

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

*Melody Sauerborn,  
Senior expert Immunogenicity,  
TNO Triskelion BV*



*Probability of a protein drug to induce*

*adverse immune response*

# IMMUNOGENICITY

*formation of anti-drug*

*antibodies (ADAs)*



## *Why is immunogenicity such a hot topic?*



### The New England Journal of Medicine

Volume 346:469-475

February 14, 2002

Number 7

#### **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.*

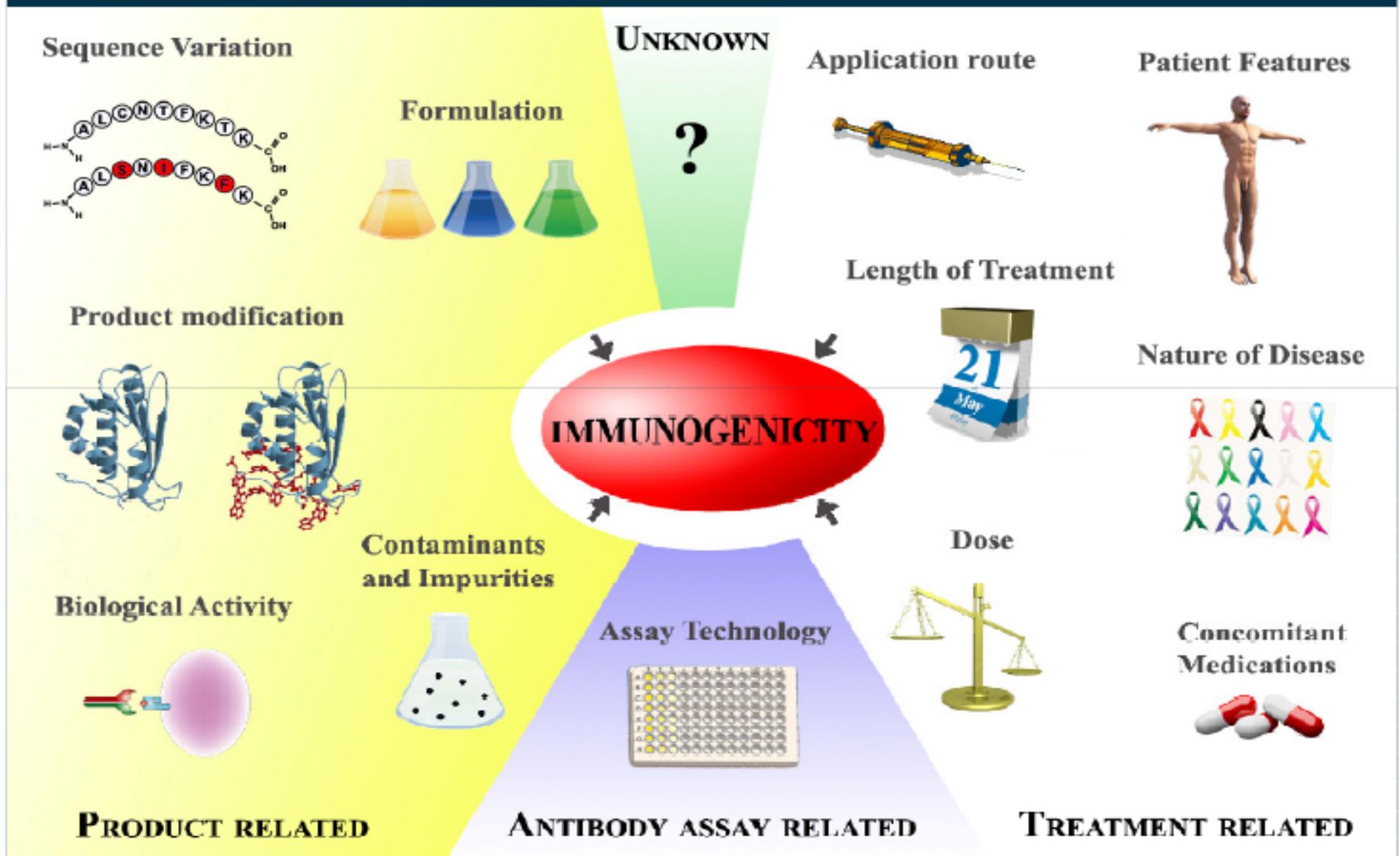
Dept of Haematology, Hôtel-Dieu, Paris, France



***Biologics can have life-threatening  
adverse immune responses,  
even leading to death!***

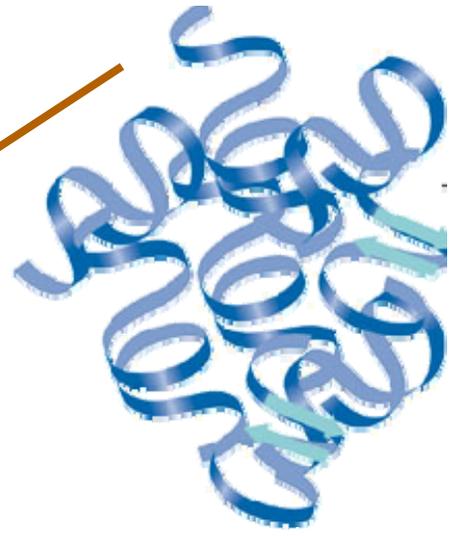
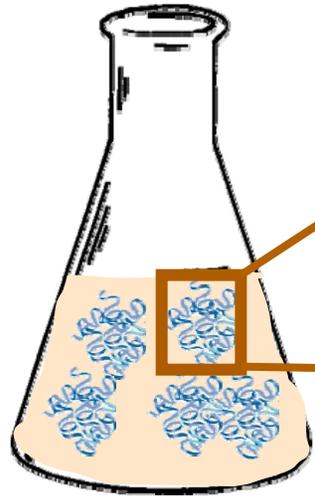


# Factors influencing immunogenicity of proteins



Courtesy of Prof. Wim Jiskoot, LACDR Leiden, the Netherlands

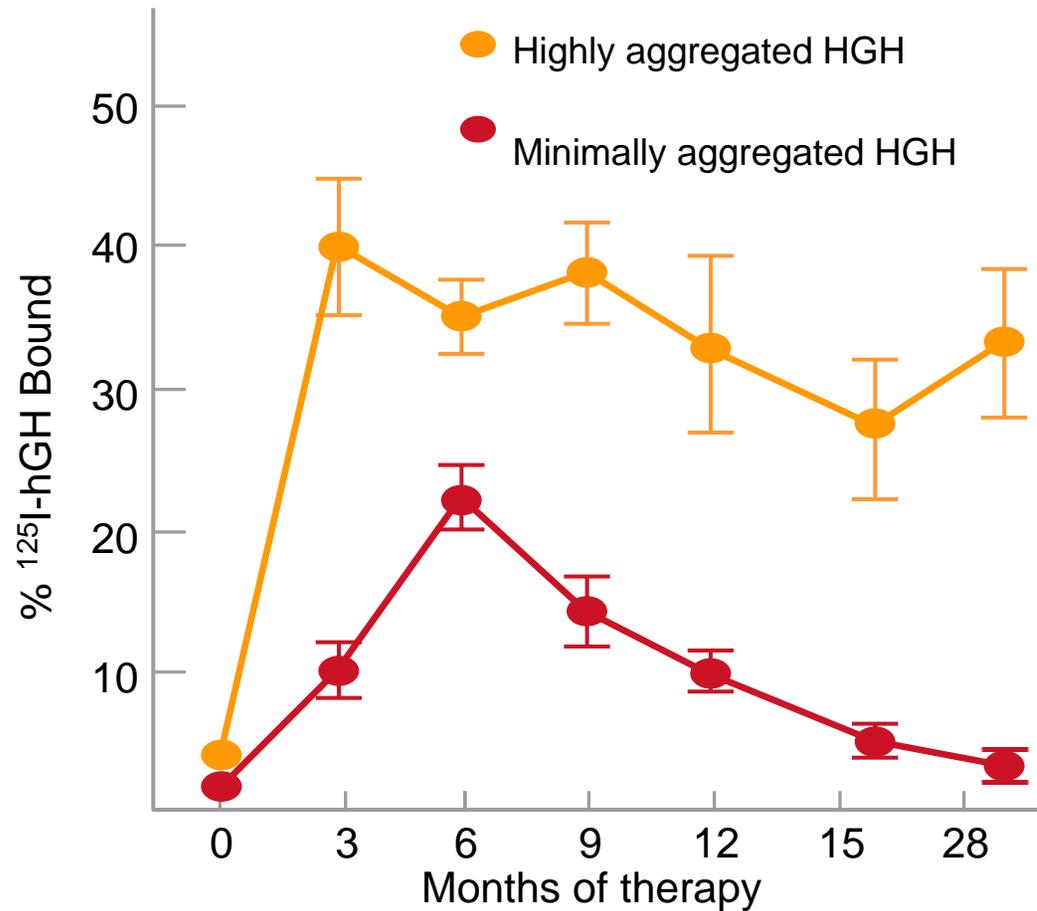
*Contaminants  
&  
Impurities*



**AGGREGATES**



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



**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 –***

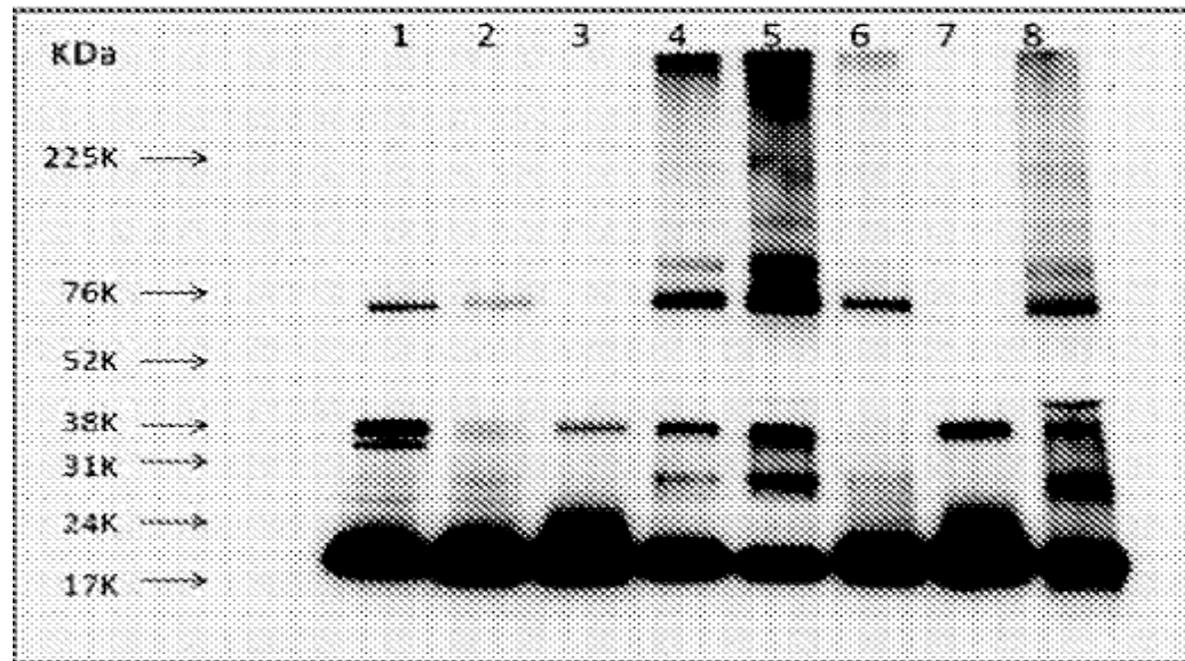
***Impact on stability –***

***Lead to increased aggregation***



## An Assessment of Biological Potency and Molecular Characteristics of Different Innovator and Noninnovator Interferon-Beta Products

Anthony Meager,<sup>1</sup> Carl Dolman,<sup>1</sup> Paula Dilger,<sup>1</sup> Chris Bird,<sup>1</sup> Gavin Giovannoni,<sup>2</sup>  
Huub Schellekens,<sup>3</sup> Robin Thorpe,<sup>1</sup> and Meenu Wadhwa<sup>1</sup>



Pharm Res (2011) 28:386–393  
DOI 10.1007/s11095-010-0288-2

RESEARCH PAPER

## 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

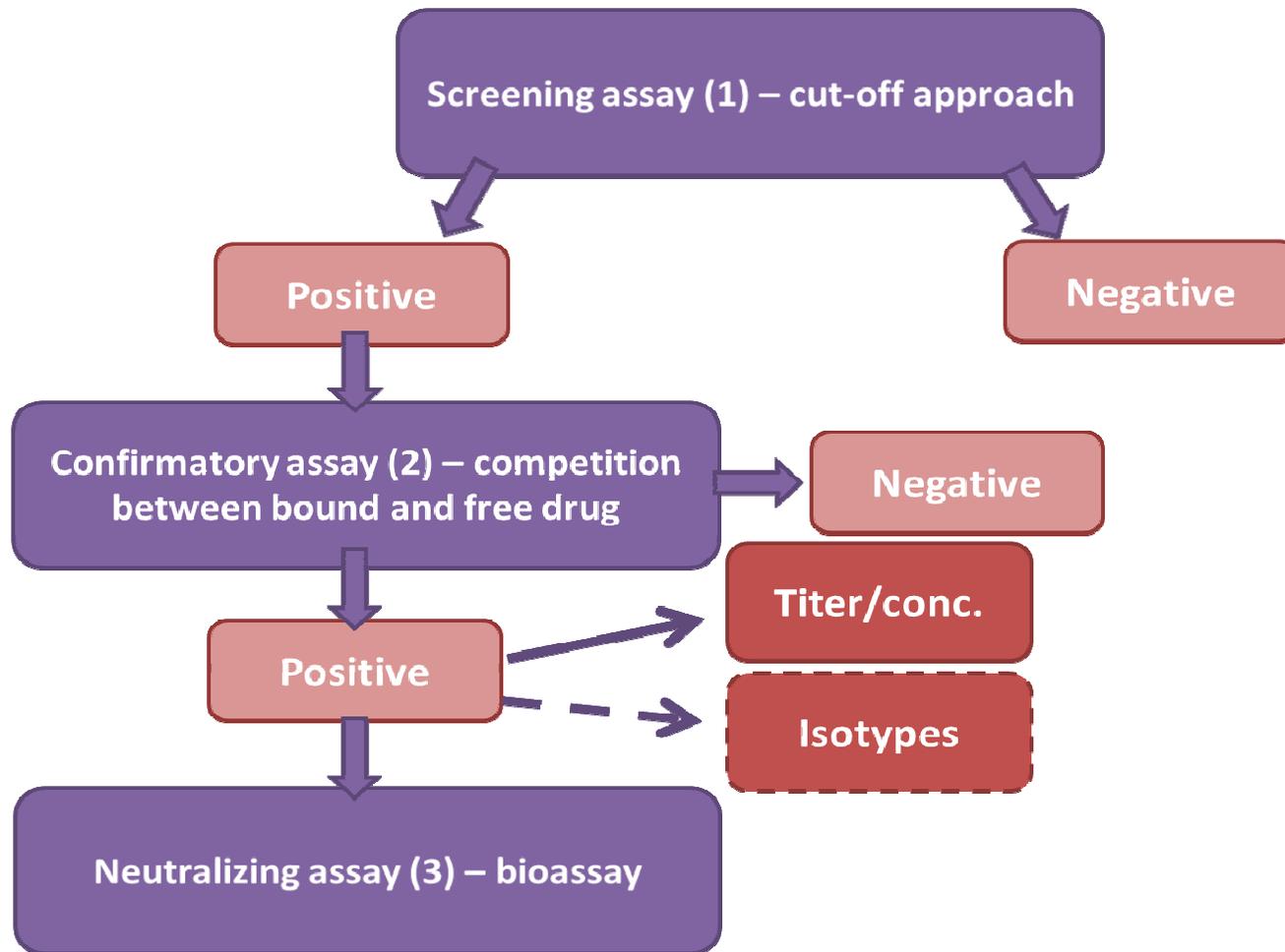
Within this study, we showed that Eprex, Binocrit, Retacrit and Dynepo differ in content, isoform profiles and potency. What these differences mean for clinical efficacy and safety of EPO products is speculative (4,5).



# *How to measure immunogenicity?*



## *The tiered approach to measure immunogenicity*



***Screening 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?***



*New Biological*

*How immunogenic?*

***Biosimilar***

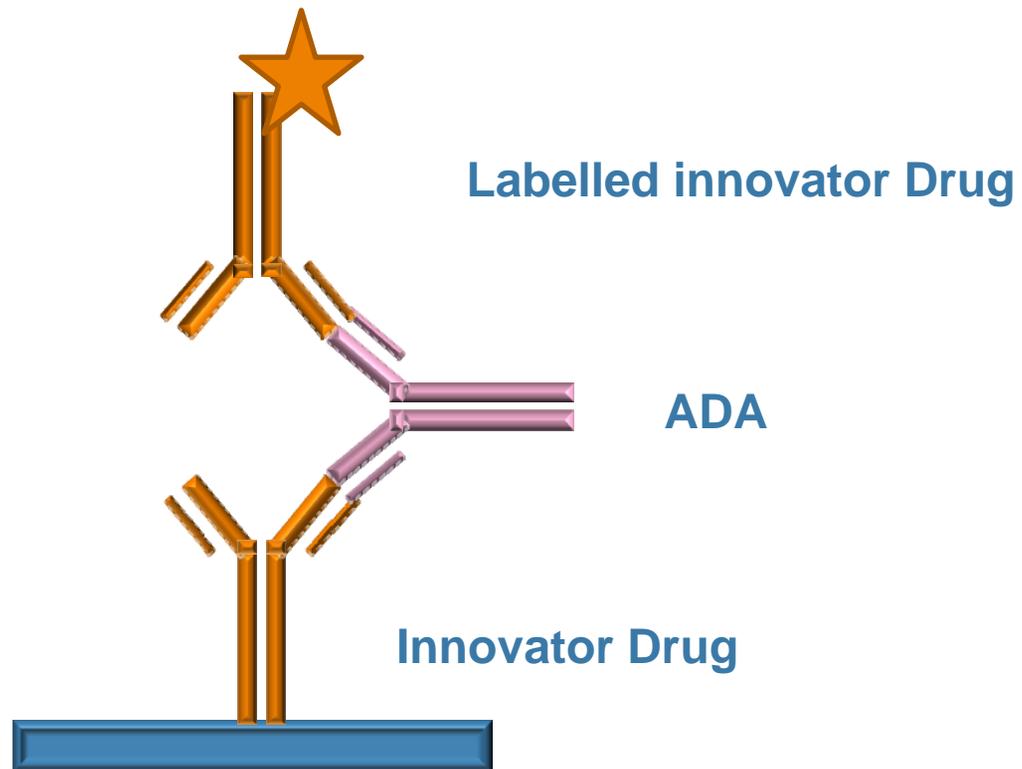
- *Is my biosimilar as immunogenic as the innovator?*
- *Is it maybe even a biosuperior?*
- *What about interchangeability?*

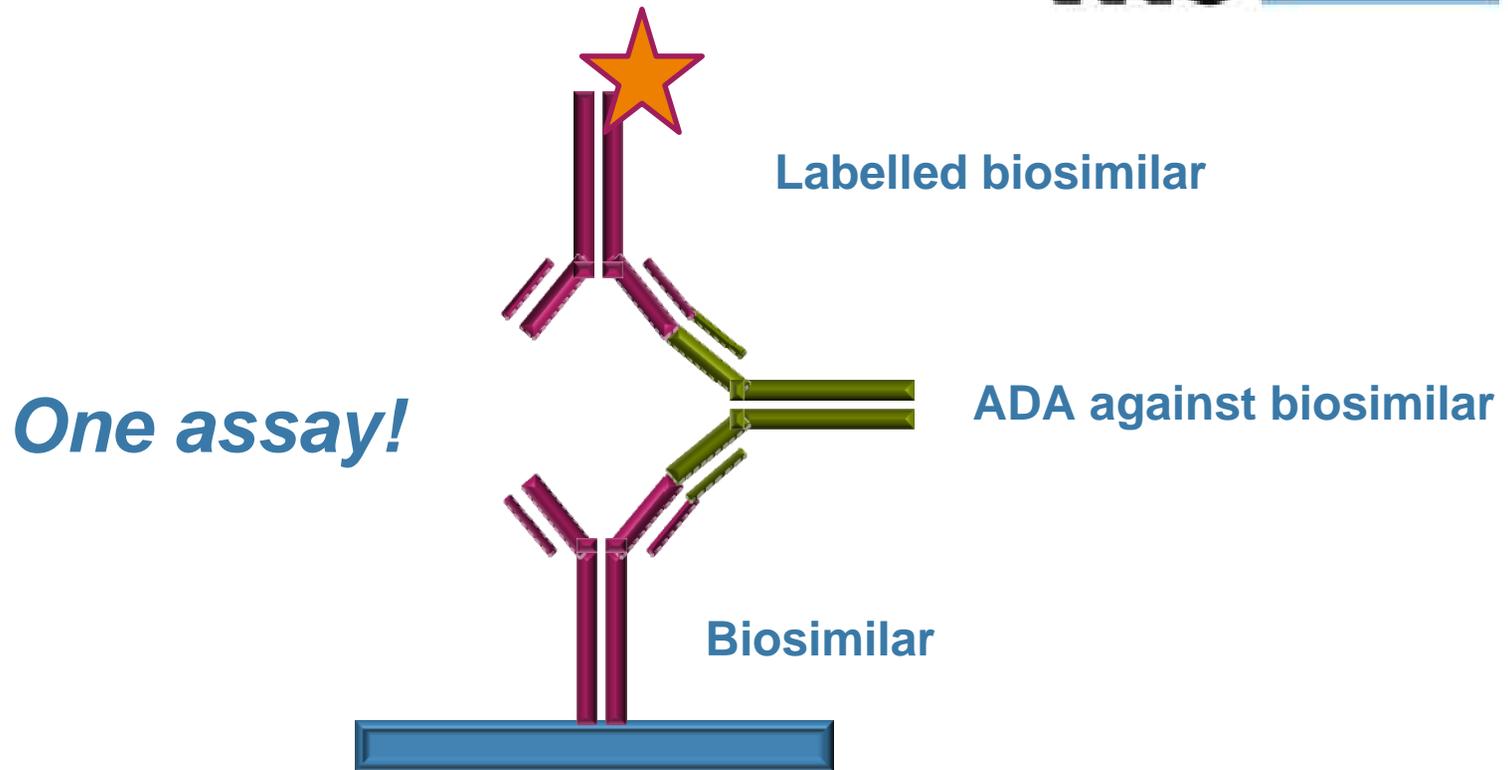


*Discussion in biosimilar immunogenicity  
program:*

- *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*

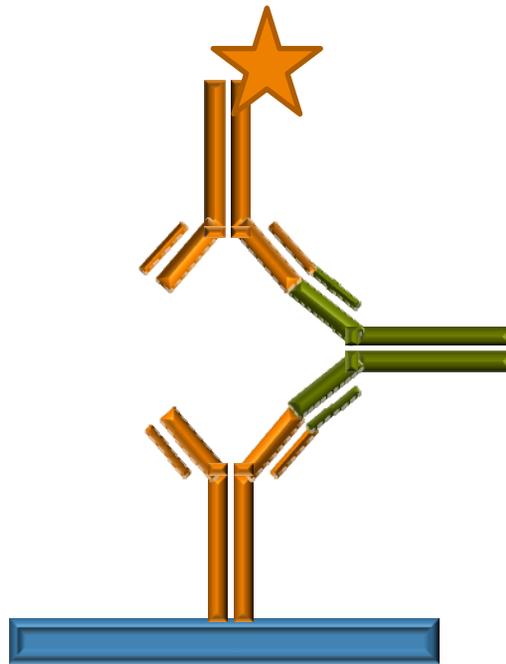




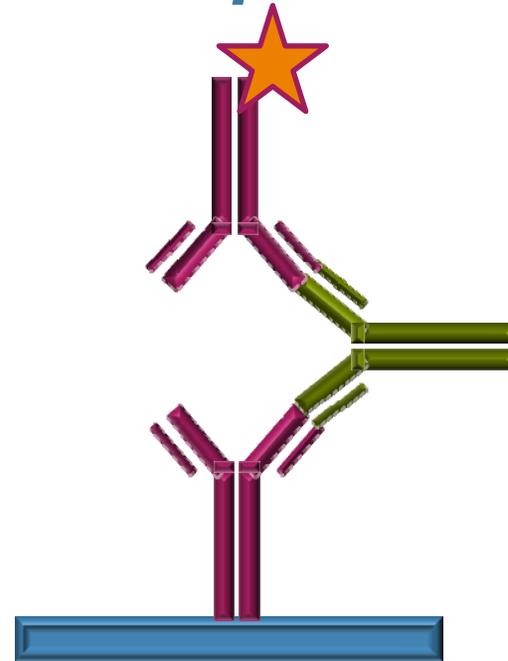
*Pro: cheap and fast; reduction of inter-assay variability  
due to use of same reagents*

*Con: cross-reactive antibodies against innovator are missed*

*Two assays – same sample!*



Innovator



Biosimilar

*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!**

