How safe is your biosimilar?
The tiered approach to measure immunogenicity of biologics – also the right approach for biosimilars?

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Probability of a protein drug to induce adverse immune response

IMMUNOGENICITY

formation of anti-drug antibodies (ADAs)
Why is immunogenicity such a hot topic?

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Pure Red-Cell Aplasia and Antierythropoietin Antibodies in Patients Treated with Recombinant Erythropoietin

Nicole Casadevall, M.D., Joelle Nataf, M.D., Béatrice Viron, M.D., Amir Kolta, M.D., Jean-Jacques Kiladjian, M.D., Philippe Martin-Dupont, M.D., Patrick Michaud, M.D., Thomas Papo, M.D., Valérie Ugo, M.D., Irène Teyssandier, B.S., Bruno Varet, M.D., and Patrick Mayeux, Ph.D.

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**Biologics can have life-threatening adverse immune responses,**

**even leading to death!**
Factors influencing immunogenicity of proteins

- Sequence Variation
- Product modification
- Biological Activity
- Contaminants and Impurities
- Application route
- Patient Features
- Length of Treatment
- Nature of Disease
- Dose
- Concomitant Medications
- Assay Technology
- Treatment related

 Courtesy of Prof. Wim Jiskoot, LACDR Leiden, the Netherlands
Contaminants & Impurities

AGGREGATES
Aggregated growth hormone more immunogenic than non-aggregated (Moore et al., 1980)

![Graph showing % 125I-hGH Bound vs Months of therapy for highly aggregated HGH and minimally aggregated HGH.](#)
PROTEIN AGGREGATES ARE IMMUNOGENIC
Do we expect this to be an issue with biosimilars?
Small differences in production processes can lead to conformational or folding changes – possible large impact on immunogenicity
Change in confirmation and/or folding –

Impact on stability –

Lead to increased aggregation
An Assessment of Biological Potency and Molecular Characteristics of Different Innovator and Noninnovator Interferon-Beta Products

Anthony Meager,1 Carl Dolman,1 Paula Dilger,1 Chris Bird,1 Gavin Giovannoni,2 Huub Schellekens,3 Robin Thorpe,1 and Meenu Wadhwa1
Quality of Original and Biosimilar Epoetin Products

Vera Brinks • Andrea Hawe • Abdul H. H. Basmeleh • Liliana Joachin-Rodriguez • Rob Haselberg • Govert W. Somsen • Wim Jiskoot • Huub Schellekens

Within this study, we showed that Eprex, Binocrit, Retacrit and Dynepo differ in content, isoform profiles and potency. What these differences mean for clinical efficacy and safety of EPO products is speculative (4,5).
How to measure immunogenicity?
The tiered approach to measure immunogenicity

- **Screening assay (1) – cut-off approach**
  - Positive
  - Confirmatory assay (2) – competition between bound and free drug
    - Positive
    - Neutralizing assay (3) – bioassay
    - Titer/conc.
    - Isotypes
  - Negative
Screening and confirmation assays (ligand binding assays/ binding antibodies)

- Enzyme-linked immunosorbent assay (ELISA; direct, bridging, indirect)
- Optional: acid disassociation pre-treatment of samples
- Radioimmunoassay (RIA)
- Electrochemiluminescence; Meso Scale Discovery (MSD)
- Luminex multiplexing
- Surface plasmon resonance (Biacore)
Neutralizing assays (cell-based assays/neutralizing antibodies)

- Anti-viral assays
- Proliferation (e.g. growth factors)
- Anti-proliferation assays (e.g. interferons)
- Gene reporter assays
- Potency assays (e.g. Antibody-dependent cell-mediated cytotoxicity (ADCC))
How about measuring immunogenicity of biosimilars?
New Biologicals

How immunogenic?
**Biosimilar**

- Is my biosimilar as immunogenic as the innovator?
- Is it maybe even a biosuperior?
- What about interchangeability?
Discussion in biosimilar immunogenicity program:

- Two separate positive controls: one against innovator and one against biosimilar?

- One or two assays? Shall we just use the innovator assay to detect ADAs against the biosimilar?
Example: bridging ELISA

Labelled innovator Drug

ADA

Innovator Drug
Pro: cheap and fast; reduction of inter-assay variability due to use of same reagents

Con: cross-reactive antibodies against innovator are missed

One assay!
Two assays – same sample!

Pro: True differences in immunogenicity rates are picked up
Con: more sample volume needed, run through two assays;

Validation criteria must be identical between the assays
Considerations in choosing one or two assays:

- One assay will only reveal relative immunogenicity rates between biosimilar and innovator.
- Only two assays can reveal true immunogenicity differences.
What about neutralizing antibody assays?

- Dependent on neutralizing assay format!
- In general: for all assays – they need to have comparable sensitivity, selectivity and precision
Be aware!

• Due to low animal numbers in preclinical study, immunogenicity rates may differ

• Even if your biosimilar has reduced immunogenicity – Biological relevance need to assessed in combination especially with PK data
Thank you for your attention and enjoy the rest of the conference!