ADA-formation and its effect on Monoclonal Antibody PK

María J. Garrido
Agenda

- Definition
- ADAs production
- Types of ADAs
- mAbs and Immunogenicity
- ADME processes
- Technical assays
- PK models
- Examples
- Remarks
Definition

- **ADA**: Anti-Drug Antibody also denoted as “immunogenicity”, corresponding to an immune reaction to a therapeutic biomolecule (biological/biotechnology-derived proteins)

- **Factors**: Patient-related (genetic background, pre-existing immunity, immune status); therapy-related (immunomodulating therapy, dosing schedule and route of administration) and product-related (manufacturing process, formulation, and stability)

- **Consequences**: Loss of efficacy of the therapeutic protein and different types of side effects.

Complex process that involves the antibody formation by T and B cells activation.

*Systematic immunogenicity testing is often necessary after marketing authorization, and may be included in the risk management plan*
ADAs Formation

**ANTI-DRUG ANTIBODIES (ADAs)**

Schematic representation of the main immunogenic mechanism involved during mAbs (or other biologic agents) administration.

Types of ADAs

**Non-Neutralizing (BAb)**
- Produced earlier in high amount and persistently
- Bind to non-selective epitopes
- May increase either clearance of mAb-ADA or prolong bioavailability of the therapeutic agent
- No effect on the therapeutic efficacy

**Neutralizing (NAb)**
- Produced later and tend to disappear over time
- Bind to selective epitope
- May influence also the clearance of the complex, mAb-ADA
- Affect the therapeutic efficacy
Currently there are more than 250 approved Biotherapeutic Molecules:

*Fusion protein, bispecific antibody, PEGylated antibody, monoclonal antibody (mAb) and antibody drug conjugate (ADC)*

Types of mAbs:

“omab”: murins,
“ximab”: quimerics,
“zumab”: humanized,
“umab”: fully human
<table>
<thead>
<tr>
<th>Product</th>
<th>Antigen</th>
<th>Type</th>
<th>Route</th>
<th>ADAs</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tositumomab</td>
<td>CD20</td>
<td>Murine IgG2a1</td>
<td>IV</td>
<td>99%</td>
<td>NHL</td>
</tr>
<tr>
<td>Muromonab</td>
<td>CD3</td>
<td>Murine IgG2a k</td>
<td>IV</td>
<td>86%</td>
<td>Graft Reject</td>
</tr>
<tr>
<td>AMG-317</td>
<td>IL-4Ra / IL-13</td>
<td>Full Human IgG2</td>
<td>IV/ SC</td>
<td>45%</td>
<td>Asthma</td>
</tr>
<tr>
<td>Abciximab</td>
<td>GP IIb/IIla-R</td>
<td>Chimeric IgG2</td>
<td>IV</td>
<td>6-44%</td>
<td>Angioplasty</td>
</tr>
<tr>
<td>Daclizumab</td>
<td>CD25</td>
<td>Humanized IgG1</td>
<td>IV</td>
<td>14-34%</td>
<td>Graft Reject</td>
</tr>
<tr>
<td>AMG-x</td>
<td>Soluble Protein NA</td>
<td>Humanized IgG1 (modified)</td>
<td>IV/SC</td>
<td>17%</td>
<td>NA</td>
</tr>
<tr>
<td>Infliximab</td>
<td>TNF</td>
<td>Chimeric IgG1k</td>
<td>IV</td>
<td>10%</td>
<td>RA, CD, IBD</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>α4-Integrin</td>
<td>Humanized IgG4k</td>
<td>IV</td>
<td>10%</td>
<td>MS</td>
</tr>
<tr>
<td>Certolizumab</td>
<td>TNF</td>
<td>PEG-Humanized Fab’</td>
<td>SC</td>
<td>9%</td>
<td>CD</td>
</tr>
<tr>
<td>MTRX1011A</td>
<td>CD4</td>
<td>Humanized IgG1 (modified)</td>
<td>IV/SC</td>
<td>7%</td>
<td>RA</td>
</tr>
<tr>
<td>Efalizumab</td>
<td>CD11a</td>
<td>Humanized IgG1 k</td>
<td>SC</td>
<td>6.3%</td>
<td>Psoriasis</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>Humanized IgG1 k</td>
<td>IV</td>
<td>2-8.3%</td>
<td>CLL</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>TNF</td>
<td>Human IgG1 k</td>
<td>SC</td>
<td>1-12%</td>
<td>RA</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGFR</td>
<td>Chimeric IgG1 k</td>
<td>IV</td>
<td>5%</td>
<td>Colorectal CA</td>
</tr>
<tr>
<td>Golimumab</td>
<td>TNF</td>
<td>Human IgG k</td>
<td>SC</td>
<td>4.1%</td>
<td>AS</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>IL-12 / IL-23</td>
<td>Human IgG1 k</td>
<td>SC</td>
<td>3.2%</td>
<td>Psoriasis</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>EGFR</td>
<td>Human IgG1 k</td>
<td>IV</td>
<td>3%</td>
<td>Colorectal CA</td>
</tr>
</tbody>
</table>

IV: Intravenous; SC: Subcutaneous; TNF: Tumor necrosis factor; NHL: Non-Hodgkin lymphoma; NA: Not available; RA: Rheumatoid arthritis; CD: Chron’s disease; IBD: Inflammatory bowel disease; MS: Multiple sclerosis; CLL: Chronic Lymphocytic Leukemia; AS: Ankylosing spondylitis

mAb structure and PK

Structure IgG

Target mediated disposition; charge/PI mediated clearance; off-target binding

IgG recycling for long half-life

Glycan mediated clearance and tissue distribution
Mechanism of mAbs

Degradation of antigens bound to mAb, while antibody is recycled.

“Fab binding to antigens pH dependent, strong binding at pH 7.4 and no or weak at pH 6”
**ADME processes: overview for mAbs**

**Absorption:**
Bioavailability (F): 100-30% depending on route IV, SC or IM
Presystemic degradation by proteolitic enzymes → Non linear F

**Distribution:**
High MW and poor lipophilicity:
- Small Volume of distribution (V): Vc from 2-3 L; Vs-s from 3.5- 7 L
- Tissue: blood ratio < 0.5
  mAb high affinity to extravascular sites > 0.5 increasing the V
  Efalizumab exhibits V dose dependence due to cell internalization

**Elimination:**
- Linear or non-specific mediated by Fc
- Non-Linear or specific mediated by Fab’ promoting IC endocytosis
  Target-mediated drug disposition (TMDD), saturable (dose and target expression).

\[
CL = CL_{linear} + CL_{TMDD}
\]
Factors target-related affecting the non-linear PK

- **Localization**: soluble antigens
  - low endogenous levels: PK linear
  - high endogenous levels: PK non-linear
  - cell surface: PK non-linear

- **Affinity**: high affinity binding seems to act as barrier to distribution

- **Turnover**: overexpression may produce by proteolysis circulating serum target able to influence the PK of mAb
Immune complexes (ICs) between ADA-therapeutic protein influence:

*Therapeutic exposure or hypersensitivity reactions.*

Comparison of the impact of ADAs on PK, safety and efficacy across mAbs therapies can be misleading due to the difference in the protocols, study design and analytical assays.
Techniques for detecting ADAs

- **Detection** of ADA formation is highly dependent on the sensitivity and specificity of the assay.
- **Factor influencing**: method; sample handling; timing of sample collection; concomitant medications and even disease condition.

*Testing for ADA is a regulatory requirement and samples are commonly tested in a tiered approach to evaluate and confirm ADA.*

ADA positives go to NAb assays:

- ligand-binding assays that measure neutralization of binding
- cell-based assays that measure the neutralization of a biological effect of the drug:
  - **Cell-based assays**, in contrast to ligand-binding assays, are considered to provide a readout that is most representative of the biological effect elicited by neutralizing antibodies.
1. **Drug soluble target**
   
   Phase I: Therapeutic humanized antibody (TA) for binding to soluble target present in serum ADA incidence > 70% but with a competitive assay only 6.6-7.8% were positive.

2. **Outlier removal or Inappropriate CP (Cut Point) establishment**
   
   Biological and analytical outliers

---

**Clinical relevance:**

Adalimumab [rheumatoid arthritis]: 272 patients treated for 3 years

76 (28%) developed ADAs:

- 45 (72.4%) low ADA titer
- 31 (27.6%) high ADA titer;

*only these had lower mAb concentrations and lower clinical remission*
Technologies to detect immunogenicity based on ligand-binding assays:

• ELISA
• RIA
• Meso Scale Discovery (MSD);
• Electrochemiluminescence (ECL),
• Gyros
• Immuno CAR
• SQI Diagnostics

Liu. Protein Cell (2017)
Assumptions used in mAb and ADA modeling:

1.- Production of ADA:
   - Constant
   - mAb dose-dependent (*)

2.- Onset of ADA production:
   - Constant lag-time
   - a delay represented by a transit compartment

3.- Formation of ADA-mAb complex:
   - Reversible binding
   - Affinity maturation (polyclonal ADA)

4.- Biodistribution of mAb and ADA:
   - Compartmental models
   - Physiological PBPK models

5.- Elimination of mAb and ADA:
   - Linear process
   - target/ADA-mediated elimination

Utility of M&S to evaluate immunogenicity effects: mAb dose-dependent

PK model

No ADAs

ADAs

Perez Ruixo et al. AAPS J (2013)
Elotuzumab

Elotuzumab, a humanized IgG1, is an immunostimulatory mAb indicated in multiple myeloma (MM).

**Target:** glycoprotein (*SLAMF7*) expressed on more than 95% of myeloma cells (MM) and on natural killer (NK) cells.

**Dual mechanism:**

1. Direct activation NK by binding to *SLAMF7*
2. Cellular cytotoxicity antibody-dependent activating NK via CD16, killing MM with minimal effects on normal tissue

**Preclinical:** Elotuzumab was enhanced by a pretreatment with sub-therapeutic doses of lenalidomide and bortezomib.

**Clinic:** Phase I and II studies mAb combined with lenalidomide and bortezomib or lenalidomide and dexamethasone.
ECL assay for anti-Elotuzumab in serum samples

• A solid-phase extraction with acid pretreatment (SPE/AD) to dissociate the ADA bound to drug followed by an electrochemiluminescent detection.

• ADA positives further characterized with functional NAb cell-based bioassay.

   Bioassay: cells for binding and activation of the CD16α receptor by elotuzumab.
**Elotuzumab**

- $C_{\text{min,1}}$: minimum serum concentration after the first dose
- $C_{\text{min,ss}}$: minimum serum concentration at steady state

**M-protein levels at baseline**

- **ADA status**
  - **Negative**
    - $n=283$
    - $74.1 \, \mu g/mL$
  - **Positive**
    - $n=61$
    - $38.8 \, \mu g/mL$

- **ADA status**
  - **Negative**
    - $n=283$
    - $424 \, \mu g/mL$
  - **Positive**
    - $n=61$
    - $331 \, \mu g/mL$
No clear relationship between positive ADA responses and elotuzumab exposure

1. Vmax increased for those with high M-protein basal levels, decreasing drug exposure
2. May ADAs interfere with the detection of elotuzumab?
Elotuzumab

Study Design

Time-varying clearance using $\rho$ with a sigmoid function to provide smooth transition CL. $CL_i$ is the individual clearance, $T_{onset}$ time of onset, $T_{offset}$ time of offset

- ADAs and NAbs occurred early and resolved after 2–4 months.
- Early ADAs associated with an apparent increase in CL, returned to baseline when ADAs were no longer detected.
- No effect on drug exposure, then no change in efficacy or safety in the combination.
Olaratumab a humanized IgG1 that binds to platelet-derived growth factor receptor-α (PDGFRα) applied to metastatic cancer.
Study design

**Olaratumab 15 mg/kg**

- **STS**
  - mAb iv 60 min days 1 and 8 of 21 day/cycle
  - mAb + Dox 75 mg/m² at day 1 every cycle

- **NSCLC**
  - mAb iv 30 min days 1 and 8 of 21 day/cycle + 200 mg/m² paclitaxel + carboplatin

Assay: **ELISA**

ADAs no effect on PK

No effect on PK

No effect on PD
Lanreotide is a somatostatin analog used for hormone-related syndromes associated with neuroendocrine tumors (NETs).

Studies:
290 Patients from four clinical trials phase II, III and IV enrolled in different centers worldwide provided 1541 serum lanreotide concentrations measured by RIA.

ADAs no effect on PK

PK model

0 order process

1st order process

F₂, D₀

F₁, ka

Depot

Central V/F

CL/F
Remarks

- No rules for Immunogenicity (IM) prediction
- IM represents a complex scenario and depends on many factors: patients characteristics, disease progression, type of biomolecule, formulation....

Questions still opened:

- How drug exposure and/or drug efficacy may be limitated by ADAs generation, in particular by Nabs?.
- May be the current technical assays considered enough for the quantification of ADAs levels?

“PK characterization of mAbs highly depends on the data”
Thank you !!!

www.unav.edu/psp