Immunogenicity of mAbs
Industry perspective

AM Autere, Roche, May 2010
Background

Experience on industry commenting on the recent regulatory guidance documents related to immunogenicity & mAbs

- EMA CONCEPT PAPER ON IMMUNOGENICITY ASSESSMENT OF MONOCLONAL ANTIBODIES INTENDED FOR IN VIVO CLINICAL USE 2009
- EMA CONCEPT PAPER ON THE DEVELOPMENT OF A GUIDELINE ON SIMILAR BIOLOGICAL MEDICINAL PRODUCTS CONTAINING MONOCLONAL ANTIBODIES 2009
- EMA GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY- DERIVED THERAPEUTIC PROTEINS 2008
- EMA GUIDELINE ON PRODUCTION AND QUALITY CONTROL OF MONOCLONAL ANTIBODIES AND RELATED SUBSTANCES 2009
- Ph. Eur. monograph on monoclonal antibodies for human use
- .....
Agenda

• Impact of sequence/humanisation
• Impact of product quality
• Role of models to reduce and predict immunogenicity
• Clinical trials
• Industry feedback on the EMA concept paper on mAb immunogenicity
• Examples
• Summary

*Immunogenicity in this presentation: anti therapeutic antibodies (ATAs) to mAbs*
Humanisation?

“The expectation for such a humanised monoclonal antibody would be improved pharmacokinetic properties, as well as decreased immunogenicity, compared with the original murine antibody.”

Source: Ulrich Kalinke 3rd Annual Biosimilars, February 18 and 19, 2009 Amsterdam
Humanisation does not eliminate potential for immunogenicity – also other factors are involved

**Immunogenicity may be an issue even with “fully human“ mAbs**

<table>
<thead>
<tr>
<th>Product Name / INN</th>
<th>Format</th>
<th>Approval</th>
<th>Immunogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthoclone (muromonab)</td>
<td>murine</td>
<td>1986</td>
<td>53%</td>
</tr>
<tr>
<td>Zevalin (ibritumomab tiuxetan)</td>
<td>murine</td>
<td>2002</td>
<td>30%</td>
</tr>
<tr>
<td>Bexxar ($^{131}$I-tositumomab)</td>
<td>murine</td>
<td>2003</td>
<td>9%</td>
</tr>
<tr>
<td>Rituxan (rituximab)</td>
<td>chimeric</td>
<td>1997</td>
<td>0% (NHL); 67% (RA)</td>
</tr>
<tr>
<td>Remicade (infliximab)</td>
<td>chimeric</td>
<td>1998</td>
<td>8-61%</td>
</tr>
<tr>
<td>Simulect (basiliximab)</td>
<td>chimeric</td>
<td>1998</td>
<td>0-1%</td>
</tr>
<tr>
<td>Erbitux (cetuximab)</td>
<td>chimeric</td>
<td>2004</td>
<td>5%</td>
</tr>
<tr>
<td>Reopro (abciximab)</td>
<td>chimeric Fab</td>
<td>1994</td>
<td>4-21%</td>
</tr>
<tr>
<td>Zenapax (daclizumab)</td>
<td>humanized</td>
<td>1997</td>
<td>8-34%</td>
</tr>
<tr>
<td>Herceptin (trastuzumab)</td>
<td>humanized</td>
<td>1998</td>
<td>0.1%</td>
</tr>
<tr>
<td>Synagis (palivizumab)</td>
<td>humanized</td>
<td>1998</td>
<td>0-1%</td>
</tr>
<tr>
<td>Mylotarg (gemtuzumab)</td>
<td>humanized</td>
<td>2000</td>
<td>0%</td>
</tr>
<tr>
<td>MabCampath (alemtuzumab)</td>
<td>humanized</td>
<td>2001</td>
<td>50%</td>
</tr>
<tr>
<td>Raptiva (efalizumab)</td>
<td>humanized</td>
<td>2003</td>
<td>2-6%</td>
</tr>
<tr>
<td>Xolair (omalizumab)</td>
<td>humanized</td>
<td>2003</td>
<td>0%</td>
</tr>
<tr>
<td>Avastin (bevacizumab)</td>
<td>humanized</td>
<td>2004</td>
<td>0%</td>
</tr>
<tr>
<td>Tysabri (natalizumab)</td>
<td>humanized</td>
<td>2004</td>
<td>7%</td>
</tr>
<tr>
<td>Humira (adalimumab)</td>
<td>human</td>
<td>2002</td>
<td>5-12%</td>
</tr>
<tr>
<td>Vectibix (panitumumab)</td>
<td>human</td>
<td>2006</td>
<td>1-4%</td>
</tr>
</tbody>
</table>


**Disclaimers..**
- Immunogenicity method, product, condition, study specific – not to be compared
- Type of ATAs and clinical consequences more relevant than the incidence of ATAs
Impact of product quality on immunogenicity?

Impurities, PTMs, heterogeneity, formulation

Presence of aggregates/ particulates is unwanted

EMA BWP draft mAb guideline:

<table>
<thead>
<tr>
<th>Lines 38-40 (ROCHE)</th>
<th>The statement &quot;natural tendency...to aggregate&quot; is troubling. This seems to imply antibodies are just waiting to jump out of solution. Reword this sentence: &quot;..., and therefore the concentration of antibody in the final formulation may be higher than normally experienced for other biotechnology products, especially in the case of sub-cutaneous delivery forms. The formation of sub-visible and visible particulates is therefore of importance and should be closely monitored on lot release and during stability studies. The presence of visible particulates is unwanted....&quot;</th>
</tr>
</thead>
</table>

Some feedback:
Impact of product quality on immunogenicity?

*Example aggregates*

Final EMA BWP mAb guideline:

Multimers and aggregates should also be appropriately characterised using a combination of methods. The formation of aggregates, sub-visible and visible particulates in the drug product is important and should be investigated and closely monitored on batch release and during stability studies. In addition to the pharmacopoeial test for particulate matter, other orthogonal analytical methods may be necessary to determine levels and the nature of particulates.

Particular attention should be paid to the demonstration of the suitability of the analytical methods used to control multimers and aggregates.

Industry is minimising aggregate formation:

added to achieve the correct toxicity. The amount of Polysorbate 80 was optimized to minimize the formation of aggregates, as well as sub-visible and visible particles during agitated and freeze-thaw conditions.

“Levels of aggregates should be kept as low as possible, although it was not known whether aggregates of mAbs are more immunogenic than non-aggregated mAbs”

Risk minimisation by good, consistent quality
Predictability of immunogenicity?

• (Lack of) Immunogenicity cannot be predicted based on the sequence or glycosylation of the mAb
  – Minimisation of potential immunogenic epitopes

• Immunogenicity cannot be predicted based on quality characteristics of the product

• Predictive value of animal studies is low
  – “Cynomolgus monkeys are not recognized as an adequate model to predict human immunogenicity due to species differences in the response…”

• Emerging *in vitro* and *in silico* technologies
  – These technologies not validated and cannot be used in prediction or evaluation of immunogenicity
  – May be used in lead optimisation

*Appropriate human data needed in relevant indications*
Human data
Analytics

Specific issues on mAb immunogenicity testing

• MAbs have long half life and high trough levels. MAbs and ATAs have similar structure
  – Drug interference relevant
    • Adequate and feasible sampling & follow-up period/ removal of the therapeutic mAb from the samples
  – Methodology may (and seem to be) problematic

• MAbs may have several mechanisms of action
  – What is binding only ATA?
  – Appropriate assays needed

• ...
Multifunctionality of mAbs – implications also on immunogenicity testing?

The Mode of Action of mAbs is complex and may involve Contributions from multiple Mechanisms

- Activation of Effector Mechanisms
  - Antibody-dependent cellular Cytotoxicity (ADCC) (Examples: Rituximab, Trastuzumab)

- Complement Activation (CDC) (Example: Rituximab)

- Activation of T-Cells (Example: Catumaxomab)

- Inhibition of Signal Transduction or Receptor Activation
  - Inhibition of Ligand Binding (Example: Cetuximab)
  - Induction of Receptor Internalization (Example: IGF-1R-Abs)
  - Inhibition of Receptor Dimerization (Example: Pertuzumab)
  - Inhibition of Receptor Shedding (Example: Trastuzumab)

- Induction of Apoptosis (Example: Rituximab)

- Targeting of Toxins (Example: T-DM1)

- Blocking Ligand Binding (Example: Bevacizumab)

The in-vivo net contribution of different modes of action described for one mAb is often incompletely understood and may also be different in different indications.

- Human data from relevant conditions & populations
- Interpretation of results

**Case-by-case:**

- Different mAbs are used in different indications; Same mAbs are used in different conditions
  - Immunocompetent/ hypersensitive/ immunocompromised patients
  - In life threatening diseases vs. in less severe diseases
  - Short vs. often chronic treatment
  - Age, concomitant medication, dose, route of administration may have impact

- ATA response may be persistent or transient; Induction of tolerance possible

**Results from immunogenicity assays cannot directly predict clinical consequences:**

- **Impact of ATAs on safety and efficacy relevant:** also immunogenic mAb may be appropriate

- Relevance of clinical risk management plans
Main points of the EBE feedback on the EMA concept paper on mAb immunogenicity (1)

• Not apparent to all EBE members why a specific guideline on mAb immunogenicity needed
  – Guidance on immunogenicity of mAb derivatives/2nd generation products may be useful

• Immunogenicity cannot be predicted
  – Human data necessary for each product, in all relevant indications
  – Case-by-case risk based approach needed
  – Clinical impact of ATAs may vary (although immunogenicity of conventional mAbs not often adverse/common?; relatively low risk of neutralisation of endogenous proteins or induction of autoimmune disorders expected?; main impact decreased efficacy?)
  – Does humanisation reduce induction of adverse ATAs?
  – Animal, in vitro and in silico approaches currently not relevant for human immunogenicity, regulatory requirements for these approaches would not be appropriate
Main points of the EBE feedback on the EMA concept paper on mAb immunogenicity (2)

<table>
<thead>
<tr>
<th>Potential specific issues on mAbs and mAb related therapeutics to be addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Quality related issues:</td>
</tr>
<tr>
<td>• Immunogenicity potential of Ig-structure based products</td>
</tr>
<tr>
<td>o Likelihood and clinical sequelae of antibodies to mAbs. In comparison to other therapeutic proteins such as growth factors the ADAs to mAbs are generally anti-idiotypic and do not have the potential to neutralize endogenous proteins or induce autoimmune disorders</td>
</tr>
<tr>
<td>• Identifying, characterization and relevance of antibodies to different regions of the mAb molecules</td>
</tr>
<tr>
<td>o However, epitope mapping should not be a routine part of immunogenicity testing strategy</td>
</tr>
<tr>
<td>• Relevance of addressing specific structure of the given mAb to immunogenicity</td>
</tr>
<tr>
<td>o amino acid sequence</td>
</tr>
<tr>
<td>o impact of minimizing immunogenic epitopes and “humanization” – does humanization reduce ADA rates (with examples on observed, and on relevant differences in formed neutralizing antibodies or the differences in pre-existing antibodies to human/humanized vs. chimeric products)</td>
</tr>
<tr>
<td>o posttranslational modifications including glycosylation, oxidation, deamidation, aspartate cyclisation</td>
</tr>
<tr>
<td>o chemical modifications</td>
</tr>
<tr>
<td>• If it would be important to consider the Fc isotype and allotype in</td>
</tr>
</tbody>
</table>

• Include specific examples of immunogenicity related to change in mAb manufacturing/ packaging/aggregate percentage etc., if available
Main points of the EBE feedback on the EMA concept paper on mAb immunogenicity (3)

• Effector functions and MOAs to be taken into account in the selection of methods and evaluation
  – Antigen binding is not always the only relevant function of mAbs
  – MAbs that deplete B-cells etc

• Discussion on ATA analytics welcomed
  – Interference due to high dose, long half-life and repeated dosing, and mAb structure specific challenges in measuring ATAs to mAbs
  – Not agreed that competitive ligand based assays are better than bioassays in evaluating neutralising ATAs – case by case approach needed

• Relevance of isotype and subtype information on ATAs not clear

• Relevance of determining neutralising activity on each sample not clear

• Benefit/ risk of mAb products vs. other therapies important
Immunogenicity of biosimilar mAbs  
EBE feedback Jan 2010 on the EMA concept paper on biosimilar mAbs  

- “Immunogenicity is an important parameter in determining similarity. Studies to assess immunogenicity should be comparative and designated to evaluate long-term immunogenicity using the same analytical standards expected for an innovator mAb.”

- “Immunogenicity can differ across indications for an individual mAb and the reasons for these differences are not always understood but may include different populations, disease states, concomitant medications, dose and route of administration. The biosimilar mAb guidance should emphasise that human clinical data on immunogenicity in each indication are required and that only in exceptional circumstances could extrapolation of immunogenicity be considered”

- “It should be considered that while rates of immunogenicity may be similar between innovator and mAb cross reactivity of the ADAs produced may be different. A requirements for characterisation of the response should be included in the guidance including an assessment of isotype and determination of cross reactivity of the subject’s antibody between the innovator or other biosimilar..”
Examples (1)

**Approved fully human mAbs, inflammation, sc, repeated dosing**

- ATA incidence up to ~10%, most neutralising. “EIA was used for detection of ATAs. This method measures only the non-complexed antibodies. Furthermore, occurrence of mAb in the serum samples interferes with the method and results in false negative results. It is difficult to identify individuals with a positive immune response and the practical usefulness of the present EIA method is low, the impact of being antibody positive on efficacy and safety is difficult to evaluate. The presence of neutralizing antibodies was determined by functional cell-based bioassay applied for samples identified as immune response positive by the EIA only in one clinical study”

- “Efficacy reduced in subjects positive for ATAs. Slightly higher incidence of injection reactions in subjects positive for antibodies”

- “Co-administration of mAb with MTX reduces the clearance of mAb and the likelihood of ATA formation.” “The neutralising effects of ATA may be reversed with increasing the dose intensity”

Source: EPAR
Examples (2)

Approved fully human mAb, rare inflammatory disease (scarcity of patients), sc, repeated dose

• Long term safety data limited. No ATAs detected (n<200). Immunogenic potential concluded as low. Good local tolerability regarded as clinical evidence that immunogenicity could be low. “However, due to methodological insufficiencies (drug interference) underestimation of antibody incidence could not be excluded. An improved assay should be developed (post approval)”. Pharmacovigilance activities regarding immunogenicity.

**Typical:** Improved assays should be developed post approval. Pharmacovigilance activities regarding immunogenicity.
Approved humanised mAb in oncology

• “As with all therapeutic proteins, there is a potential for immunogenicity. The incidence of antibody development in patients has not been adequately determined because the assay sensitivity was inadequate to reliably detect lower titers. ELISAs were performed on sera from approximately 500 patients treated, primarily in combination with chemotherapy. High titer ATAs were not detected.”

• Post marketing commitments regarding immunogenicity

• Contraindications: Hypersensitivity to the active substance, to any of the excipients, to CHO cell products or other recombinant human or humanised antibodies
Examples (4)

Approved mAb derivative

“Due to the small number of subjects across studies that developed an immune response, conclusions concerning the impact of immunogenicity on safety and efficacy cannot be made. However, the development of immunogenicity in subjects did not appear to be associated with adverse safety and efficacy outcomes. Further assessment of immunogenicity and possible clinical consequences remains important and is currently addressed in the context of the Risk Management Plan (immunogenicity potential safety risk). The current ELISA assay is complex and not amenable to large-scale use. As a follow-up measure, the applicant will report the results from clinical samples with a new ATA assay (compared to the old ELISA assay).”

“Satisfactory consistency of relevant product quality characteristics demonstrated”
Summary – mAb immunogenicity

Risk minimisation and estimation

Amino acid sequence
- Optimise

Good Quality
- Glycosylation, PTMs, heterogeneity

- Impurities, degradation products
  - Reduce/ justify

- Consistent process

- Formulation
  - Optimise

Clinical use, target population
- Indications; immune status; concomitant therapies; previous therapies; route of administration, duration;

Reality

- Immunogenicity cannot be predicted
- Need to be studied in human
- Clinical trial design with appropriate and feasible sampling and sufficient analytical methods
- Risk based evaluation of the results
  - Neutralising/ binding only ATAs - clinical consequences?
- Management of immunogenicity in clinical practise

Impact of ATAs on safety and efficacy
Benefit/risk in the given indication