemia or growth
- In vitro PD marker
  - aPTT, HbA1c or IGF1
- Drug activity unchanged
- Drug concentration unchanged

In vitro neutralizing ADA: Positive OR Negative



Clearing antibody *versus* neutralizing antibody

Clinically relevant NAbs to be reported in product labels - that can guide ‘doctors’

Similarities	Differences
Active drug is not present/available	NAbs may work faster as no large immune complexes needs to be formed
Decreases clinical efficacy due to absence of active drug	NAbs may work at lower concentration as the Ab:drug ratio for inactivation is closer to 1. Clearing Ab in ~5+ fold excess to drug (1)
	Clearing Ab needs to be polyclonal or target a repetive antigen and in excess to drug (1)
	NAb prevents binding to target (mode of action)

Ref. 1 – McSweeney et al. 2018 - J Control Release. 2018 August 28; 284: 171–178

Case Story – Integrated data approach versus *in vitro* neutralizing antibody assessment

Investigational drug – Low immunogenicity risk:

- mAb targeting a soluble ligand, no risk of ADCC
- Drug is used on top of standard care
- No adverse events related to anti-drug antibodies in phase 2
- Low frequency of ADA formation in phase 2
  - One case <1% with an effect on PK, PD
- Rare event trial => ADA correlation to clinical effect impossible on patient level

Case Story – Integrated data approach versus *in vitro* neutralizing antibody assessment

At End of Phase 2 meetings (US, EU, Japan, Canada):

Do you agree that an integrated data approach to assess neutralizing antibodies can be used in phase 3?

- Immunogenicity risk is low
- PD biomarker that reflects the presence of the active drug target (ligand)
- Ligand binding PK assay – MSD, likely ADA interference
- Binding antibody assay – bridging format, MSD, no drug interference at relevant conc. of drug
- Long drug halflife in µg/mL levels likely to compromise *in vitro* nAb during and post treatment
- ADA, PK and PD samples will be assessed at the same time points for an optimal integrated data approach
- Back-up samples will be stored should further ADA characterization be considered valuable

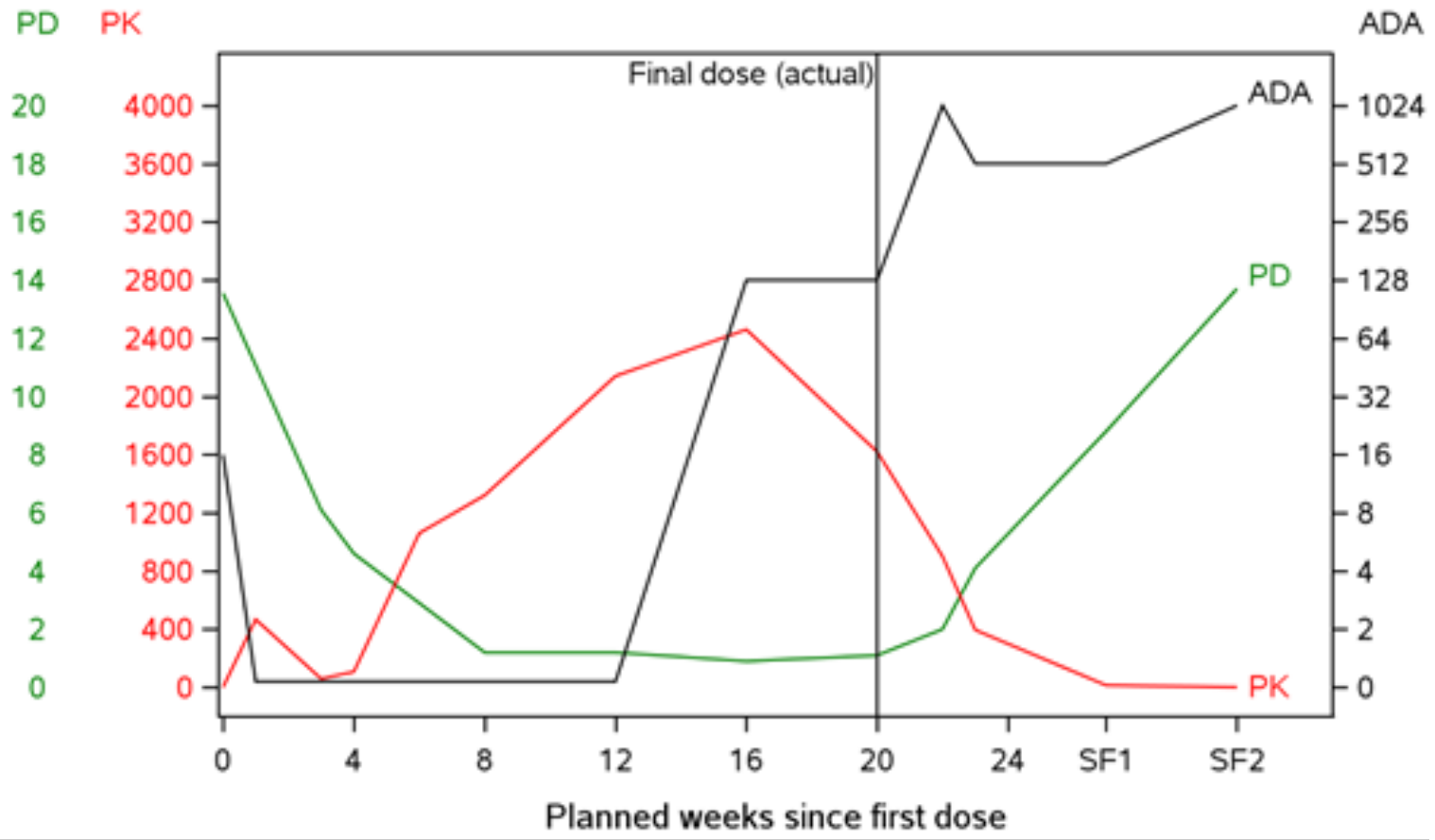
- Presentation of a text book example of high ADA leading to reduced PK, thereby impacting the PD activity

Regulators are open for an integrated data approach

FDA 2019 guideline: Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

In selected cases, where there is a highly sensitive PD marker or an appropriately designed PK assay or both that generate data that inform clinical activity, it may be possible to use these in lieu of a NAb assay. This determination should be done in consultation with the Agency.

Case Story – ADA's effect on PK, PD



Event cascade:

High titer ADA development =>

Reduced PK =>

Increased PD (no effect of drug)

Note: Individual patient profile. Only case with a titer >128
SF visits 1 and 2 scheduled 7 and 11 weeks after final dose, respectively.
PD concentration in green.
PK concentration in red
ADA titers in black (MRD not multiplied, sensitivity 30 ng/mL)
Abbreviations: ADA = anti-drug antibodies; PD = pharmacodynamics; PK = pharmacokinetics; SF= safety follow-up

Case Story - a highly sensitive PD marker?

Clinical PD

- Reflects the clinical response at any given time point
- Not interfered by circulating drug
- Directly shows the impact of ADA – Clearance/Neutralisation

Biomarker PD

- Reflects circulating drug activity
- Unbound target (free)

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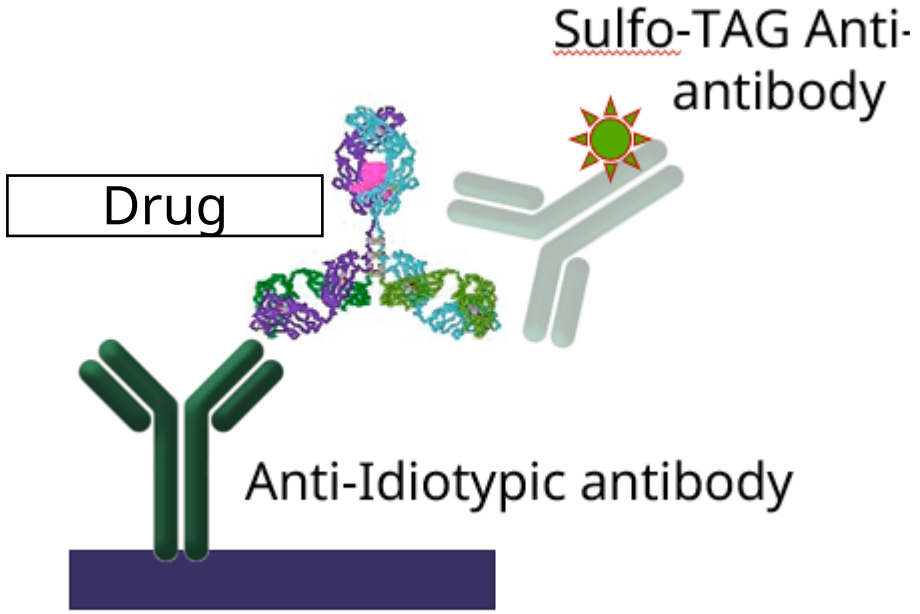
Case Story – An appropriate PK Assay?



Free: Can be inhibited by ADA – NAb LBA like and clearance

Total: Not inhibited by ADA - clearance

Consequence on interpretation of data using the integrated approach

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Case Story – Regulatory feedback

- **One example** to evaluate the neutralising effect by an integrated data approach is not sufficient
  - *That makes it difficult to build a case for non-immunogenic molecules*

- **The PD marker is not the only down stream effect** caused by removal the ligand, hence the drug effect cannot exclusively be related to the suggested PD marker
  - *If the ligand activity can be directly measured by the PD marker, why should other PD markers be required?*

- **Other factors may influence the PD marker**
  - *Fair point, but those factors will be transitory, and we still have the PK and ADA to guide the evaluation.*

Case Story – Regulatory feedback

- Recommend to **include free ligand** in the integrated data approach
  - Free ligand is consumed within minutes. The free ligand assay is difficult to evaluate due to extensive accumulation of ligand on the drug.
    Dilution linearity is compromised due to change in equilibrium during dilution.

- Recommend to **include clinical effect** in the integrated data approach
  - Clinical effect can in rare event studies only be addressed on population level.

Case Story – Conclusion

- Regulators strongly supported the integrated data approach to evaluate the neutralizing effect of ADA+ subjects, but
- Most regulators continues to require an *in vitro* NAb assay even for drugs with low immunogenicity risk, despite having both a reasonable PK and PD marker

- Future perspective:
  - Continue to use integrated data approach for evaluation of *in vivo* NAb
  - At some point *in vitro* NAb may not be needed for low immunogenicity risk drugs