# Challenges of Critical Quality Attributes (CQA) Assessment in biosimilar mAbs development

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- The field
- Inherent Variability of biologics
- Continuum of comparability
- Case study
- Biosimilarity
- Orthogonality & redundancy
- Critical Quality Attributes (CQA)
- Quality by design (QbD)
- Criticality & Uncertainty
- Conclusion

# TOP BIOLOGIC PATENT EXPIRATIONS

| Biologic | Global sales $ bn | Expiry Date | |
|---|---|---|---|
| | | EU | US |
| Adalimumab (Humira) | 11.8 | 2018 | 2016 |
| Insulin Glargine (Lantus) | 10.3 | 2014 | 2014 |
| Etanercept (Enbrel) | 8.7 | 2015 | 2028 (extended) |
| Infliximab (Remicade) | 8.1 | 2015 | 2018 |
| Rituximab (Mabthera) | 6.6 | 2013 | 2016 |
| Bevacizumab (Avastin) | 5.6 | 2019 | 2017 |
| Insulin Aspart (Novomix, Novorapid) | 5.4 | 2015 | 2015 |
| Interferon Beta-1A (Avonex, Rebif) | 5.4 | Expired | Expired |
| Trastuzumab (Herceptin) | 5.1 | 2014 | 2019 |
| Glatiramer Acetate (Copaxone) | 4.7 | 2015 | 2014 |
| Pegfilgrastim (Neulasta) | 4.2 | 2015 | 2014 |
| Ranibizumab (Lucentis) | 4.2 | 2016 | 2016 |

IMS Health - 2015

# Biosimilars in EU ~ 35

**as 7/2017**

- Omnitrope (somatropin) – EU first biosimilar approved in 2006.
- To date, **EMA has approved 38 biosimilars** - product classes of human growth hormone, granulocyte colony-stimulating factor, erythropoesis stimulating agent, insulin, follicle-stimulating hormone (FSH), parathyroid hormone and tumour necrosis factor (TNF)-inhibitor
- **Three** approvals **have been withdrawn**; two for filgrastim: Filgrastim ratiopharm in April 2011 and Biograstim in December 2016, and one for a somatropin biosimilar (Valtropin) in May 2012.
- This leaves a total of **35 biosimilars approved** for use in Europe.

# Biosimilars in US

**as 10/2017**

**Zarxio**/Sandoz, Inc. => **Neupogen**/Amgen (3/15)
**Inflectra**/Celltrion/Hospira => **Remicade**/Janssen (4/16)
**Erelzi**/Sandoz => **Enbrel**/Amgen (8/16)
**Amjevita**/**Amgen** => **Humira**/AbbVie (9/16)
**Renflexis**/Samsung => **Remicade**/**Janssen** (4/17)
**Cyltezo**/ **Boehringer Ingelheim** => **Humira**/AbbVie (8/17)
**Mvasi**/**Amgen** => **Avastin**/ **Genentech** (9/17)

# FDA March 6, 2015

**Zarxio (filgrastim-sndz)**
the first biosimilar in US

Sandoz, Inc.'s Zarxio is biosimilar to Amgen Inc.'s Neupogen, originally licensed in 1991. Zarxio is approved for the same indications as Neupogen, as "interchangeable"

**FDA 2015**

# FDA April 5, 2016

**Inflectra (infliximab-dyyb)**
**second biosimilar approved by the FDA**

- Inflectra is manufactured by Celltrion, Inc, for Hospira, It is biosimilar to Janssen Biotech, Inc.'s Remicade

- Multiple indications - Crohn's disease, ulcerative colitis, rheumatoid arthritis, arthritis of the spine, psoriatic arthritis & chronic severe plaque psoriasis).

**FDA 2016**

# FDA July - 2016

**Erelzi, (etanercept-szzs)**

**Third biosimilar approved by the FDA**

- Erelzi manufactured by Sandoz Inc., is a biosimilar to Enbrel originally licensed to Amgen in 1998.

- Multiple indications - moderate to severe rheumatoid arthritis, moderate to severe polyarticular, active psoriatic arthritis, active ankylosing spondylitis and chronic moderate to severe plaque psoriasis

**FDA 2016**

# FDA September - 2016

## Amjevita (adalimumab-atto)

**Fourth biosimilar approved by the FDA**

- Amjevita manufactured by Amgen as a biosimilar to Humira approved in 2002 and is manufactured by AbbVie Inc.

- Multiple indications - rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis; and plaque psoriasis.

**FDA 2016**

**FDA**

"Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product "

**April 2015**

**EMEA**

"Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1) "

**December 2014**

**EMEA**

"Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues"

**30 May 2012**

# Variability is inherent in biologics

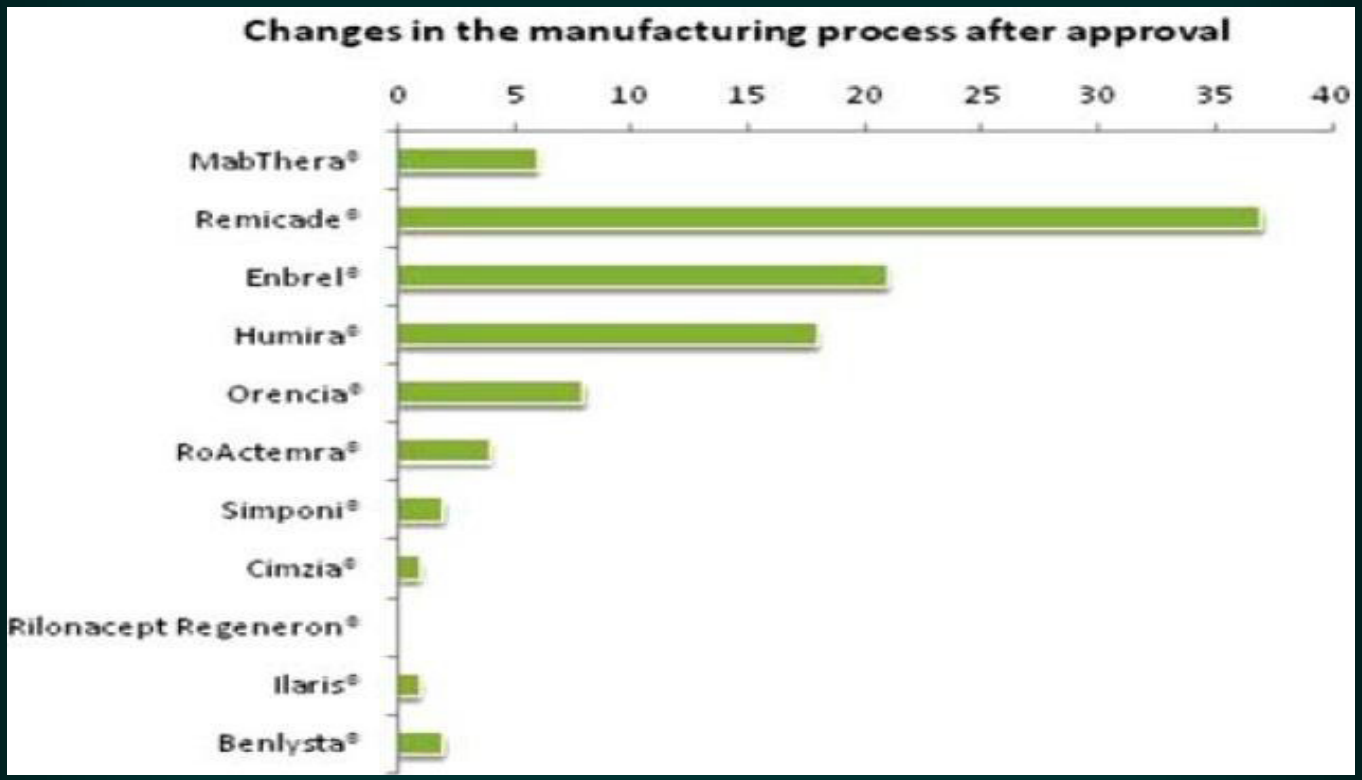
Batch-to-batch

- Non-identicality is a normal principle in biologics
- No batch of any biologic is “identical” to the other batches
- Variability is natural even in the human body and usually not problematic

Manufacturing changes

- Manufacturing changes occur due to process improvements, scale up, etc
- Differences in attributes sometimes significantly larger than batch-to-batch variability

# Manufacturing changes are made frequently




- **manufacturing changes are well understood by means of comparability exercises (ICHQ5E) and tightly controlled by regulators**

Jan Visser, **et all** Comparability to establish Biosimilarity 2014 **Sorrento, Italy**

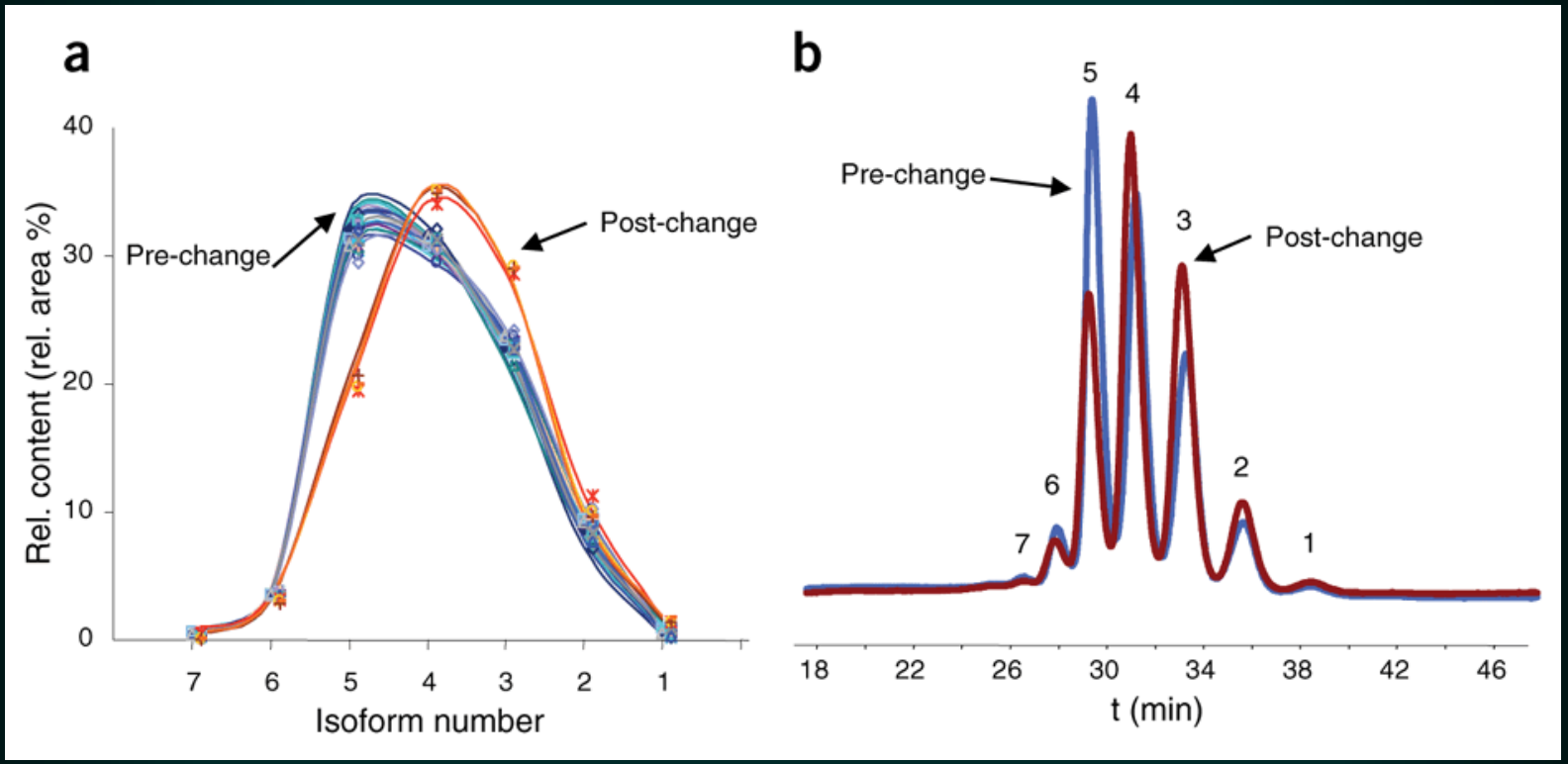
# Continuum of comparability allows control of variability in biologics

- Comparable ≠ Identical

- Biologic after an approved manufacturing change is as safe and efficacious as the pre-change product!
- Biosimilar is as safe and efficacious as its reference product!

# Aranesp - manufacturing changes

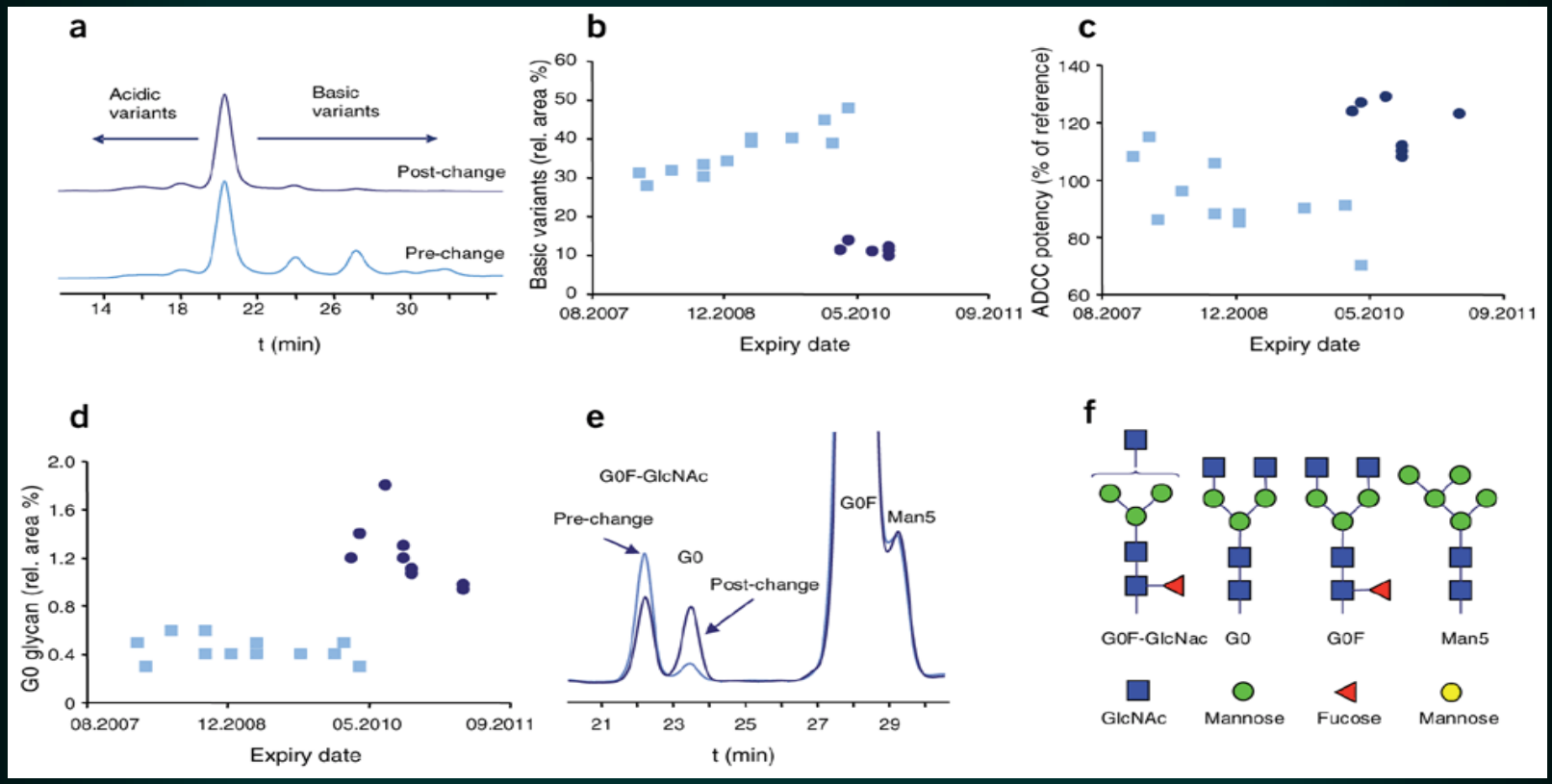
## Comparison of the pre- and post-change batches measured by capillary zone electrophoresis.




**(a)** Relative content of the individual isoforms of the pre-change ($n$ = 18) and thepost-change ($n$ = 4) batches. **(b)** Representative electropherograms; peaks are labeled with the isoform number.

**Martin Schiestl, at all, *Nature Biotechnology* 29, 310–312 (2011)**

# Rituxan - manufacturing changes
## Comparison of the different pre- and post-change batches

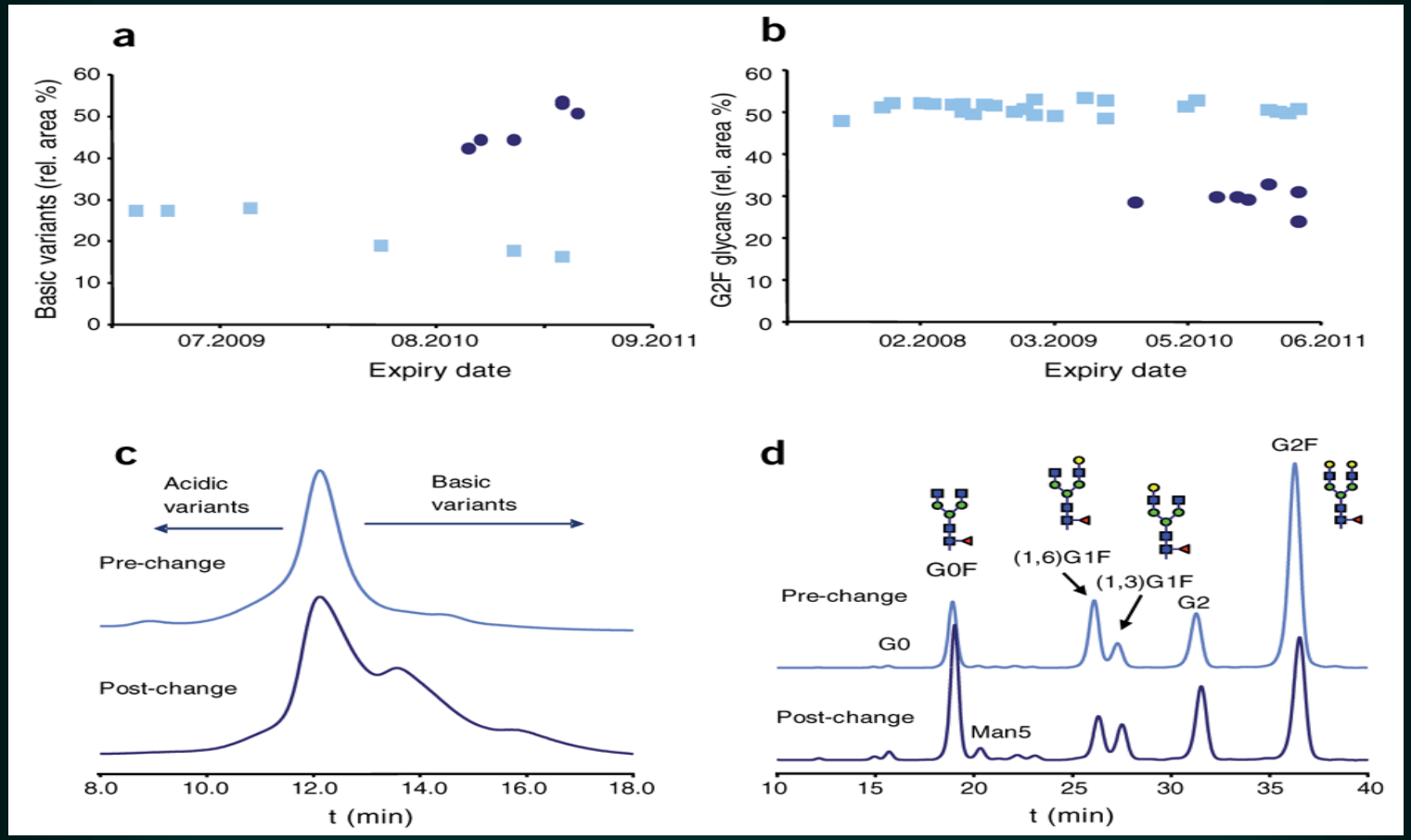



**(a)** Exemplary CEX chromatograms. **(b)** Amount of basic variants of the pre-change ($n$ = 12) and post-change ($n$ = 6) batches as measured by CEX. **(c)** ADCC potency of the pre-change ($n$ = 11) and post-change ($n$ = 8) batches. **(d)** Relative amount of the G0 glycan of the pre-change ($n$ = 13) and post-change ($n$ = 11) batches. **(e)** Exemplary glycan mapping chromatograms. **(f)** Glycan legend.

**Martin Schiestl, at all, *Nature Biotechnology* 29, 310–312 (2011)**

# Enbrel - manufacturing changes

## Comparison of the different pre- and post-change batches




**(a)** Relative amounts of basic variants of the pre-change ($n = 6$) and the post-change ($n = 6$) batches as measured by CEX. **(b)** Relative amount of the G2F glycan of the pre-change ($n = 25$) and the post-change ($n = 9$) batches. **(c)** Exemplary CEX chromatograms. **(d)** Exemplary glycan mapping chromatograms.

**Martin Schiestl, at all, *Nature Biotechnology* 29, 310–312 (2011)**

**(Aranesp)**

- Decrease in sialylation rate in post-change batches

**(Rituxan/Mabthera)**

- Reduction # of variants from 12 to 6
- Reduction (C-terminal Lys and N-terminal Glu) from 30–50% to 10%
- Fucosylated glycans G0 up three-fold, => up ADCC potency

**(Enbrel)**

- Major differences - glycosylation and in the ratio of basic variants present in the molecule.
- Variants containing N-glycan G2F down from 50% to 30% and C-terminal Lys up from 15% to 30%.

# Rituximab & Herceptin biosimilars in development

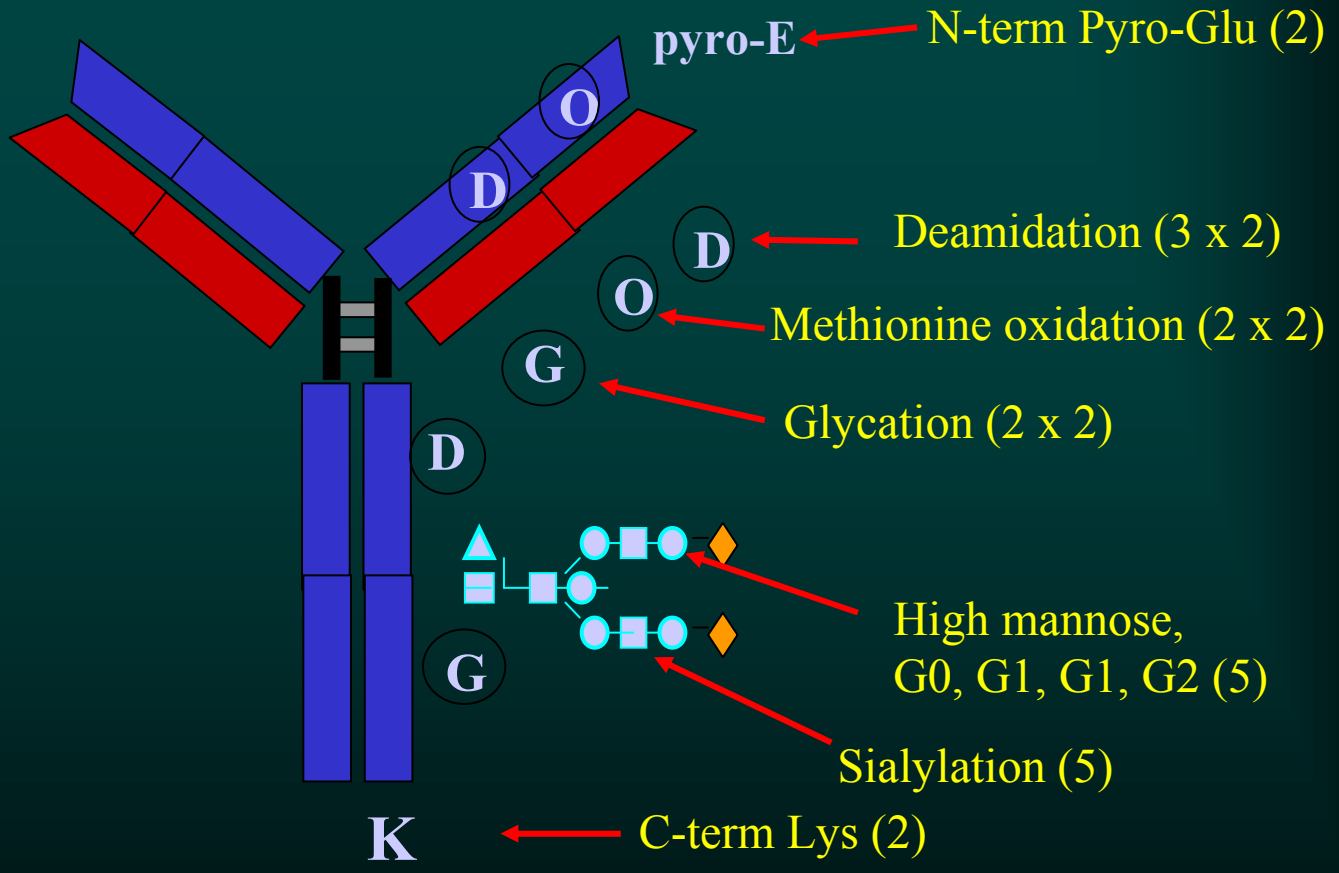
"Physicochemical and Functional Comparability Betweenthe Proposed Biosimilar Rituximab GP2013 and Originator Rituximab"

Jan Visser • at all BioDrugs (2013) 27:495–507

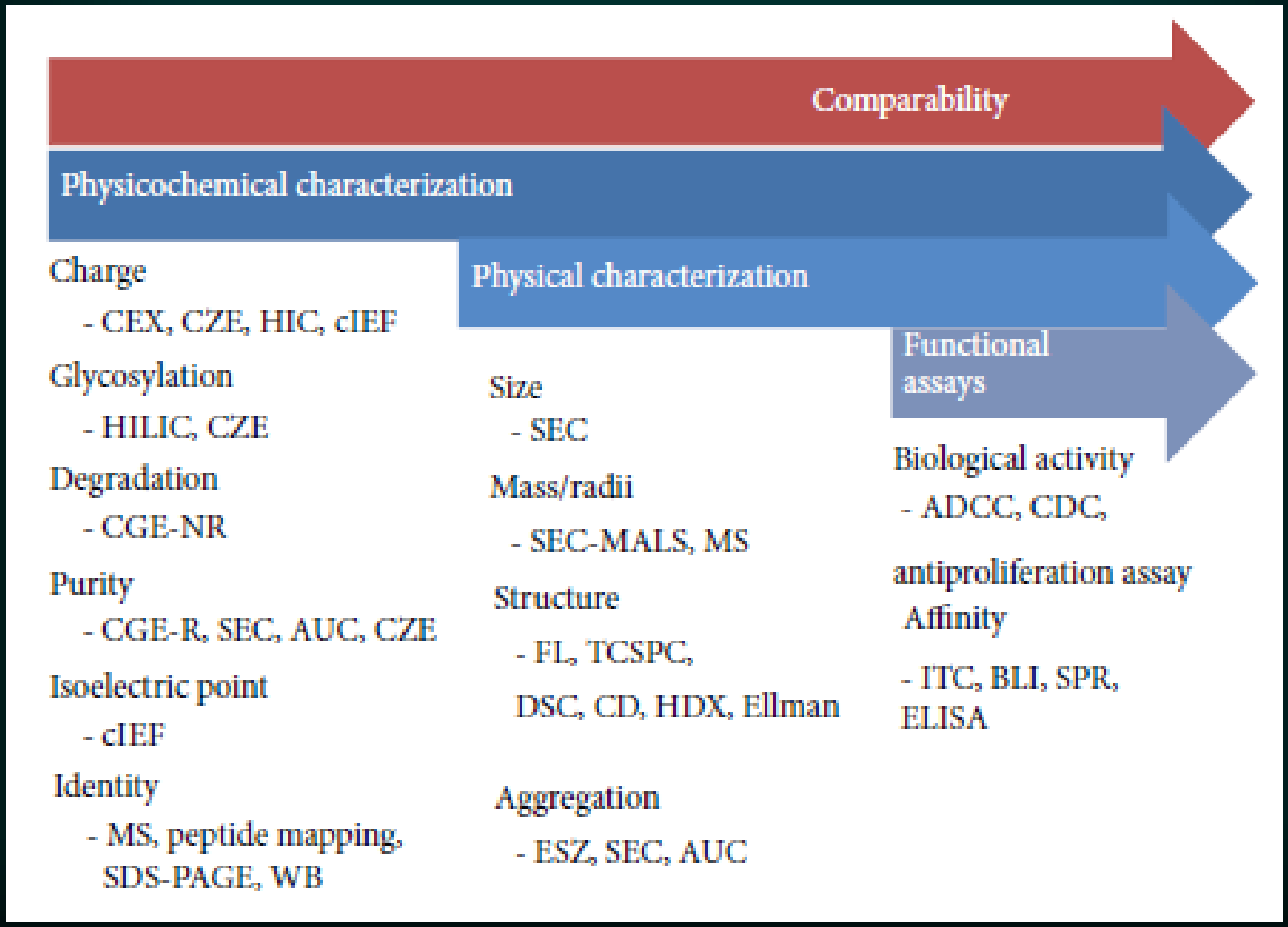
"Physicochemical and Biological Characterization of a Biosimilar Trastuzumab"

Carlos A. López-Morales, at all , BioMed Research International, Volume 2015, Article ID 427235

# mAb's are a heterogeneous mixtures - the hot spots of change



# Characterization strategy performed for Trastuzumab-Probiomed & Herceptin-F. Hoffmann, La Roche Ltd.




Carlos A. López-Morales, at all , BioMed Research International, 2015

# Impact of CQAs on safety and efficacy- (Herceptin /Trastuzumab)

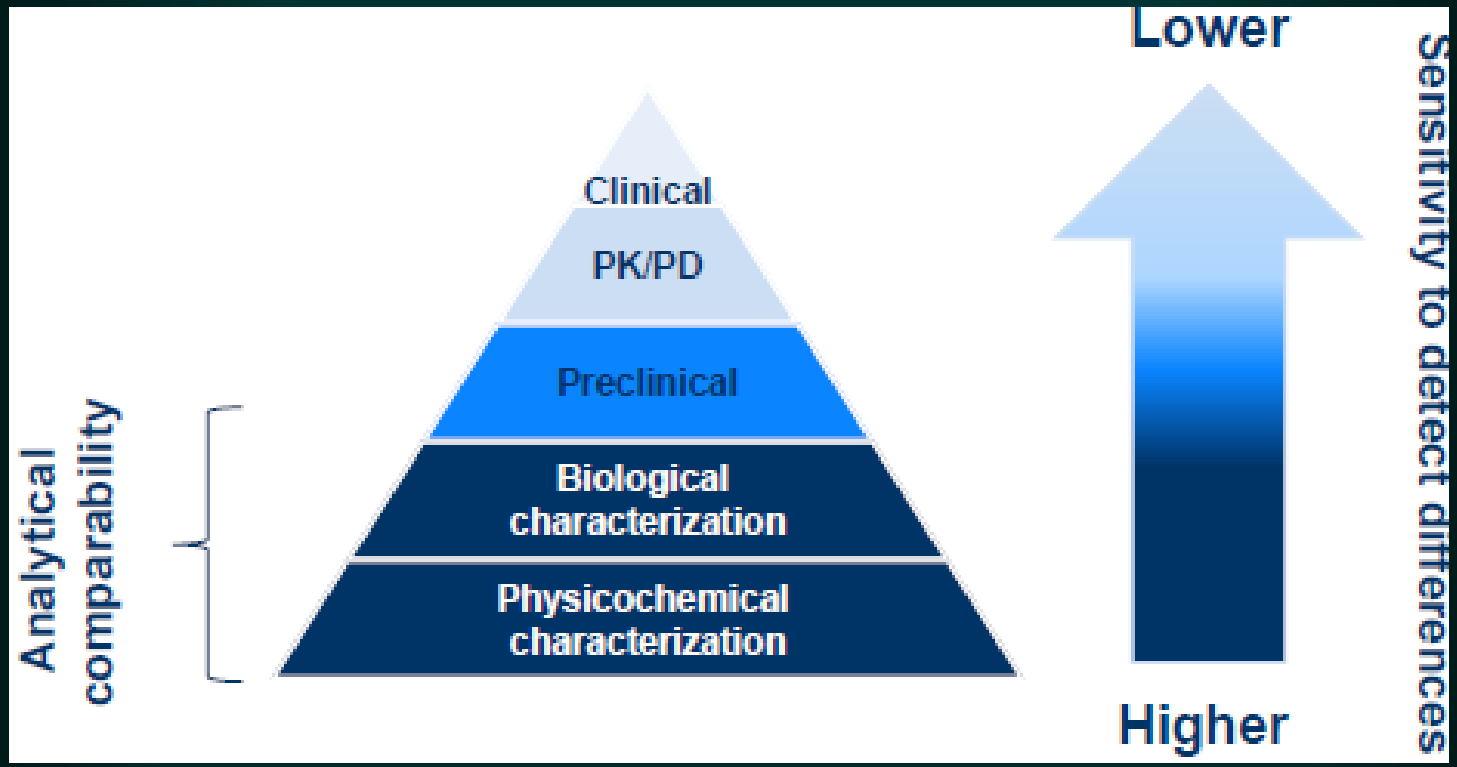
| Attribute | PD | PK | Immunogenicity |
|---|---|---|---|
| Sequence | Nonspecific | Nonspecific | Different response due to sequence modifications |
| Higher order structure | Nonspecific | Nonspecific | Determined by MW & structure complexity |
| Glycosylation | Fucosylated, highly mannosylated, and sialylated variants could alter efficacy | Highly mannosylated => higher clearance<br><br>Highly Sialylated => lower clearance | Sialic acid can hide Antigenic determinants. Highly mannosylated & nonglycosylated variants => up immunogenicity |
| Charge heterogeneity | Altered if pI differences are >1 unit | Major Δ alter volume of distribution and clearance | Acidic variants are prone to elicit immunogenicity |
| Aggregates | Lower biological activity | Lower absorption & bioavailability | ADAs presence |
| FcγRI affinity<br>FcγRII affinity<br>FcγRIII affinity | Affects endocytosis, phagocytosis, antigen presentation ADCC, | Not determined | Not determined |
| FcRn affinity | Not determined | Lower affinity to acidic & oxidized methionine variants No Δ in variants with 3- to 4-fold changes in FcRn Affinity | Not determined |

Carlos A. López-Morales, at all , BioMed Research International, 2015

# 2013 EU Remsima® vs infliximab Δ

- Differences were noted, but of no clinical consequence:
- Higher level part-assembled antibodies - no impact on binding affinity or potency
- C-terminal Lys levels were transient (Lys is rapidly cleaved following administration)
- Glycans lacking fucose down => lower binding affinity to (FcγRIIIa)
- discrepancies were addressed through a series of complex and well-conceived in vitro studies

# The evaluation of biosimilarity is based on comparability gained at all levels

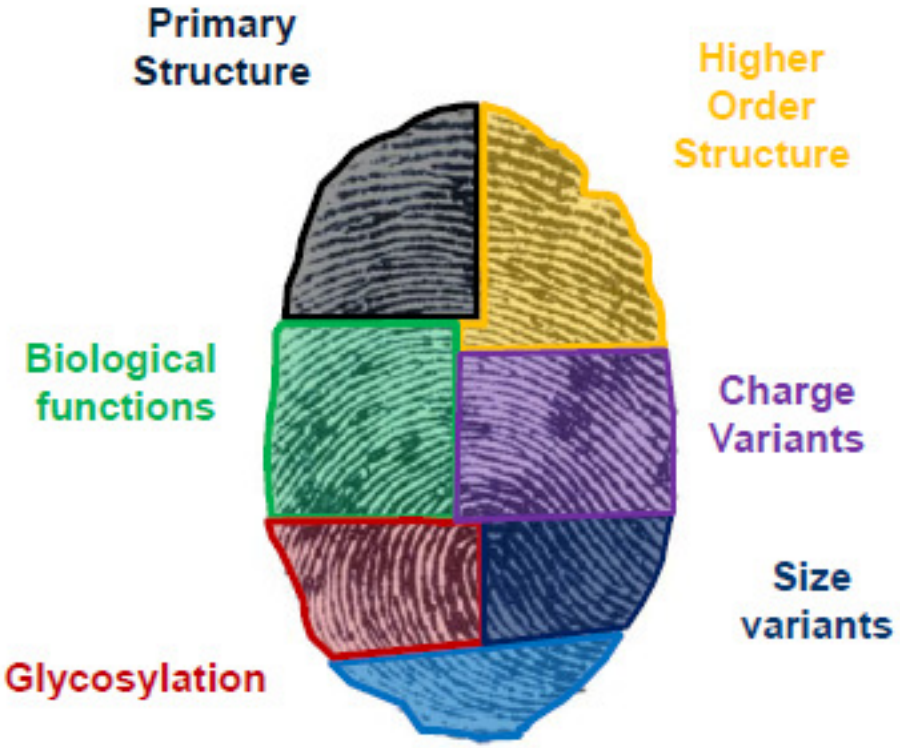



Jan Visser, **et all** Comparability to establish Biosimilarity 2014 **Sorrento, Italy**

# Quality Attributes analysis

**Attributes e.g.:**

- Primary structure
  - Mass
- Disulfide bridging
  - Free cysteines
  - Higher order structure
- N- and C-terminal heterogeneity
  - Glycosylation
  - Glycation
- Fragmentation
  - Oxidation
- Deamidation
- Aggregation
  - Particles
- Target-binding
  - Fc effector functions




**Methods e.g.:**

- MS
- Peptide mapping
  - Ellman's
  - CGE
- SDS-PAGE
- CD, FT-IR
- H-D exchange
- NMR, X-ray
- HPLC
- HPAEC
- IEF
- 2AB NP-HPLC
- SE-HPLC
- FFF
- AUC
- DLS
- MALLS
- Bioassays
- SPR

Jan Visser, **CMC Strategy Forum Europe 2014, Sorrento, Italy**

# Orthogonality & redundancy is the key

- There is no single type of assessment that can verify biosimilarity.
- Even for functional assays, there ought to be an orthogonal array of cell based and ELISA based potency assessments, as well as binding kinetic determinations.
- Orthogonal approach that combines physicochemical and functional analysis

# Orthogonality ??

- Secondary structure - fourier transform infrared spectroscopy (FTIR) and circular dichroism (CD) - FTIR is stronger with β-sheets, while CD is better with α-helices.
- Aggregation - size exclusion chromatography, (SEC) multi angle laser light scattering (SECMALS) and sedimentation velocity analytical ultracentrifugation (SVAUC)

# Orthogonality & redundancy is the key

There are two principles here:

- Redundancy
- Orthogonality
- 50–60 methods to analyze structure
- 15 methods to test function

For mAbs an array of binding assays to assess both :

- Fab/antigen interaction
- Fc/Fc receptor interaction, binding kinetics, -- surface plasmon resonance (SPR; e.g., Biacore) or biolayer interferometry (BLI, e.g., Octet).

# Quality by design (QbD)

- Identifying CQAs - most difficult step in quality by design (QbD) of biopharmaceuticals
- Structural & functional data are the key to assess CQAs of biopharmaceuticals
- Product risk assessments - As the molecule progresses through development and more is learned about the relationship between product attributes and their impact (or nonimpact) on potency, (PK), or safety

Anurag S. Rathore, et all BioPharm International 2014

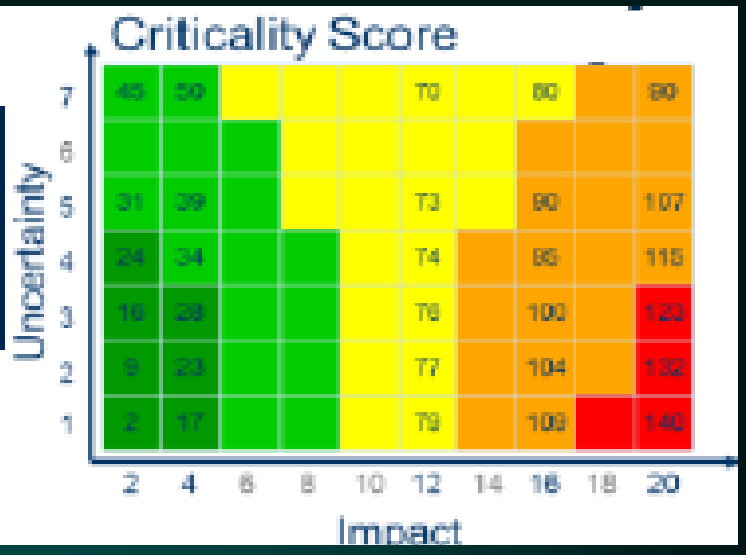
# Quality by design (QbD)

- Product risk assessment team should include experts in PK, toxicology, *invivo* biology, and clinical
- Attributes assessment of on potency, PK, PD, immunogenicity, off target effects => direct impact on safety. The data come from *structure activity relationship* (SAR) studies, nonclinical studies, clinical exposure history, and toxicology reports (e.g., Fc fusion proteins, pegylated proteins) etc.
- Impact & uncertainty scores are assigned, the product of these two values constitutes the *risk priority number* (RPN) for the attribute

Anurag S. Rathore, et all BioPharm International 2014

# QbD - Criticality assessment of Quality Attributes

| Criticality | Criticality Score |
|---|---|
| Very High | 121 – 140 |
| High | 86 – 120 |
| Moderate | 56 – 85 |
| Low | 31 – 55 |
| Very Low | 2 – 30 |




**Criticality Score (2-140)**
Quantitative measure for an attribute's impact on safety and efficacy. Using best possible surrogates for clinical safety and efficacy

**Impact (2-20)**
Known or potential consequences on safety and efficacy, considering, biological activity, PK/PD, immunogenicity, safety

**Uncertainty (1-7)**
Relevance of information e.g. literature, prior knowledge, *in vitro*, preclinical clinical or combination of information

# Criticality

- Highly critical CQAs which directly impact safety or efficacy => residual host cell proteins, endotoxin, aggregates, & potency
- Uncertain criticality main focus on CQAs with unknown impact on efficacy. Vary, typically posttranslational modifications => glycosylation, charge isoforms, phosphorylation, oxidation, & deamidation.

Anurag S. Rathore, et all BioPharm International 2014

# How comparable do biosimilars need to be?

- Biosimilar needs to be as safe & efficacious as the reference product
- The more critical a quality attribute is, => more comparable it should be
- The more comparable a biosimilar is to the reference analytically, the smaller the residual uncertainty, the more tailored the non-clinical and clinical program

# How comparable do biosimilar mAbs need to be? some FDA ideas

U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

## Examples of Reasons: Cannot be biosimilar

- Differences in primary amino acid sequence
- Differences in other glycan structures if produced in a different cell substrate
- Differences in antigen, C1q or FcγR binding affinity
- Differences in fucose and galactose profiles for mAbs with effector function
- Differences in size variants if variant not understood
- Differences in charge profile if variants not understood
- Differences in potency assays (effector function or other)

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# How comparable do biosimilar mAbs need to be? some FDA ideas

U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

## Examples of Reasons:

**Highly similar**  ≈

- No differences in antigen or FcγR binding affinity
- Minor differences in fucose and galactose profiles, whether cell surface or soluble target, but characterized
- Differences in charge profile limited to C-term lys, N-term p-Glu)
  - Acceptable differences may depend on context such as route of administration/site of action
- No differences in size variants or lower levels of variants
- No differences in potency assays (effector function or other)

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Jan Visser, **CMC Strategy Forum Europe 2014, Sorrento, Italy**

# Conclusions

- The scientific principles behind the comparability in manufacturing changes and assessment of biosimilarity are the same!
- The analytical comparability is the key in establishing biosimilarity – the most sensitive to differences!
- The QA's of the reference product, and the QA criticality assessment are key elements in directing biosimilar development!
- The closer the biosimilar and it's reference are analytically, the less residual uncertainty and the more tailored the (non)clinical program should be!

# The End