How to monitor and mitigate immunotoxicity during early phase clinical trials in inflammatory diseases?

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London, May 19 2017
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Inflammatory diseases

Inflammatory bowel diseases (IBD)
- Crohn’s disease
- Ulcerative colitis

Rheumatological diseases
- Spondyloarthritis
- Rheumatoid arthritis
- Psoriatic arthritis

Dermatological diseases
- Plaque psoriasis

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Inflammatory diseases

**Concept:**
elevated Tumor Necrosis Factor alpha (TNF) concentrations at the sites of inflammation drive disease pathology

**Therapeutic goal:**
removal of excess TNF from sites of inflammation

**Development of anti-TNF biologicals**
anti-TNF biologicals

Approved for treatment of IBD in Europe

Induction followed by maintenance therapy

Treatment cost: 8.000-15.000€/year
Pharmacokinetics/Pharmacodynamics

Pharmacokinetics
- Absorption
- Distribution
- Elimination

Pharmacodynamics

Dose → Exposure of patient to drug? → Inflammation

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Pharmacokinetic variability-IV drug

Therapeutic window concept:
- supratherapeutic concentrations
- subtherapeutic concentrations
Pharmacokinetic variability-SC drug

Therapeutic window concept:
- supratherapeutic concentrations
- subtherapeutic concentrations

Next administration

Trough concentrations

Serum concentration vs. Time
What causes PK variability?

- Patient
  - Stability
  - Storage and handling
  - Complexation and aggregation
  - Level of humanization
  - Route of administration
  - Comedication
  - Dosage regimen and duration
  - Immunogenicity...

- Disease
  - Disease severity
  - Disease type
  - FcRn expression
  - TMDD
  - Protein-losing enteropathy
  - Catabolic state...

- Drug regimen

- PK

- PD
Pharmacokinetics/Pharmacodynamics

Dose → Exposure of patient to drug? → Inflammation

Pharmacokinetics
- Absorption
- Distribution
- Elimination

Pharmacodynamics

Therapeutic Drug Monitoring (TDM)
TDM: How to measure? ELISA

- infliximab, adalimumab, golimumab: both TNF coated and MA/MA ELISA have been developed. Van Stappen T *et al.*, TDM 2015; Bian S *et al.*, JBPA 2016, Detrez I *et al.*, JCC 2016
- TNF coated ELISA is used in CE-labelled kit (Ridascreen-R-biopharm)
TDM: How to measure? rapid assays

**ELISA:**
- Requires time: approximately 2h
- Requires laboratory equipment or transport to central laboratory
- Requires multiple samples in order to be cost-efficient
- Long time to result time

**Rapid assays:**
- Lateral flow technology  
  Van Stappen T *et al.*, Clinical and Translational Gastroenterology 2017
- Fiber optic surface plasmon Resonance: FO-SPR  
  Lu J *et al.*, Biosens Bioelectron 2016  
  Lu J *et al.*, Anal Chem 2017

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Increase the speed of TDM: rapid assays

- **Lateral flow technology** (Van Stappen *et al.*, Clin Transl Gastro 2017)

- **Fiber optic Surface Plasmon Resonance (FO-SPR)** (Lu *et al.*, Biosens Bioelectron 2016; Lu *et al.*, Anal Chem 2017)

**ESSENTIAL**
- Head to head comparisons with existing assays
- Commercially available

Ridaquick infliximab monitoring (R-biopharm)

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Increase the speed of TDM: rapid assays

Improvement possible?

- **Full blood** *versus* **serum**
  - Validation with existing assays using serum

Other alternative forms

- **Dry blood spots** collection followed by extraction: increases speed and accessibility of collecting samples

- **FO-SPR technology**: all matrices (full citrated blood, plasma, serum, dry blood spot extracts) validated (Lu J *et al.*, Anal Chem 2017)

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Relationship between high serum infliximab concentrations and risk of infections

Estimated hazard ratios and 95% confidence intervals for a first infection episode in spondyloarthritis patients in each quartile of trough serum IFX concentration (A) or mean of the last 3 trough serum concentrations (B) Bejan-Angoulvant T et al., Arthritis & Rheumatology 2017

➢ High IFX concentration (>15-20 µg/ml) are correlated with a higher risk of first infection episode

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Relationship between immunogenicity and low concentrations of anti-TNF biologicals

• All biologicals can evoke an immune response
  o infliximab: chimeric
  o adalimumab & golimumab: fully human

• Resulting in a formation of anti-drug antibodies (ADA)
  o Infliximab: > 90% neutralizing ADA
  o Adalimumab: 97% neutralizing ADA

Van Schie K et al., Ann Rheum Dis 2015
nonneutralizing *versus* neutralizing ADA
The effect of (non)neutralizing ADA?

Carrascosa JM, Actas dermisifiliogr. 2013

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Relationship between immunogenicity and low concentrations of anti-TNF biologicals

- Transient *versus* persistent ADA
  - persistent ADA are associated with loss of clinical response whereas transient ADA are not
    Vande Casteele N *et al.*, Am J Gastroenterol 2013

- Size and titer of immune complexes
  - large immune complexes: favor further ADA induction through complement activation and larger uptake by antigen presenting cells: large immune complexes are found in patients with acute severe infusion reactions
    Van Schouwenburg P *et al.*, Nat Rev Rheumatol 2013
How to measure ADA: bridging ELISA

Drug sensitive: in presence of excess of IFX, ATI can not be detected only determine ATI when IFX is below detection limit

Van Stappen T et al., IBD 2015

IFX: infliximab; ATI: antibodies towards infliximab

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Drug-sensitive *versus* drug-tolerant assay

- **No** detection of ADA in presence of drug
- **Detection** of ADA in presence of drug

Pretreatment steps: removal of excess of drug & dissociation of drug/anti-drug complex

**Bridging assay**

**Drug tolerant protocol**

1. Apply sample on TNF plate
2. Transfer supernatants in acid
3. Neutralize sample
4. Analysis in bridging ELISA

**ADA assays: bridging ELISA: drug tolerant?**

Van Stappen T *et al.*, IBD 2015
Van Stappen T *et al.*, DTA 2016

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ADA assays: bridging ELISA: drug tolerant?

Van Stappen T et al., DTA 2016

Comparison of the bridging ELISA with and without the sample pre-treatment protocol for the detection of anti-drug antibodies (ADA) in two patients treated with infliximab.

The infliximab concentration is presented as a dotted line with triangles indicating the date of infusion. ADA levels are represented with a dashed line (circles indicating the date of infusion) and a full line (diamonds indicating the date of infusion) for the bridging ELISA without and with the sample pretreatment protocol, respectively.

➢ predict formation of ADA providing opportunities for early treatment optimization
ADA assays: Affinity Capture ELISA: increase drug tolerance

Van Stappen T et al., Clin Transl Gastro 2017, Van Stappen T et al., Gut 2017

➢ No bridging format
➢ Increased drug tolerance
**ADA assays: drug resistant**

- **No bridging format**
- **Drug resistant assay**

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1. Addition of excess adalimumab to form adalimumab/anti-adalimumab antibody complexes

2. Precipitation using PEG to get total ADA to adalimumab; Spin and wash samples to get the pellets

3. Acid dissociation and coating of reconstituted pellets in an acidic solution on an empty plate

4. Specific detection of total ADA levels using adalimumab-biotin

**Anti-adalimumab antibody**

**Adalimumab**

**Polyethylene glycol**

**Streptavidin-HRP**

Adalimumab-biotin 1% BSA

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How to monitor ADA formation

**Drug-sensitive bridging assay**
(Bian S et al., J Pharm Biomed Anal 2016; Van Stappen T et al., Inflam Bow Dis 2016; Detrez I et al., J Crohn Col 2016)

**Drug-tolerant bridging assay.**
(Van Stappen T et al., Drug Test Anal 2016)

**Drug-tolerant ACE assay**
(Detrez I et al., JCC 2016; Van Stappent T et al., Clin Transl Gastro 2017, Van Stappen T et al., Gut 2017)

**Drug-resistent PANDA assay**
(Bian S et al., AAPS 2016)

- Increased incidence of anti-drug antibody detection
- Occurrence of transient and persistent antibodies
- Detection of ADA is not always associated with loss of response

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How to mitigate ADA formation

• De-immunizing the molecule
  o Removing or masking or substitution known immunogenic epitopes Jawa V et al., Clinical Immunol 2013

• Avoid patients with high disease activity and comorbidities
  o In patients with acute bacterial infections or systemic inflammatory activity, dendritic cells may express higher levels of co-stimulatory molecules, decreasing threshold for T cell activation Atzeni F et al., Autoimmunity reviews 2013

• Add concomitant drugs
  o Methotrexaat reduces ADA due to immune suppressive nature or due to anti-inflammatory effect Ungar B et al., AP&T 2017
How to mitigate ADA formation

• Adapt therapeutic regimen
  o tolerance induced by high dosing van der Maas A et al., BMC Musculoskelet Disord 2012

• Desensitization or tolerance techniques
  o Gradually increasing doses to reach the full treatment dose over approximately 4 to 6 hours Mourad A et al., 2015 Ann Allergy Asthma Immunol
Acknowledgements

Laboratory for Therapeutic and Diagnostic Antibodies http://pharm.kuleuven.be/biotech
Leuven IBD research group http://www.ibd-kuleuven.com

Prof Séverine Vermeire
Prof Marc Ferrante
Prof Gert Van Assche
Prof Paul Rutgeerts