Assays for Immunogenicity: Are We There Yet?

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Goals: Know Some AntiDrug Antibody Lab Methods & Implications

- Classical ‘sandwich’ assay
- Capture assay
- Homogenous mobility shift assay
- Functional reporter gene assay
- Clinical predictive value
Potential Effects of Anti-Drug Antibodies (ADA)

• ADA alter drug clearance, pharmacokinetics
  – Measurement: altered drug levels/PK

• ADA alters drug activity
  – Measurement: drug-cell interaction or drug-receptor interaction

• ADA promotes toxicity
  – Serum-sickness, rashes, arthralgias, fever, etc.
‘Standard’ Immunoassay Formats

- Classic fluid phase competitive radioimmunoassay (RIA)
  \[ \text{Precipitate Abs} = \]  

- Classic bridging ‘sandwich’ enzyme linked immunosorbent assay (ELISA)
Drug Concentration Assays & Anti-Drug Antibody Assays – Lab Aspects

- Rarely RIA (because use of radioactivity is a regulatory and logistic burden)
- Frequently classical ELISA (sandwich assay).
- For both sandwich assays and competitive inhibition assays, there are other ways to amplify signal/readout
  - Chemiluminescence
  - Electrochemiluminescence
  - Fluorescence
  - Obsolescence….. Newer techniques as well.
- Often, the description is not very informative.
- Looking at method details can be informative.
Measurement Challenges

• In assays to measure drug, anti-drug antibodies may interfere with ability of analytical reagent to bind or recognize the drug (competitive inhibitor).

• In assays to measure anti-drug antibody (ADA), drug may interfere with ability to measure ADA (competitive inhibitor).

• For therapeutic IgG monoclonal antibody drugs, additional challenges:
  – Anti-human IgG reagent will detect both drug and anti-drug antibody
  – Rheumatoid factors (anti-IgG Fc) may augment or interfere with binding assays.
Anti-Drug Antibody Measurements
- Simple Sandwich

Antigen: Drug (IgGκ antiTNFα)

Antibody1: Pt Anti-Drug Ab

Antibody2 = Detection Antibody (anti-IgG), labeled
Anti-Drug Antibody Measurements - Simple Sandwich: Problem for IgG Drug

Antibody2 = Detection Antibody (anti-IgG), labeled (reacts with drug)

Antibody1: Pt Anti-Drug Ab

Antigen: Drug (IgGκ antiTNFα)
Anti-Drug Antibody Methods

- Problem in measuring ADA’s when the drug is a human(ized) antibody
  - Anti-IgG Fc problem:
    - will detect drug (e.g. monoclonal IgG antiTNF such as adalimumab)
  - Solution: Use antibody that does not react with therapeutic drug IgG (most IgG1-kappa)
    - Detecting antibody options:
      - anti-lambda
      - Antibodies against other IgG subclasses
Anti-Drug Antibody Measurements - Simple Sandwich

Antigen: Drug (IgG_κ antiTNF_α)

Antibody1: Pt Anti-Drug Ab

Antibody2 = Detection Antibody (anti-λ), labeled
Anti-Drug Antibody Methods

Another Solution: Assume that the anti-drug antibodies are bivalent, i.e., both F(ab) binding sites of the antibodies can bind drugs at the same times.
Anti-Drug Antibody Measurements – Simple Sandwich Assay

Antigen2 = Drug (antiTNFα), labeled

Antibody: Pt Anti-Drug Ab

Antigen: Drug (antiTNFα)
Anti-Drug Antibody Methods

• Potential problem in measuring ADA’s when the drug is a antibody to a circulating, soluble ligand, using the labeled drug detection method:
  – Ligand (drug target, eg TNF) could serve as bridge between 2 drug molecules.

Intent: Detect anti-drug Abs

Possibility: Detect drug ligand/target
**Anti-Drug Antibody Measurements**

– Isotype Capture Method

**Antigen** = Drug (antiTNF), labeled

**Antibody1**: Anti-Human Ig (isotype specific, e.g. IgG1, IgG4, IgM, etc)

**Antibody2**: Anti-Drug Ab
Advantages of Capture Method

- Avoid interference of ligand/target of drug (e.g. presence of circulating TNF would not give false-positive result)
- Determine the isotype of the anti-drug antibody
  - Perhaps advantage in measuring IgG4 ADAs
Anti-Drug Antibody Measurements – Isotype Capture Method: Problem of Polyclonal Hypergammaglobulinemia

Antigen = Drug (antiTNF), labeled

Antibody2: Anti-Drug Ab

Antibody2b: Other Ig

Antibody1: Anti-Human Ig (isotype specific, e.g. IgG1, IgG4, IgM, etc)
Isotype Capture Method - Commercialized

Streptavidin Bead
Biotinylated Drug
ADA (IgG4)
Anti-Isotype Capture
Photonic Sensor Ring

*This document refers to the MaverickTM Detection System in a Research Use Only application.
Homogeneous Mobility Shift Assays: Size Matters

(A) IFX-488 (~150kD) + IgG or IgE (~150kD) + IgM (~900kD) + IgA (~320kD) → Incubation → 300kD to >2000kD Immune Complexes → SE-HPLC

(B) IFX (~150kD) + TNF-488 (~51kD) → Incubation → >200kD Immune Complex → SE-HPLC

S-L. Wang et al. / Journal of Immunological Methods 382 (2012) 177–188
Size Exclusion Chromatography

Separate molecules based on their molecular size.

Small molecules go into the gel interstices, spend more time coming out of a column.

Larger molecules go around the gel interstices, ‘channel’ with faster flow.

Look at the time of small vs large molecules to come off the chromatography column.

Drugs attached to anti-drug antibodies are functionally much larger (faster off column)
Size Exclusion Chromatography

Retention Time
Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum

Shui-Long Wang, Linda Ohrmund, Scott Hauenstein, Jared Salbato, Rukmini Reddy, Patrick Monk, Steven Lockton, Nicholas Ling, Sharat Singh*

Department of Research and Development, Prometheus Laboratories, Inc, 9410 Carroll Park Drive, San Diego, CA 92121, USA
Homogeneous Shift Assay for ADA

- Use drug labeled with fluorescent dye
- Mix and incubate with serum.
- Apply to chromatography column
- If the labeled drug comes off the column early (i.e. is ‘shifted’), it must have bound to another large molecule, i.e., anti-drug antibody.
- ‘Homogeneous’ = all happening in solution, no precipitation capture on a surface.
Size Exclusion Chromatography with Labeled Infliximab & ADA

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Correlation of ADA Measured by HMSA vs Sandwich ELISA

Fig. 8. Correlation of the ATI-HMSA and bridging ELISA on the measurement of ATI in IBD patient serum samples.

S-L. Wang et al. / Journal of Immunological Methods 382 (2012) 177-188
Many ADA Methods Challenged By Presence of Drug

- Competition between drug in serum specimen competing with drug used to measure anti-drug antibody.
- How to measure ADA even when drug is present?
Anti-Drug Antibody Measurements – Problem of Drug Competition

Antigen2 = Drug (antiTNF), labeled

Antigen: Drug (antiTNFα)

Antibody: Pt Anti-Drug Ab
Preformed Immune Complexes: Drug+Anti-Drug Antibody > Acid Dissociation Step
Acid Dissociation Step

- Used in some, but not all methods.
- Does not totally solve the problem of drug interference, but usually effective.
Anti-Drug Antibodies – Functional Interference

- Some ADAs may interfere with drug function, whether or not drug levels (i.e. drug clearance) is effected
- Various functional assays, not widely used with rheumatic disease drugs.
Neutralizing ADAs: Prevent Drug from Binding to Ligand

Bioactive TNF-α

Idiotype

CDRs/ FRs of murine Fabs

Idiotypic

-umab

-ximab

-zumab

Neutralizing ADAs
Reporter Gene Assay (Cell-Based) Assay for Neutralizing Abs to Inhibitory Therapeutic Abs

Wu, et al

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<th>Antibody</th>
<th>Drug</th>
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**Assess disease activity**

- **Consider increasing dose or decreasing dose interval**
- **Active: Consider switching to drug out of class**
- **Inactive: Explore other reasons for symptoms**

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<th>Drug</th>
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**Based on clinical experience, experts suggest if antibodies are positive, consider adding an immunomodulator in addition to other changes in therapy.**

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**Consider switching to drug out of class**

- **Consider switching to drug within class**
Randomized Trial: Outcome of Using Infliximab & ADA Levels in Crohn’s Disease

Clinical Implications of Measuring Drug and Anti-Drug Antibodies by Different Assays When Optimizing Infliximab Treatment Failure in Crohn’s Disease: Post Hoc Analysis of a Randomized Controlled Trial

Casper Steenholdt, MD, PhD¹, Klaus Bendtzen, MD, DMSc², Jørn Brynskov, MD, DMSc¹, Ole Ø Thomsen, MD, DMSc¹ and Mark A. Ainsworth, MD, PhD, DMSc¹

OBJECTIVES: Cost-effective guidance of therapeutic strategy in Crohn’s disease patients with secondary infliximab (IFX) treatment failure may be achieved by serum IFX and anti-IFX antibody (Ab) measurements by radioimmunoassay (RIA). This study investigated implications of using other techniques for this purpose.

Do Different ADA Assays Agree? Detection of Anti-Infliximab

Figure 3. Detection of anti-IFX Abs by RIA, ELISA, HMSA, and RGA in patients with secondary IFX treatment failure (n=66). Anti-IFX Abs were undetectable in the same 43 patients by all assays (not shown on figure).

Anti-Drug Antibodies – Conclusions

• Complicated assays
• Poor concordance between assays
• Not highly standardized
• My view: interpret with caution.
  – Very high levels with absence of drug in patient taking drug: switch to another drug.
  – Is there evidence for this?
Does ADA Classification Predict Response?

Table 4. Clinical outcomes after 12 weeks of interventions defined by the treatment algorithm presented in Figure 1, and based on classification by each of four different assays for therapeutic monitoring.

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<th>– Response on ADL</th>
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<tbody>
<tr>
<td>Group 1 RIA</td>
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<td>4</td>
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<tr>
<td>Group 1 RGA</td>
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$\chi^2$-test; $P=0.724$