# Inferring Neutralizing Antibodies – When is data integration appropriate?

Shobha Purushothama

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# Talk Outline

- Why do we need to detect neutralizing antibody (NAb)?
- 3 Case Studies – where potential NAb impact is on efficacy
  - Regulatory input received
- Closing thoughts

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# Why do we need to detect NAb?

Provide safe and efficacious therapy to patients

- Impact of NAb
  - Safety (e.g. homology with endogenous protein)
    - NAb in Phase 1
  - Efficacy – later phases
    - Stand alone NAb assay?
    - Leverage data from other assays?

**Understand clinical relevance**

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# Scientific Considerations

The decision to deploy a standalone NAb assay or other assay(s) that inform NAb impact should occur on a case by case basis

Considerations

- The particular therapeutic molecule and its MOA
- **Clinical Impact** of NAb
- The suite of assays available to interrogate its physiological impact
- The quality of those assays

**The assay(s) that can best inform NAb clinical impact should be deployed**

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# Testing Strategy for “low risk” /Category 1 mAb

| | SAD | MAD | Phase II and III |
|---|---|---|---|
| ADA Assay Format | Screen + confirm | Screen + confirm | Screen + confirm |
| ADA Sample Collection | At minimum, baseline and end of study | Frequent | Frequent |
| Execution of ADA testing | At end of study, If required | At end of study, batch wise | At end of study, batch wise |
| Neutralization Antibody Assay | NA | NA | PD; CLBA, CBA if of added value |

**Loss of efficacy or PD can be considered as in-vivo indicator of NAb**

Reference: Kloks et al, J. Immunological Methods, 2015, 217;
Goodman J et al, Bioanalysis, 2018, 10(4)
CLBA = Competitive Ligand Binding Assay, CBA = Cell Based Assay

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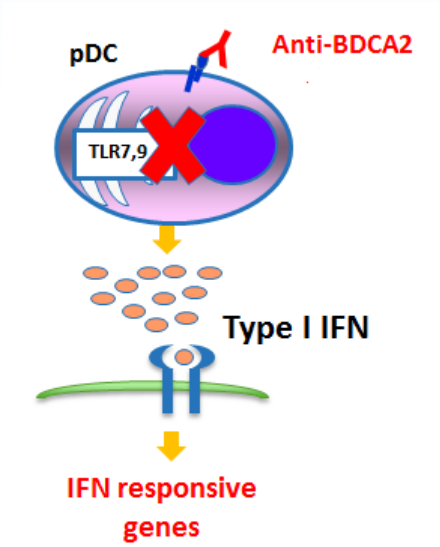
# Case Study 1

**Proximal PD**
**BDCA2 internalization**




**Molecule:** fully humanized $IgG_1$ anti-BDCA2 mAb

**MOA –** antagonistic mAb; drug binding leads to rapid internalization of BDCA2

**NAb Impact** – **loss of efficacy**

**Assays:**

- **PK:** anti-id capture sandwich ELISA (free PK) Assay range: 0.2-10 μg/mL
- **ADA:** solution bridging ELISA; sensitivity 62.5 ng/mL; drug tolerance 125:1 (drug:Ab)
- **Proximal PD** – flow cytometry assay measuring BDCA2 on pDCs (whole blood)

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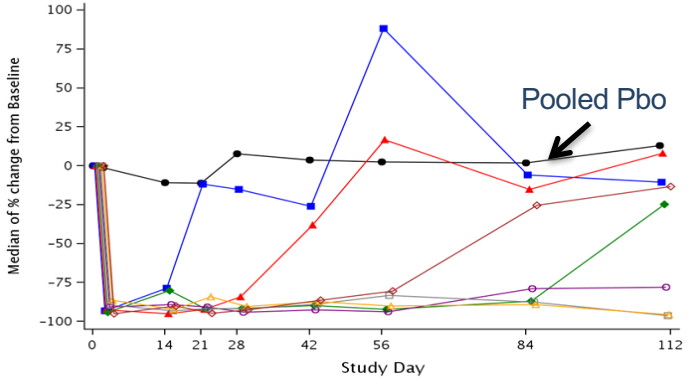
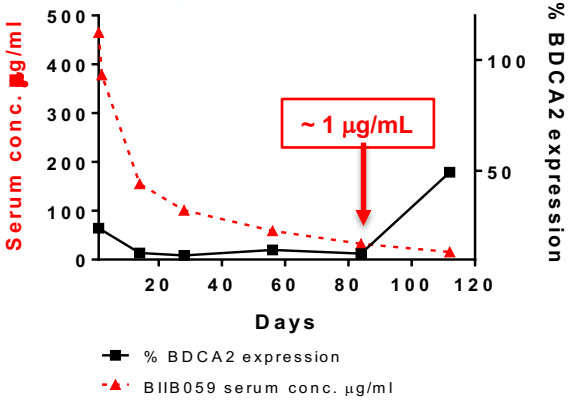
# Phase 1 PK-PD Data

## Proximal PD




## PK-PD Relationship



# Potential Data Scenarios….

| | | | | | | |
|---|---|---|---|---|---|---|
| PK | + | | + | - | | - |
| PD | + | | + | - | | - |
| ADA | + | | - | + | | - |
| NAb | + | - | NA | + | - | NA |
| NAb Conclusion | Yes | No? | No | Yes | No? | No |
| Clinical Impact on Efficacy | | No | | | Yes | |
| PK-PD-ADA NAb Conclusion | No? | | No | Yes | | No? |
| Clinical Impact on Efficacy | | No | | | Yes | |

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# Case study 1 – Show me the data

Measured drug concentrations μg/mL

| subject | W2 | W4 | W6 | W8 | W12 | W16 | W20 |
|---|---|---|---|---|---|---|---|
| 1 | 19.6 | 34.0 | 50.6 | 39.1 | 17.3 | 7.51 | 3.32 |
| 2 | 27.7 | 50.7 | 59.5 | 47.6 | 20.1 | 7.74 | 3.49 |
| 3 | 29.0 | 21.9 | 32.7 | 20.0 | 3.45 | BLQ | BLQ |

**PD**

**Complete target internalization**

**Target returned to baseline levels**

**ADA Positive**

Evidence of NAb activity…

# Conclusions

PK assay data provided earliest indicator of presence of neutralizing activity

- Confirmed by PD assay data when drug levels fell below 1 μg/mL

If dosing had continued with current Phase 2 regimen (Q4W):

- Exposure would have remained above levels for target saturation
- No impact on PD would have occurred
- Do not expect to impact efficacy

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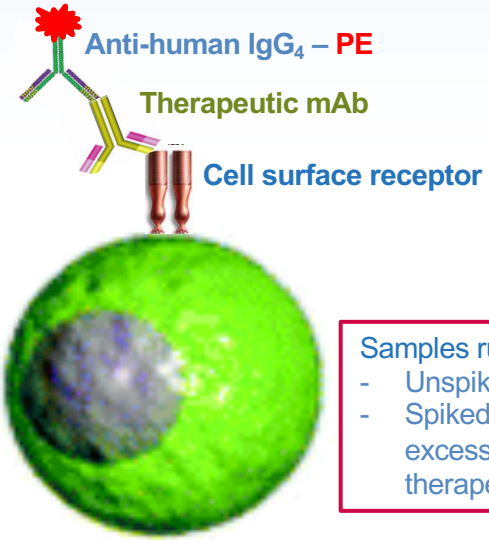
# Case Study 2




Samples run:
- Unspiked
- Spiked with excess mAb therapeutic

$$\frac{\text{MFI } \alpha\text{hIgG4-PE [unspiked]}}{\text{MFI } \alpha\text{hIgG4-PE [spiked]}} \times 100 = \text{\% Receptor Saturation}$$

**Molecule:** fully humanized antagonistic IgG$_4$ mAb against cell surface receptor

**NAb risk** – loss of exposure/efficacy

**Assays:**

- **PK:** anti-id capture sandwich ELISA (free PK) Assay range: 0.5-32 μg/mL
- **ADA:** Bridging ELISA with established clinically relevant 500 ng/mL sensitivity
- **PD/TE:** Whole blood flow cytometry assay measuring target receptor saturation on leukocytes

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# What about NAb assays?

- NAb assay format: flow cytometry assay using cell line expressing target receptor – essentially reconstructed Target Saturation Assay

- The advantages of a mature asset…
  - Lots of historical data
    - Historically, all ADA+ demonstrated neutralizing activity
    - Have not included NAb assay in on-going studies
  - Target saturation assay has been demonstrated to be a good predictor of clinical efficacy (50-70% saturation)

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Reference: Muralidharan et al. J. Clinical Pharmacol. 2017, 57 (8)

# Clinical Study Design

- PK-PD study in treatment naïve patients
- Part 1: 8 weeks - PK-PD after single dose administration
- Part 2: additional 24 weeks with standard dosing schedule (Q4W)

- Note: All subjects tested ADA (-) at Screening/Baseline

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Reference: Plavina T et al. J. Clin Pharmacol. 2016, 56(10)

# Data Snapshot: PK, PD, ADA

| Time Point | S1 | S2 | S3 | S4 | S5 | |
|---|---|---|---|---|---|---|
| | Neg | Neg | Neg | Neg | Pos | ADA |
| **W4** | 9.08 | 4.85 | 3.39 | 10.8 | 5.55 | PK (μg/mL) |
| | 80% | 78% | 86% | 91% | 81% | PD (% Sat) |
| | Pos | Pos | Pos | Pos | Pos | |
| **W8** | BLQ | BLQ | BLQ | BLQ | BLQ | NAb? |
| | 14% | 13% | 4% | 5% | 5% | |
| | | | Pos | | Pos | |
| **W12** | BLQ | 0.536 | BLQ | BLQ | BLQ | |
| (+ 1 dose) | 37% | 68% | 32% | 9% | 9% | |

ADA (-) population: W8 trough PK > 1 μg/mL; PD ~50% receptor saturation

# Data Snapshot: PK, PD, ADA

| Time Point | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| | NA | NA | NA | NA | NA |
| **W16** | 3.77 | 9.93 | 0.526 | BLQ | BLQ |
| (+2 doses) | 59% | 84% | 66% | 12% | |
| | Neg | Neg | Neg | Pos | W/D |
| **W24** | 8.31 | 14.2 | 5.66 | BLQ | |
| (+4 doses) | 67% | 83% | 83% | 6% | |
| | Neg | | | | |
| **W28/32** | 13.7 | 11.3 | | | |
| (+5/6 doses) | 93% | 73% | | | |

NAb!

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# Conclusions

- Persistent positive subjects showed sustained impact on PK and PD
  - **Neutralizing Impact = ADA(+) + PK(-) + PD(-)**
- PK did not tell the whole story
  - Saturation assay was more insightful

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# Case Study 3

**Molecule:** fully human $IgG_1$ mAb with antagonistic MOA, administered IV

**NAb Risk:** loss of exposure/efficacy

**Assays:**

- **PK:** anti-id capture sandwich ELISA (free PK); Assay range: 0.1-10 μg/mL
- **ADA:** solution bridging ELISA; Sensitivity = 15.6 ng/mL; Drug tolerance = 2000:1 drug:ADA

**Immunogenicity Data to date:**

- In 3 completed early phase single- and multiple-dose studies, no treatment emergent immunogenicity was observed
- The highest measured drug concentration in ADA samples well within drug tolerance

# Proposed NAb Approach

Monitor ADA(+) and exposure data in an integrated fashion to inform presence of neutralizing activity

# Interpreting clinical impact of NAb

## Proposed Approach

| ADA Status | Exposure | Efficacy | NAb | Impact |
|---|---|---|---|---|
| (+) | ✓ | ✓ | ✗ | ✗ |
| (+) | ✓ | ✓ | ✓ | ✗ |
| (+) | ✗ | ✗ | ✓ | ✓ |

## Stand Alone NAb Assay

| ADA Status | NAb Status | Impact |
|---|---|---|
| (+) | (+) | Review exposure/efficacy |
| (+) | (-) | Review exposure/efficacy |

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# Scientific Rationale

- A standalone NAb assay:
  - Will not provide data that would lend additional insight into clinically meaningful neutralizing activity
- An integrated data approach:
  - Provides a more conservative means to indicate the presence of neutralizing activity

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# Regulator Response

They did NOT say…..
“We still expect a NAb assay”

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# Closing Thoughts

- Not simply a case of developing or not developing a NAb assay
- Examine all available assays to determine which assays can best inform presence and impact of neutralizing activity
  - Expanding the definition of NAb assay

Biogen. Reference: Stevenson, L. 2016 *Bioanalysis:* 8, 2287-2289

# Acknowledgments


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# Back up slides

# Phase 1 Immunogenicity Summary

Completed Phase 1

- Total of 75 subjects (healthy volunteers and patients)
- Single and multiple doses; IV and SC

Overall, 11/75 were positive for ADA at ≥ one time point

- Of those, 5 showed persistent, but low titer responses
- One patient in the highest multiple dose cohort administered SC was ADA (+) with impact on exposure
- Exposure similar between ADA(-) population and all other ADA(+) subjects

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