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Disclosure and Disclaimer

Unless specified as FDA Guidance or Regulations, this speech reflects the views of the author and should not be construed to represent FDA’s views or policies. I have no financial relationships to disclose.
“Immunogenicity is defined as the propensity of the therapeutic protein product to generate immune responses to itself and to related proteins or to induce immunologically related adverse clinical events”
Immunogenicity Risk Assessment for Therapeutic Proteins and Peptides Requires Knowledge of Consequences and Probabilities (modified from Stirling and Ghee 2002)

Knowledge about likelihoods
- Some basis for probabilities
- No basis for probabilities

Knowledge about Consequences
- Consequences well-defined
- Consequences poorly-defined

Incertitude
- Risk (I)
- Ambiguity (III)
- Uncertainty (II)
- Ignorance (IV)
Immune Responses to Therapeutic Proteins and Peptides: Consequences for Safety

Fatality/Severe Morbidity

• Anaphylaxis—clinically defined, does not imply mechanism

• **Neutralization of endogenous protein with non-redundant function generated by antibodies to the therapeutic homolog:**
  Endogenous factor with non-redundant function resulting in a deficiency syndrome e.g., Pure Red Cell Aplasia from Anti-Drug Antibodies (ADA) to erythropoietin;

• **Neutralizing Antibodies to Life Saving Therapeutics**
  Enzyme replacement therapy for lysosomal storage diseases
  Coagulation factors for hemophilias

• Immune Complex Mediated Disease: delayed hypersensitivity
  serum sickness, nephropathy: observed in the context of administration of high doses of therapeutic protein in setting of robust antibody response; “dosing over”
Diminished efficacy of highly effective therapeutics
mAbs: e.g. TNF blockers

Alterations in PK
Antibodies to protein therapeutics may diminish or enhance PK/PD
Changes in dosing level to exceed antibody level, “dosing over,” may lead to worsened infusion reactions, epitope spread, generation of neutralizing antibodies, and circulating immune complex mediated hypersensitivity responses

No apparent effect
But sustained response may lead to epitope spread and generation of neutralizing responses, e.g. IL-2
Immunogenicity of Self Proteins Not Dichotomous

FOREIGN

- Abundance
- Alteration
- Adjuvants

SELF

- Expect Immunogenicity
  - No tolerance
  - Neutralize Product
  - Hypersensitivity

- Potential Immunogenicity
  - Incomplete tolerance
  - Altered structure/
    Antigen Present
  - Epitope spreading

- Rare Immunogenicity
  - Robust tolerance
  - Novel Route of
    Administration
  - Adjuvants
  - HLA Haplotype Specific
### Immunogenicity Risk Assessment: Consequences Based on Biological Function and Redundancy of Activity

<table>
<thead>
<tr>
<th>ADA Consequences</th>
<th>LESS SERIOUS</th>
<th>SEVERE AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous homolog?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Redundant/Unique Biology?</td>
<td>Redundant</td>
<td>Unique</td>
</tr>
<tr>
<td>Impact of Autoimmune/KO</td>
<td>Minimal</td>
<td>No SAEs</td>
</tr>
<tr>
<td>Intended Disease IND</td>
<td>Not Life-threatening</td>
<td>Life-threatening</td>
</tr>
<tr>
<td>Intended Disease Post AP</td>
<td>Not Life-threatening</td>
<td>Life-threatening</td>
</tr>
<tr>
<td>Treatment Options</td>
<td>Available</td>
<td>Not available</td>
</tr>
</tbody>
</table>

*Modified by Barry Cherney and Amy Rosenberg from Holly W. Smith, Eli Lilly*
# Immunogenicity Risk Assessment: Probabilities Based on Patient and Protocol Factors

## Clinical/Protocol Factors

<table>
<thead>
<tr>
<th>Clinical/Protocol Factors</th>
<th>Single</th>
<th>Chronic</th>
<th>Intermittent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing Frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose Concentration</td>
<td>Very High</td>
<td>Low-Average</td>
<td></td>
</tr>
<tr>
<td>Route of Administration</td>
<td>Oral</td>
<td>i.v.</td>
<td>s.c.</td>
</tr>
<tr>
<td>Patient Immune Status</td>
<td>Suppressed</td>
<td>Healthy</td>
<td>Activated</td>
</tr>
<tr>
<td>Immunomodulatory Action</td>
<td>Immunosuppressant</td>
<td>Immunostimulant</td>
<td></td>
</tr>
<tr>
<td>Endogenous Protein Level</td>
<td>High</td>
<td></td>
<td>Low</td>
</tr>
</tbody>
</table>

PROBABILITY = LOW UNKNOWN HIGH

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Immunogenicity of Therapeutic Proteins and Peptides: Consequences for Safety

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Multiple Changes in Approved rhu Erythropoietin (Eprex) Associated with Increased Incidence of PRCA

Endogenous Erythropoietin:
*Sole* factor mediating red blood cell production
Low abundance protein; levels in nanomolar range (sea level); spikes in levels due to changes in oxygen levels: altitude changes, anemia.

Tolerance to therapeutic, widely used in chronic kidney disease, relatively robust:
Few cases of PRCA related to Epo usage prior to 1998: associated with autoimmune disease

Increased incidence in PRCA following changes in formulation, container closure and administration route in 1998
Dramatic Increase in PRCA Cases Associated with Changes to Eprex: Formulation, Container Closure, Route of Administration  
(Boven K et al 2005)
PRCA in Development of *Biosimilar* Epo: Suspect Lineup in the Search for the Smoking Gun

- Three cases of NABs and one case of PRCA in development of a biosimilar Epo: **two implicated batches** thoroughly analyzed for suspect PQAs
  - Aggregates of Epo arising from:
    - Micelles of erythropoietin: polysorbate generated micelles
    - *Tungsten leachates* from tungsten used in needle hub formation in the form of reactive tungsten oxides caused Epo aggregation; shown previously to aggregate other therapeutic proteins
  - adjuvant material leaching from rubber stopper:
    - *vultac* responsible for cross linking of rubber protein; but cannot cross link other proteins; vultac has adjuvant properties (Sharma et al 2004)
What if?
Lessons from high risk therapeutic proteins as applicable to peptide/generic peptide therapeutics

*Approved peptide based on activity of a non-redundant endogenous protein*→* Generic Peptide ANDA*

Approved Peptide based on activity of a life saving Enzyme Replacement Therapy → Generic Peptide ANDA

Approved Peptide based on activity of a mAb to TNF-a with high efficaciy in autoimmune disease → Generic Peptide ANDA

*No room for significant differences in immunogenicity of approved peptide products with established clinical safety profile vs. generic peptides not tested clinically*
Case Example of a High Risk Approved Peptide Therapeutic: Teriparatide as Replacement for Parathyroid Hormone a Non-Redundant Endogenous Hormone

- Parathyroid hormone: Endogenous non-redundant 84-amino acid hormone; primary regulator of calcium and phosphate metabolism in bone and kidney
- Teriparatide: approved peptide therapeutic derived from the N-terminal 34 amino acids of human parathyroid hormone as replacement therapy for PTH to treat osteoporosis
- Teriparatide relatively non-immunogenic per clinical study: 2.8% of treated patients developed anti-drug antibodies after twelve months of treatment

Generic versions of Teriparatide must not have significant differences in immunogenicity or patients risk loss of calcium homeostasis
Immunogenicity of Therapeutic Proteins and Peptides

Consequences for Safety

Fatality/Severe Morbidity

**Neutralizing Antibodies to Life Saving Therapeutics**

- Enzyme replacement therapy for lysosomal storage diseases
- Coagulation factors for hemophilias

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Neutralizing Antibodies to Life Saving Enzyme Replacement Therapy (Myozyme) in Infantile Pompe Disease (IPD) Patients Leads to Respiratory Failure and Death (Kishnani PS et al 2011)

High Titer ADA CRIM+
Low Titer ADA CRIM+
High Titer ADA CRIM−

(Kishnani PS et al 2011)
ERT Tolerant Pompe Patients Experience Prolonged Survival

(Kazi ZB et al JCI Insight 2017)

Kaplan-Meier Survival Estimates

Age in Months

ERT monotherapy (n = 10)  ERT + ITI (n = 19)
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No room for significant differences in immunogenicity of approved peptide products with established clinical safety profile vs. generic peptides not tested clinically
Immunogenicity Assessment for High Risk Peptide Generics

• Differences in impurities, formulation components and aggregates may have a profound effect on immunogenicity with the additional caveat that peptides are generally more prone to aggregation than full length proteins.

• Robust non-clinical evaluations of proposed peptide generics must be performed for generic peptides in general but are especially critical for generic peptides that are life saving or therapeutic counterparts of non-redundant endogenous proteins/peptides; animal studies may be informative especially for evolutionarily conserved proteins (eg thrombopoietin).

• Residual uncertainty as regards potential for immunogenicity following comprehensive evaluation of a proposed high risk generic peptide should prompt consideration of whether the ANDA route is appropriate for the proposed generic product.
Acknowledgements

- Daniela Verthelyi, Ph.D., M.D, OBP, CDER
- Zuben Sauna, Ph.D., OTAT, CBER
- Steven Kozlowski, M.D., OBP, CDER
Speakers
Valerie Quarmby

Valerie Quarmby has contributed to IND, BLA and related filings for many approved medicines at Genentech.

She has presented and published extensively in the areas of bioanalysis and biopharmaceutical development, and she has extensive knowledge of the strategies and methods than can be used in risk based assessments of immunogenicity for protein therapeutics.
Vibha Jawa

Vibha brings more than 20+ years of experience in supporting biologics, vaccine development and gene therapy with contributions to multiple IND, BLA and MAA filings. She is a recognized leader in the area of Bioanalysis and Immunogenicity with more than 50 peer reviewed publications. In her current role as an Executive Director for Biotherapeutics Bioanalysis at Bristol Myers Squibb, Vibha is responsible for leading biotherapeutic and cell therapy bioanalytical (BA) function.

Her research interest has focused on streamlining preclinical immunogenicity assessments by using algorithms and invitro human derived assays and use those outputs to drive a risk based clinical strategy. She is an active member of multiple industry groups like AAPS and Industry Innovation and Quality (IQ) Consortium for Cell/Viral/Gene therapies.
Sophie Tourdot

Sophie has over 20 years of experience in vaccine and immunotherapy pre-clinical development for infectious diseases, oncology and allergy in positions held in academia and industry.

Prior to joining Pfizer, Sophie was a key member of the leadership team of the IMI-funded ABIRISK project, a consortium program focused on the analysis of underlying biological mechanisms, measurement and clinical relevance of unwanted immunogenicity of therapeutic proteins.

In her current role, Sophie leads the Immunogenicity Sciences group in charge of immunogenicity risk assessment of Pfizer biologics portfolio at all stages of development. A major activity of her group is the use of pre-clinical immunogenicity screening tools for therapeutic protein drugs, molecular design, and lead selection. She is also Director of Scientific Affairs for the European Immunogenicity Platform.
Dr Tim Hickling, D.Phil., Immunosafety Science Lead, Roche, Switzerland
Tim leads the Immunosafety group in Roche that includes responsibility for immunogenicity risk
evaluations and developing predictive methods for immune responses. Tim joined Roche in 2020 after
leading Pfizer’s Immunogenicity Sciences group, where he developed mathematical models of immune
responses to vaccines and therapeutic proteins. He had previously obtained his Biochemistry degree and
Immunology Doctorate from the University of Oxford, U.K. and was an Assistant Professor in Virology at the
University of Nottingham, U.K.