Safety Considerations in Biologics

By: Jan Fourman

Objectives

• Gain a better understanding of biologic agents, risk factors and potential MOA’s as it relates to safety
• Discuss complexities in clinical evaluation of and reporting of safety considerations in the era of biologics
• Provide case examples and discuss clinical cases illustrative of drug safety in the development and use of biologics
Safety Requirements

• Sensitivity and need for safety evaluation is consistent between biologics and small molecules, though biologics offer the added challenge of immunogenicity
• While study methods are generally comparable, approaches taken may vary considerably with biologics
• Biologic activity, scientific rationale, clinical relevance should drive program design

Special Considerations for Biologics

• Nature and size of molecule
  – Monoclonal antibodies (Mabs), fusion proteins, cytokines, hormones, growth factors, enzymes, thrombolytics, etc.
  – MW range 1,000 (peptides) to > 140,000 (Mab) Daltons
• Structure:
  – Complex and heterogeneous
• Molecular target and expression
  – High specificity and selectivity
  – potential for exaggerated pharmacology or off target effects
  – Species specificity of molecule in evaluating preclinical safety and efficacy studies
Differing Types of Biologics

• Antibodies
  – Polyclonal, monoclonal, chimeric, humanized, fully human
    • Remicade, Rituxan, Xolair, Avastin, Humira, etc

• Recombinant Proteins
  – Mostly ligands and enzymes that stimulate processes
    • Insulin, EPO, Thrombin

• Fusion Proteins
  – Created through the joining of two or more genes that were originally coded for separate proteins
    • Enbrel

• Peptides
  – Smaller than proteins
  – Consist of between 2 and 50 amino acids
    • Glucagon

Safety Consideration for Biologics

• Immune System
• Cardiovascular and cardiopulmonary
• Central Nervous System
• Gastrointestinal System
• Renal
• Hematologic
Assessing Immunogenicity Risk: Clinical Perspectives

Immunogenicity

- Immunogenicity = immune response to a therapeutic protein (biologic)
  - Wanted immunogenicity: The injection of an antigen (e.g., vaccine) leads to an immune response against the pathogen.
  - Unwanted immunogenicity: The organism mounts an immune response against an antigen which is undesired.
- Immunogenicity is generally not a concern for small molecules, but is a significant concern for biologics
- Clinical consequences of unwanted immunogenicity can range from redness at site of injection to rash to anaphylaxis and even death.
Case Studies

• The Eprex Story: pure red cell aplasia

• Thrombopoietin (TPO): thrombocytopenia

Eprex Story

• Eprex is a recombinant erythropoietin (EPO) that is used to treat anemia (due to chronic kidney disease or chemotherapy)

• Pure red cell aplasia (PRCA) = rare condition in which the body no longer makes red blood cells, resulting in severe anemia. Symptoms include: pale skin, unusual bleeding or bruising, or unusual tiredness or weakness.

• Prior to 1998, EPO-induced antibody PRCA was very rare

• From 1999 to 2002, there was a large increase in the number of global cases

WHY?
Eprex Story continued

• Possible Causes:
  – Manufacturing change: substitution of human serum albumin with polysorbate 80 (a synthetic stabilizer)
  – Container change: leaching from uncoated rubber stoppers

• Resolution:
  – Switching to IV administration
  – Use Teflon-coated rubber stoppers

Drug given to treat anemia in very ill patients made them significantly worse
Due to changes in manufacturing (Product Attributes)

Thrombopoietin Story

• TPO = growth factor required for normal production of platelets (which are required for clotting of blood)
• In 1995, two recombinant TPOs entered clinical trials
  – recombinant human TPO (rhTPO), administered IV
  – pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), administered SQ
• 1995 to 1999: several small studies established proof of concept in treatment of immune thrombocytopenic purpura (ITP) and myelodysplastic syndrome (MDS)
• Large study in healthy volunteers with PEG-rHuMGDF – for platelet donation
• Several volunteers experienced dramatic decreases in platelets

WHY?
TPO Story continued

• 4% of healthy volunteers developed anti-drug antibodies to PEG-rHuMGDF, leading to increased bruising and heavy menses
• 3 healthy volunteers (<1%) developed antibodies to Peg-rHuMGDF that cross-reacted with normal full-length, endogenous TPO
  – These subjects did not respond to steroids or IV immunoglobulin
  – Cyclosporin therapy eliminated antibodies and restored platelet count in 1 of the subjects
• Consequences: These symptoms did not occur in subjects treated with recombinant human TPO (rhTPO); however, clinical development of both biologics was halted

Lessons Learned from Case Studies

• Unintended consequences
• Impact of manufacturing changes
• Impact of route of administration
• Cross-reactivity with endogenous proteins
• Different consequences for healthy volunteers and patients
  – Healthy volunteers – no benefit, significant clinical harm
  – Patients – significant benefit, little clinical harm
Clinical Consequences of Unwanted Immunogenicity

- None
- Minor
  - Injection site reactions (local redness, irritation)
- Moderate
  - Rash
  - Loss of efficacy due to neutralizing antibodies
  - Loss of efficacy due to altered PK of therapeutic agent
- Major
  - Anaphylaxis
  - Death
  - Neutralizing antibodies that cross-react with and neutralize a critical endogenous substance

Clinical Considerations

- Patient status
- Treatment history
- Dosing route
- Duration of treatment
- Intended disease state
- Available treatment options
How do we know if immunogenicity is a problem?

- We look at…
- Adverse events over time
  - Occur early or late in treatment?
  - Review clusters of events representative of immunogenicity
- Pharmacodynamic response over time
  - Loss of efficacy
- Concentration of drug over time
- Formation of antibodies to drug (ADA)
- Formation of neutralizing antibodies

And patients fail to benefit!

Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up

Geertje M. Bartelds, MD; Charlotte L. M. Krieckaert, MD; Michael T. Nurmohamed, MD, PhD; Pauline A. van Schouwenburg, MSc; Willem F. Lems, MD, PhD; Jos W. R. Twisk, PhD; Ben A. C. Dijkmans, MD, PhD; Lucien Aarden, PhD; Gerrit Jan Wolbink, MD, PhD

Sampling and Assays

- Assays
- Interpreting Results
- Sampling Schedules

Screening Assays

1. Patient Sample
2. Tier 1 - Anti-Diag Antibody Screening
   - Signal below cutoff: Report as not detected
   - Signal above cutoff: Confirm
3. Tier 2 - Anti-Diag Antibody Confirmation
   - Not confirmed: Report as not detected
   - Confirmed: Tier until signal below cutoff
4. Tier 3 - Anti-Diag Antibody Titration
   - Tier until signal below cutoff: Report as detected with titer
   - Postbaseline sample is ≥4-fold higher than baseline titer
5. Tier 4 - Neutralizing Anti-Diag Antibody Assay
   - Below cutoff: Report as not detected for neutralizing antibody
   - Above cutoff: Report as detected for neutralizing antibody
Interpreting Assay Findings

Sample Level

- ADA Sample
  - Positive
    - Treatment Emergent
    - Not-treatment Emergent
  - Negative
    - Truly Negative OR INCONCLUSIVE
    - Need drug level to assess whether ADA assay drug tolerance has been exceeded!

ASSESS as Transient or Persistent

Sampling in Early Phase Studies

- Suggested Sampling Schedule
  - Lower risk molecules
    - Pre-dose, 4 weeks after first dose, at time of last dose, washout sample
    - Typically batch and analyze samples at end of trial
  - High risk molecules
    - Pre-dose, 2 weeks, 4 weeks, and 12 weeks after the first dose, then every 12 weeks until last dose, washout sample
    - Typically batch and analyze samples at end of trial
    - Assess positive samples with neutralization assay
Sampling in Phase 2 and 3 Studies

• Multiple factors will influence sampling schedule for later phases of development – discuss with experts and regulators!
  – It is imperative that the phase 2 and 3 study design have a sample collection and storage plan that will be used for assessment.
  – Assays that will be utilized in these phases include sensitive and drug tolerant ADA screens and potentially (if a positive ADA) a sensitive and drug tolerant neutralizing ADA screen (that is usually cell based).

What are regulators looking for?

• They want to understand the clinical consequences – safety and efficacy
• They want to be able to predict who is at risk
• They want to understand the mechanism by which the immune system is activated
### Immunogenicity Labeling for Approved Biologics in the US

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>US Approval</th>
<th>% ADA</th>
<th>Clinical Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adcetris (brentuximab vedotin)</td>
<td>Hodgkin's Lymphoma</td>
<td>2011</td>
<td>7%</td>
<td>Warning for Infusion reactions, including anaphylaxis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>transient (30% transient)</td>
<td>Recommend premedication</td>
</tr>
<tr>
<td>Yervoy (ipilimumab)</td>
<td>Metastatic melanoma</td>
<td>2011</td>
<td>1.1-6.9%</td>
<td>Boxed Warning for Immune-Mediated Adverse Reactions</td>
</tr>
<tr>
<td>Benlysta (belimumab)</td>
<td>Lupus</td>
<td>2011</td>
<td>1-4.8%</td>
<td>Warnings for Hypersensitivity Reactions, Including Anaphylaxis and Infusion Reactions</td>
</tr>
<tr>
<td>Krystexxa (pegloticase)</td>
<td>Gout</td>
<td>2010</td>
<td>92% anti-pegloticase, 42% anti-PEG</td>
<td>Boxed Warnings for Anaphylaxis and Infusion Reactions</td>
</tr>
<tr>
<td>NPlate (romiplostim)</td>
<td>Idiopathic thrombocytopenia purpura</td>
<td>2008</td>
<td>8% (extensive section)</td>
<td>Warning for Lack or Loss of Response to NPlate</td>
</tr>
</tbody>
</table>

### Immunogenicity-Related PMCs/PMRs for Approved Biologics in the US (#1)

<table>
<thead>
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<th>Drug</th>
<th>PMC/PMR</th>
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<tr>
<td>Adcetris (brentuximab vedotin)</td>
<td>• Perform additional experimental work to understand the impact of soluble CD30 in serum samples on the determination of anti-drug antibodies</td>
</tr>
<tr>
<td>Yervoy (ipilimumab)</td>
<td>• To develop a validated, sensitive, and accurate assay for the detection of binding antibodies to ipilimumab, including procedures for accurate detection of antibodies to ipilimumab in the presence of ipilimumab levels that are expected to be present in the serum or plasma at the time of patient sampling.</td>
</tr>
<tr>
<td></td>
<td>• To develop a validated, sensitive, and accurate assay for the detection of neutralizing antibodies to ipilimumab, including procedures for accurate detection of neutralizing antibodies to ipilimumab in the presence of ipilimumab levels that are expected to be present in the serum or plasma at the time of patient sampling. In the event such an assay can not be developed, evidence of due diligence in attempting to develop the assay will be provided.</td>
</tr>
<tr>
<td></td>
<td>• To conduct an assessment of anti-drug antibody (ADA) response and neutralizing ADA responses to ipilimumab with a validated assay (required in PMR 2 and 3) capable of sensitively detecting ADA responses in the presence of ipilimumab levels that are expected to be present at the time of patient sampling. The ADA response will be evaluated in at least 300 ipilimumab-treated patients enrolled in the required postmarketing trial (PMR 6) comparing 3 mg/kg versus 10 mg/kg of ipilimumab monotherapy. The final report will include information on the level of ipilimumab in each patient’s test sample at each sampling time point</td>
</tr>
</tbody>
</table>
## Immunogenicity-Related PMCs/PMRs for Approved Biologics in the US (#2)

<table>
<thead>
<tr>
<th>Drug</th>
<th>PMC/PMR</th>
</tr>
</thead>
</table>
| Benlysta (belimumab) | • Develop improved immunogenicity assays that are less sensitive to product interference that are capable of detecting human anti-human antibodies (HAHA) in the presence of belimumab at ranges that would be expected to occur in patients receiving both high and low doses  
• Conduct a randomized, placebo-controlled clinical trial with Benlysta (belimumab) in 5000 patients with active, autoantibody-positive systemic lupus erythematosus to evaluate Benlysta’s long term safety profile including adverse events of special interest (e.g., mortality, malignancy, serious and opportunistic infections and depression/suicidality). |
| Krystexxa (pegloticase) | • An observational safety study enrolling 500 patients treated with Krystexxa (pegloticase) for one year duration; specifically an evaluation of the frequency and severity of infusion reactions, anaphylaxis, and immune complex-related adverse events, and identification of serious adverse events associated with Krystexxa (pegloticase) therapy  
• The current anti-PEG antibody ELISA shows a very high degree of intra-and inter-assay variability possibly related to the PEG coating of the ELISA plate. This indicates either that the assay is not sufficiently optimized or that the format is unsuitable. Redevelop the anti-PEG antibody assay to address these concerns  
• The sensitivity of your IgE assay, as currently designed, is insufficient to detect IgE antibodies to the product. For an antigen-specific IgE assay to be useful, it should have sensitivity in the nanogram to sub-nanogram range, and there are technologies currently available that can meet this criterion. Develop a more sensitive antigen-specific IgE assay. Consider using ECL technology.  
• Your IgE assay was not properly validated due to a lack of positive control antibody. Develop a suitable positive control for the IgE ELISA. Cross-linking the current rabbit polyclonal to a human IgE may be an option |

## Immunogenicity-Related PMCs/PMRs for Approved Biologics in the US (#3)

<table>
<thead>
<tr>
<th>Drug</th>
<th>PMC/PMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nplate (romiplostim)</td>
<td>• To conduct an “Antibody Registry Study” that will enroll subjects who have received romiplostim and whose blood samples contain antibodies to either romiplostim or thrombopoietin. The antibody assays will be performed by Amgen in response to spontaneously submitted requests for the post-marketing blood tests. As described in the Romiplostim prescribing information, a lack or loss of response to romiplostim should prompt the healthcare provider to search for causative factors, including neutralizing antibodies to romiplostim. In these situations, healthcare providers are to submit blood samples to Amgen for detection of antibodies to romiplostim and thrombopoietin. The Antibody Registry Study will collect follow-up platelet count and other clinical data sufficient to assess the long term consequences of the detected antibodies. Patients will be followed until the detected antibodies resolve or stabilize in titer over a several month period of time.</td>
</tr>
</tbody>
</table>
Challenges in Recognizing Adverse Effects from Biologics

- Limited confirmatory testing to confirm causality and at times is a diagnosis of exclusion
- Presence of confounders in the underlying disease state for which the biologic is being administered
- Adverse drug affects may be quite diverse with varying symptoms and severity
hERG assay for MAb’s?

- MAb’s have very low potential for interacting with the extra- or, intracellular pore domains
- Do not have ability to cross plasma membrane directly; no access to intracellular “pore”
- QTc assessment: integrate into repeat-dose toxicology studies in appropriate species

Radiologic Example of Drug Induced Pneumonitis
Patterns of Pulmonary Toxicity with Biologics

- Infection/immune suppression
- Pulm edema/ DAH
- Pleural and parenchymal lung involvement
- Pulmonary vasculature
- Bronchospasm
- Mediastinal Involvement
- Neuromuscular dx
- Interstitial and infiltrative lung dx

Drug induced Changes in Pulmonary Vasculature

- May affect both arterial and venous circulation
- Pathologic Affects:
  - Pulmonary arterial Hypertention
  - Pulmonary Thromboembolic disease
  - Pulmonary Veno-occlusive disease
Clinical Case of Tracheal Erosion Following Immune Modulation

Bevacizumab Induced Tracheal Wall Erosion

Pulmonary Vascular changes in Drug toxicity of the Lung

Pulmonary Embolic Dx  PVOD
**Histopathology in PVOD**

- Extensive fibrosis and occlusion of pulmonary veins
- Poor prognosis
- Etiology:
  - Genetic
  - Infx: post-viral,
  - CT Dx,
  - Drug induced-bleomycin, mitomycin, and carmustine (BCNU)

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**Normal Lung Histology**
Histopathology with ILD

Nonspecific Interstitial Pneumonia (NSIP)  Diffuse Alveolar Damage

Pathology Findings in ILD

Bronchiolitis Obliterans (BO)  Bronchiolitis Obliterans with Organizing Pneumonia (BOOP)
Progressive Multi-focal Leukoencephalopathy (PML)

- PML is a rare and usually fatal disease characterized by progressive damage (pathy) of the white matter (leuko-) of the brain (encephalo-) at multiple locations (multifocal).
- Caused by reactivation of the latent JC virus
- Medications that suppress the host cellular immune response have been associated with this severe brain disease

Angioedema/ Hypersensitivity/ Immunologic Concern with Biologics

- Exposure to any foreign protein or substance may illicit an immune response
  - Hypersensitivity/ anaphylaxis
  - Development of circulating antibodies which may affect drug efficacy and safety
Infection Concerns with Biologics

Varicella Zoster  Oral Thrush

 Concern for Racial and Ethnic Disparities in the use of Biologics

- Racial and ethnic differences in phenotypic expression of cytokines been identified
- Significant implications for metabolism/efficacy/safety and possible disease predilection

Delaney NL, Hum Immunol. 2004
Lederer DJ, J of Transplantation 2006
Racial and Ethnic Differences

– Significant differences in gene frequency and susceptibility to drug induced pathogenesis in Chinese descent
– Demonstrated inheritance of certain cytokine gene polymorphisms is strongly assoc with ethnicity
– Significant differences in gene polymorphisms for TNF, IL-6, IL-10 and TGF-B1 in South and South East Brazil
  
  Zhang JI, Resp Medicine 2012
  Hoffman et al, Amer J of Tranplant 2002
  Visentainer JE, Intern J of Immunogenetics 2008

Potential Familial Genetic Link to ILD

• Familial patterns of autosomal inheritance have been described:
  – DNA mutations in Surfactant Protein C gene
  – Similar Genetic mutations also described for genes encoding for TNF, Interleukin receptors and Transforming Growth Factor Beta-1 (TFFB1)
• Results may suggest predilection for development of ILD

  Chibbar R., Mod Pathol. 2004 ;17(8): 973-80
Conclusions

• Adverse drug affects with biologics can be quite diverse in etiology, manifestations and severity
• Familial, racial and ethnic differences in genotypic/phenotypic expression may pose unique challenges in predicting efficacy and safety in select patient populations
• Cytokine network is extremely complex and elaborate intracellular signaling pathway in which we have only yet begun to fully elucidate its role in disease and disease modulation

Why We Care

• Most biologics will induce antibodies
• Most large pharma/biotech portfolios consist of growing numbers of biologics
• Avoid unintended consequences – biologics and immunogenic responses are complex
• Level of sampling and monitoring dependent on potential risk
• Regulators continue to raise the bar for assessment and interpretation