Immunogenicity of therapeutic antibodies

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Immunogenicity

• Assays for immunogenicity
  - Drug interference
• Characterization of anti drug antibodies

• Clinical relevance
  – Side effects
  – Effects on PK
Amsterdam READE cohort

Long-term clinical and serological follow-up of 2000 patients on biologicals*

*infliximab/adalimumab/etanercept/batacept/golimumab/tocilizumab/rituximab
Pharmacokinetic assay (drug level test anti-TNF)

Anti-idiotypic

Adalimumab/infliximab/golumimab/etanercept

Anti-TNF

TNF
Serum trough infliximab level for responders (n=21; 8.2mg/l) and non-responders (n=17; 6.3mg/l) according to the ASAS-20 response criteria, at week 54 (P=0.018)
Patients with an allergic reaction to infliximab have low serum levels of infliximab

<table>
<thead>
<tr>
<th>Infliximab concentration (Mg/L)</th>
<th>2 wk</th>
<th>6 wk</th>
<th>14 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean all patients n=105</td>
<td>23.9</td>
<td>16.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of reaction</th>
<th>2 wk</th>
<th>6 wk</th>
<th>14 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt S reaction at wk 14</td>
<td>17.4</td>
<td>0.5</td>
<td>0.00</td>
</tr>
<tr>
<td>Pt R reaction at wk 14</td>
<td>37.1</td>
<td>2.8</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Detection of antibody formation against therapeutic antibodies

Different ASSAYS formats
No standards
Drug interference
Immunogenicity assay with drug interference: Bridging ELISA
ABT monoclonal therapeutics less sensitive to drug interference

Drug interference in different assays

Bridging ELISA

ABT/RIA

Antibody detection in ABT is hampered in the presence of drug

pH shift anti-idiotype
Antigen Binding Test

Rabbit-F(ab)-
anti-idiotype

acidoat treatment pH 2.5
Neutralise

Adalimumab
Anti-adalimumab

Anti-adalimumab
Acid dissociation (ARIA)

Serum

ADL

anti-ADL

Diluted in pH 3

Neutral pH 7

ADL-F(ab')2-

biotin

Streptavidin*

10 maart 2016 | 13
Drug tolerant assays detect antibodies in the presence of physiological amounts of drugs

PIA

TRIA

ARIA

ECL

Detection of anti-drug-antibodies (ADA)

<table>
<thead>
<tr>
<th>ADA detection method</th>
<th>Free anti-TNF agent</th>
<th>ADA-drug complexes</th>
<th>Free ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ABT</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
</tr>
<tr>
<td>PIA</td>
<td>–</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Pharmacokinetic assay (TNF capture)</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Characterization of Anti-Drug Antibodies
Generation of human monoclonal antibodies

Isolate PBMCs

Centrifuge 400 x g for 30 minutes

Isolate B cells

Isolate RNA, determine sequence for VH/VL

Expression Vector 1
7772 bp

Ampicillin resistance gene
Bl2 pronote
Hias III
Cla I
Xma I
Sma I
Bam III
attB I
Eca RI
Sam I
Xma I
LacZ ORF
Cle I
AmC ORF
pUC origin
Ampicillin resistance gene
Bl2 pronote
Hias III
Cla I
Xma I
Sma I
Bam III
attB I
Eca RI
Sam I
Xma I
LacZ ORF
Cle I
AmC ORF
pUC origin

Sort antigen-specific cells

Culture 1 cell/well; screening

All monoclonal antibodies are derived from different precursor B-cells

<table>
<thead>
<tr>
<th>clone</th>
<th>isotype</th>
<th>V gene</th>
<th>D gene</th>
<th>J gene</th>
<th>CDR3-IMGT</th>
<th>length</th>
<th>R</th>
<th>S</th>
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<tbody>
<tr>
<td>1.1</td>
<td>IgG1</td>
<td>1-03</td>
<td>2-02</td>
<td>4</td>
<td>ARDIVVVPVAMHPDY</td>
<td>15</td>
<td>16</td>
<td>4</td>
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<tr>
<td>1.2</td>
<td>IgG4</td>
<td>1-02</td>
<td>2-15</td>
<td>5</td>
<td>ARDKWGPAAAQYPDNWFD</td>
<td>18</td>
<td>9</td>
<td>7</td>
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<tr>
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<td>IgG1</td>
<td>1-18</td>
<td>1-14</td>
<td>4</td>
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<td>13</td>
<td>15</td>
<td>4</td>
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<tr>
<td>2.1</td>
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<td>3-09</td>
<td>4</td>
<td>ASEGLLTGFLPDLDY</td>
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<td>3-10</td>
<td>6</td>
<td>ARLAIPWGFGEAVFSYHYDMDV</td>
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<td>15</td>
<td>9</td>
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<td>2.3</td>
<td>IgG4</td>
<td>4-31</td>
<td>6-13</td>
<td>3</td>
<td>AREPAATGPSGDAFDI</td>
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<td>5</td>
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<td>2.4</td>
<td>IgG1</td>
<td>1-03</td>
<td>3-16</td>
<td>3</td>
<td>ARMGERGLDV</td>
<td>10</td>
<td>19</td>
<td>7</td>
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<td>2.5</td>
<td>IgG4</td>
<td>4-59</td>
<td>6-13</td>
<td>3</td>
<td>ARQTLLLMAADGDDAFDI</td>
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<td>16</td>
<td>11</td>
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<td>2.6</td>
<td>IgG1</td>
<td>4-39</td>
<td>1-26</td>
<td>4</td>
<td>ARRSVAAFDY</td>
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<td>14</td>
<td>6</td>
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<tr>
<td>2.7</td>
<td>IgG1</td>
<td>4-34</td>
<td>1-26</td>
<td>3</td>
<td>AREGKNSGSYYVRLGDTFDI</td>
<td>20</td>
<td>5</td>
<td>1</td>
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<tr>
<td>2.8</td>
<td>IgG4</td>
<td>1-69</td>
<td>6-19</td>
<td>5</td>
<td>ARDQKGQWFDPD</td>
<td>11</td>
<td>21</td>
<td>2</td>
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<tr>
<td>2.9</td>
<td>IgG1</td>
<td>3-48</td>
<td>2-21</td>
<td>6</td>
<td>ARVKDDIVVPTGLGMDV</td>
<td>17</td>
<td>23</td>
<td>9</td>
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<tr>
<td>2.10</td>
<td>IgG4</td>
<td>1-03</td>
<td>2-21</td>
<td>5</td>
<td>AELASSGLFDP</td>
<td>11</td>
<td>15</td>
<td>6</td>
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<td>2.11</td>
<td>N.D.</td>
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<td>6-19</td>
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<td>14</td>
<td>8</td>
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<td>2.12</td>
<td>IgG4</td>
<td>1-18</td>
<td>2-21</td>
<td>6</td>
<td>AREIAPGDMDE</td>
<td>11</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>2.13</td>
<td>IgG1</td>
<td>3-48</td>
<td>5-5</td>
<td>3</td>
<td>ARTGGHSHGPGGFDI</td>
<td>15</td>
<td>9</td>
<td>1</td>
</tr>
</tbody>
</table>
Monoclonal antibodies undergo extensive affinity maturation

<table>
<thead>
<tr>
<th>Clone</th>
<th>$K_d$ (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>9.3 ± 2</td>
</tr>
<tr>
<td>1.3</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>2.1</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>2.2</td>
<td>0.78 ± 0.08</td>
</tr>
<tr>
<td>2.4</td>
<td>50,000</td>
</tr>
<tr>
<td>2.6</td>
<td>233 ± 33</td>
</tr>
<tr>
<td>2.7</td>
<td>195 ± 5</td>
</tr>
<tr>
<td>2.8</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>2.9</td>
<td>82 ± 23</td>
</tr>
<tr>
<td>2.10</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>2.12</td>
<td>0.64 ± 0.16</td>
</tr>
</tbody>
</table>

van Schouwenburg PA, et al.
ECRF bio-assay: TNF-sensitive human endothelial cell line

TNF α → IL-8 production

TNF α + adalimumab → No IL-8 production

IL-8 pg/ml vs. Adalimumab ng/ml

- Adalimumab ng/ml: 0, 0.937, 1.875, 3.75, 7.5, 15, 30, 60
- IL-8 pg/ml: 0, 920, 3000, 2000, 1500, 1000, 500, 0

Bar chart showing IL-8 production with and without Adalimumab and IFX

TNF inhibition

Adalimumab

125I adalimumab Fab

Infliximab

125I infliximab Fab

Certolizumab

125I certolizumab

Prot A sepharose

ADA from patients serum

Wolbink, data on file
To what extent do patient ADA neutralize natalizumab?

>90.9% of patient ADA is inhibited from binding natalizumab using recombinant α4β1 as blocker. N=15
Anti-drug antibodies are anti-idiotypeic and interfere with target binding
Clinical Relevance

• Side effects
• Effects on PK
• In ADA+ adalimumab patients, small immune complexes (2 antibodies) are found weeks after adalimumab administration.

• In 1 ADA+ infliximab patient, large immune complexes (>6 antibodies) were found directly after infliximab infusion
  – ADA+ patient experienced an infusion reaction

**Hypothesis**

Infusion reactions are mediated by large immune complexes
What influences immune complex size?

- Immune complex size is ratio dependent.

- Effect of concentration on complex size is unknown
  - Infliximab can be administered in various infusion speeds
  - Patients make various amounts of ADA

>100 μg/mL infliximab
Influence of concentration ADA and IFX

Patient serum
140.000 AU/ml
34.000 AU/ml
12.000 AU/ml
3.400 AU/ml

1.5 hour incubation

HP-SEC

serum + IFX-488
Cpmplexes are small however Higher concentration of ADA and IFX gives larger complexes

3.400 AU/ml

12.000 AU/ml

34.000 AU/ml

140.000 AU/ml

>600 kDa
Summary

• Immune complex size is dependent on concentration of ADA and drug
  – The higher the concentration, the bigger the complexes
  – But in general they are small

• Risk factor for infusion reactions is a high ADA titer
Infliximab

- Low ADA/drug concentration:
  - No clear adverse effects
  - Immune cell activation
  - Clearance by liver&spleen

- High ADA/drug concentration:
  - Infusion reaction
  - Complement activation
  - Immune cell activation
  - Clearance by liver&spleen

ADA
Immunogenicity in a long-term follow-up cohort of adalimumab treated rheumatoid arthritis patients

Patients & methods

1. 272 consecutive RA patients with active disease treated with adalimumab in a prospective observational cohort study

2. Disease activity monitored at baseline and 4, 16, 28, 40, 52, 78, 104, 130 and 156 weeks using the DAS28 score

3. Trough serum samples were obtained at all visits

4. Serum adalimumab concentrations and anti-adalimumab antibody (AAA) titres determined retrospectively at the end of follow-up using an ELISA and ABT (Sanquin Research, Amsterdam)

Bartelds GM, et al. JAMA. 2011;305:1460‒1468
## Results: baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Patient with AAA detected with ABT</th>
<th>Patients without AAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=272</td>
<td>n=76</td>
<td>n=196</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>54 ± 12</td>
<td>53 ± 13</td>
<td>54 ± 11</td>
</tr>
<tr>
<td><strong>Female, no. (%)</strong></td>
<td>219 (81)</td>
<td>62 (82)</td>
<td>157 (80)</td>
</tr>
<tr>
<td><strong>RF, no. (%)</strong></td>
<td>196 (72)</td>
<td>57 (75)</td>
<td>139 (71)</td>
</tr>
<tr>
<td><strong>Prior DMARDs</strong></td>
<td>3.1 ± 1.4</td>
<td>3.4 ± 1.5*</td>
<td>3.0 ± 1.3*</td>
</tr>
<tr>
<td><strong>MTX use, no. (%)</strong></td>
<td>202 (74)</td>
<td>41 (54)*</td>
<td>161 (82)*</td>
</tr>
<tr>
<td><strong>MTX dose (mg/wk)</strong></td>
<td>25 (15–25)</td>
<td>18 (10–25)*</td>
<td>25 (15–25)*</td>
</tr>
<tr>
<td><strong>No DMARD, no. (%)</strong></td>
<td>51 (19)</td>
<td>28 (37)*</td>
<td>23 (12)*</td>
</tr>
<tr>
<td><strong>Disease duration (years)</strong></td>
<td>8 (3–17)</td>
<td>12 (5–18)*</td>
<td>8 (3–16)*</td>
</tr>
<tr>
<td><strong>Erosive disease, no. (%)</strong></td>
<td>201 (74)</td>
<td>63 (83)*</td>
<td>138 (70)*</td>
</tr>
<tr>
<td><strong>ESR (mm/h)</strong></td>
<td>23 (11–42)</td>
<td>35 (18–60)*</td>
<td>21 (11–39)*</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>12 (5–29)</td>
<td>19 (7–46)*</td>
<td>11 (4–22)*</td>
</tr>
<tr>
<td><strong>DAS28</strong></td>
<td>5.2 ± 1.2</td>
<td>5.5 ± 1.1*</td>
<td>5.1 ± 1.3*</td>
</tr>
</tbody>
</table>

Sustained remission (DAS28 <2.6) correlates with absence of AAA detected

After adjustment for confounding variables MTX dosage, ESR and CRP (HR: 3.6; 95% CI 1.8–7.2, P<0.0001)

Percentage of patients developing detectable anti-adalimumab antibodies over three years

During 156 weeks follow-up, anti-adalimumab antibodies were detected (ASSAY IV:ABT) in 76 (28%) patients.

51 of 76 patients (67%) developed AAA during the first 28 weeks of treatment.
Methotrexate reduces immunogenicity in adalimumab-treated RA

Percentage of patients developing AAA

- No MTX
- Low dose MTX
- Intermediate dose MTX
- High dose MTX

Weeks

Jan van Breemen Research Institute | Reade EULAR centre of Excellence in Rheumatology
Concomitant methotrexate (PsA)

Median adalimumab concentration over time and concomitant methotrexate (MTX) use

- Concomitant MTX use (median 20 mg/week (15-25)), n=80
- Monotherapy, n=23

Vogelzang et al ARD 2014 online first
The accumulative percentage of ADA positive patients depends on assay method used.

Accumulative percentage of patients positive for ADA assessed by pH-shift-anti-idiotype antigen binding test and antigen binding test.

Antibody detection in a small proportion of RA-treated patients using the ABT
Antibody detection in a substantial amount of the patient samples using drug tolerant assays

**PIA**

- AU/ml range:
  - < 48
  - 48 - 100
  - 100 - 1000
  - > 1000

- Positives at each time point:
  - t=0: 0/89
  - t=16: 36/88
  - t=28: 40/85
  - t=52: 25/73

**TRIA**

- AU/ml range:
  - < 33
  - 33 - 1000
  - > 1000

- Positives at each time point:
  - t=0: 0/90
  - t=16: 43/90
  - t=28: 41/87
  - t=52: 31/74

**ARIA**

- AU/ml range:
  - < 30
  - 30 - 1000
  - > 1000

- Positives at each time point:
  - t=0: 0/90
  - t=16: 49/91
  - t=28: 54/87
  - t=52: 38/74

**ECL**

- AU/ml range:
  - < 3.9
  - 3.9 - 1000
  - > 1000

- Positives at each time point:
  - t=0: 1/90
  - t=16: 41/91
  - t=28: 45/87
  - t=52: 29/74

Adalimumab concentration correlates with treatment efficacy


Mean delta DAS and adalimumab levels per 20 patients at week 28
Concentration-effect curve
adalimumumab

Psoriatic arthritis (n=103)  

Psoriasis (n=135)

Last observation carried forward

Without last observation carried forward

Vogelzang et al ARD 2014 online first; Menting et al JAMA dermatol 2015 online first

Jan van Breemen Research Institute | Reade, department of Rheumatology
Conclusions

• Anti drug antibodies to therapeutic antibodies are common and anti-idiotypic
• Detection is highly dependend on the assay and testing strategy
• The clinical relevance is within the PK
• Immunocomplexes formed are small and often do not mediate side effects
Discussion

• Availability of PK assays
• Availability of information on the concentration effect relationship
• Availability of immunogenicity assays