PRE-CLINICAL IMMUNOGENICITY ASSESSMENT – SCIENTIFIC VALIDATION VERSUS REGULATORY VALIDATION APPROACH. WHAT IS THE APPROPRIATE TIERED ANALYSIS?

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Agenda

► Introduction to the use of immunogenicity assessments in pre-clinical work

► Current regulatory landscape for pre-clinical activities

► Defining appropriate tiered analysis for pre-clinical studies

► Conclusions
Introduction to Pre-clinical Immunogenicity Assessment – What is it Used For
Immunogenicity of Therapeutic Proteins

It has been found that nearly all recombinant therapeutic proteins (or biotherapeutic) are immunogenic and can lead to the production of antibodies (Abs).

Immunogenicity of a biotherapeutic is a function of it’s “foreignness” to the recipient

Immunogenicity can be either wanted or unwanted

► Wanted: vaccine administration
► Unwanted: a major consideration for data interpretation in pre-clinical and a safety concern in clinical studies
Factors That Influence Immunogenicity

► Glycosylation pattern
► Correctly folded
► Aggregation
► Truncated
► Oxidation
► Phosphorylation

► New disulfide bonds
► Formulation
► Route of Administration
► Dose and length of treatment
► Patient factors: health status, co-medications, etc…

All of these factors can result in unwanted immunogenicity
Erythropoietin > Antibody mediated pure red cell aplasia (PRCA)

Biosimilar Hexal (HX575) – Tungsten exposure in pre-filled syringes precipitated immunogenic reactions

*Significant safety risks but how much of this is relevant to pre-clinical activities?
# Immunogenic Classes of Therapeutic Protein

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Human Protein Homology</th>
<th>Immunogenicity Frequency</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Prokaryotic</td>
<td>Low</td>
<td>High</td>
<td>Staphylokinase</td>
</tr>
<tr>
<td>B</td>
<td>Mammalian</td>
<td>Low</td>
<td>High</td>
<td>OKT-3</td>
</tr>
<tr>
<td>C</td>
<td>Novel Construct</td>
<td>Medium</td>
<td>Variable</td>
<td>High: Denileukin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low: human growth hormone</td>
</tr>
<tr>
<td>D</td>
<td>Chimeric Human</td>
<td>High</td>
<td>Variable</td>
<td>H: chMuL6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L: rituximab</td>
</tr>
<tr>
<td>E</td>
<td>Humanized</td>
<td>High</td>
<td>Variable</td>
<td>L: Campath-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H: Human anti-CD3</td>
</tr>
<tr>
<td>F</td>
<td>Human</td>
<td>Identical</td>
<td>Variable</td>
<td>H: GM-CSF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L: Human insulin</td>
</tr>
</tbody>
</table>

Reference: Bugleski and Treacy, Cur Opinion Mol Ther 6: 10-16, 2004
Animal data is not predictive of clinical outcome

<table>
<thead>
<tr>
<th>Protein Therapeutic</th>
<th>Preclinical Immunogenicity?</th>
<th>Clinical Immunogenicity?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptokinase and Staphlyokinase</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Keyhole Limpet Hemocyanin</td>
<td>High in rodents</td>
<td>High</td>
</tr>
<tr>
<td>Human interferon α-2a</td>
<td>High in rodents</td>
<td>Low</td>
</tr>
<tr>
<td>Human Growth Hormone</td>
<td>High in rodents</td>
<td>Low</td>
</tr>
<tr>
<td>Human Interferon-λ</td>
<td>High in Cynomolagus monkeys</td>
<td>Low</td>
</tr>
<tr>
<td>Human Interleukin-3</td>
<td>High in Rhesus monkeys</td>
<td>Low</td>
</tr>
</tbody>
</table>

Reference: Bugleski and Treacy, Cur Opinion Mol Ther 6: 10-16, 2004
Current Regulatory Landscape For Preclinical Activities
6. Non-clinical assessment of immunogenicity and its consequences

Therapeutic proteins show species differences in most cases. This is because humanized proteins may be recognized as foreign proteins by animals. For this reason, the predictivity of non-clinical studies for evaluation of immunogenicity in humans is considered low. Non-clinical studies aiming at predicting immunogenicity in humans are normally not required.

However, ongoing consideration should be given to the use of emerging technologies (novel in vivo, in vitro and in silico models), which might be used as tools during development or for a first estimation of risk for clinical immunogenicity. In vitro assays based on innate and adaptive immune cells could be helpful in revealing cell-mediated responses.

It is expected that (non)clinical studies are supplied with material sufficiently representative of the medicinal product that is going to be placed on the market. Since immunogenicity concerns may arise from the presence of impurities or contaminants, it is preferable to rely on purification processes to remove impurities and contaminants rather than to establish a preclinical testing program for their qualification (refer to ICH S6 (R1) Harmonised Tripartite Guideline on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals).

Measurement of anti-drug antibodies in non-clinical studies may be needed as part of repeated dose toxicity studies, in order to aid in the interpretation of these studies (as discussed in ICH S6 (R1)).

Herzmans, however, antibodies in non-clinical studies may be useful in pharmacokinetic studies, where usually higher concentrations of therapeutic protein are present in the samples, the interference of the therapeutic protein in the ADA assays needs to be considered.

In the development of similar biological medicinal products (biosimilars), the comparison of the anti-drug antibody response to the biosimilar and the reference product in an animal model is not recommended as part of the biosimilar comparability exercise, due to the low predictivity for the immunogenicity potential in humans.

An immune response to a therapeutic protein representing a counterpart to an endogenous protein may result in cross-reactivity, directed to the endogenous protein in cases where endogenous protein is still produced. Any relevant experience on the consequences of induction of an immune response to the endogenous protein or its absence/dysfunction in animal models should be discussed in the integrated assessment of immunogenicity. Both humoral and cellular immune responses (where relevant) should also be considered. Usually, safety risks would be predictable, based on existing knowledge on the biological activity of the endogenous protein and animal studies would not be required to confirm these safety risks. Only in exceptional situations where theoretical considerations are suggestive of a safety risk, animal immunisation studies with the therapeutic protein or the animal homolog may be considered in order to gain information on the potential consequences of an unwanted immune response.

Source = EMA ‘Guideline on Immunogenicity Assessment of biotechnology-derived therapeutic proteins’ (CHMP/BMWP/14327/06) – Draft guidance revision

Predictive nature is low for clinical risk

Used to aid data interpretation

Samples can be banked and only analysed if necessary for data interpretation

Different classes of therapeutic compound will have different considerations for pre-clinical immunogenicity assessment based on associated risks
Recent guidance defines development and validation for clinical trials but does not specifically cover pre-clinical activities.
Preclinical Immunogenicity

• How much preclinical immunogenicity data is needed?
  – Immunogenicity in animal models is typically not predictive of immunogenicity in humans
  – Assessment of immunogenicity in animals is primarily useful to interpret nonclinical toxicology and pharmacology data.
  – Immunogenicity in animal models may reveal potential antibody related toxicities that could be monitored in clinical trials.
  – May reveal immunogenicity differences between biosimilar and reference product.

• What level of immunogenicity assay validation and is needed for preclinical studies?
  • Assay Validation reports required for the BLA

What Does ADA Tell You About Exposure in Preclinical Studies?

PK...........pharmacokinetics (drug exposure)
ADA.........onset of immune response & kinetics

Allows Pharmacokinetics to be interpreted
Immunogenicity Analysis for Preclinical Studies--What Do We Do?

From a regulatory perspective the predictive value of pre-clinical immunogenicity is low

BUT

Analysis is not totally irrelevant and all GLP studies require validated methods
Typical Timelines For Creating a Fully Validated ADA Immunogenicity Assay

Time frame for GLP study ADA method validation ≈ 9 months

Critical reagent generation – 6 months

Assay Development – 6 weeks

Assay validation – 6 weeks

Due to the long time frame for generation of validated methods, it is very rare that we only develop and validate methods when required.
So How Can We Make Preclinical Immunogenicity Testing More Efficient Whilst Maintaining Scientific Integrity of Data Generated?

- Generic assays
- Fewer tiers of analysis
  - No Tier 2 (Confirmatory)
  - No Tier 3 (Titre, Nab etc)
Typical Bridging Assay Format Versus Generic Assay for Preclinical Work

**Typical Bridging assay format**

- Plate
  - Streptavidin
  - Biotin
- Assay specific to therapeutic

**Generic assay format for Humanised antibody**

- Plate
  - Anti-Human IgG
  - Anti-Ig tox species antibody
  - ADA
- Humanised antibody therapeutic
- Assay suitable for all IgG based therapeutics

Image source = Covance
Publications Showing the Application of Generic Assays


- Application of a Plug-and-Play Immunogenicity Assay in Cynomolgus Monkey Serum for ADCs at Early Stages of Drug Development. *Journal of Immunology Research* (2016), Article ID 2618575, Surinder Kaur et al. (Genentech)
ADA is generally only required to identify if there is an affect on exposure so we don’t necessarily need all tiers
Approach if Fewer Tiers of Analysis are Applied – Requirement for Confirmatory Analysis?

Publication:

- Statistical correlation demonstrated between the screening and confirmatory results in all evaluated assays
- The confirmatory test is of limited use to identify false positives

Therefore, indicates immunogenicity testing can be simplified to remove confirmatory tier for pre-clinical work. Data also suggests could move to a 1% or 0.1% false positive rate for screening tier.
Approach if Fewer Tiers of Analysis are Applied – Requirement for Titre Analysis?

Challenges of Titre assessment:

► Approach is semi-quantitative
► Due to the nature of the immune response the titre seen will be governed by how “foreign” the biotherapeutic is to the host species
► A high titre in pre-clinical does not predict a high titre in clinical activities
► ADA data in pre-clinical is generally only used to interpret TK exposure

Therefore, indicates immunogenicity testing can be simplified to remove titre tier for pre-clinical work.
Approach if Fewer Tiers of Analysis are Applied – Requirement for Nab Analysis?

Purpose of Nab analysis:

► Used to define if immune response neutralises mode of action
► However, an immune response in the pre-clinical setting does not necessarily predict the same effect in clinical
► Therefore, Immunogenicity risk assessment is recommended
► Low risk compounds (e.g. MAbs) would not require Nab assays but for proteins with an endogenous counterpart this may be informative
Proposed Routes for Preclinical Immunogenicity Analysis

What is the immunogenic risk?

Low Risk (E.g. Mab)

- Bank samples and use generic screening assay as required

High Risk (E.g. Epo)

Several tiers of analysis

- ADA screening
- Positive
- ADA Confirmatory Assay
- Positive
- ELISA

- ADA Screening
- Negative
- ELISA

- ADA Screening
- Positive
- ADA Confirmatory Assay
- Positive
- ELISA

- ADA Screening
- Negative
- ELISA
Conclusions

- Pre-clinical immunogenicity assessment is not fully informative for clinical studies but it does have value.
- Risk appropriate, stage appropriate and assay appropriate scientific validation needs to be conducted for immunogenicity assessments.
- Low risk compounds can be banked and ADA analysis conducted if affects on exposure observed.
- High risk compounds would still involve multiple tiers of immunogenicity assessment.
- Approach for pre-clinical Immunogenicity assessment will be case by case. No single rule fits all compounds.
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