

Quality assessment of biosimilars

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Agenda

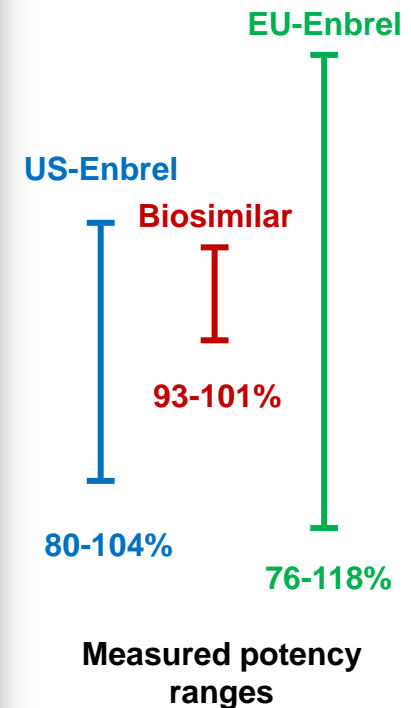
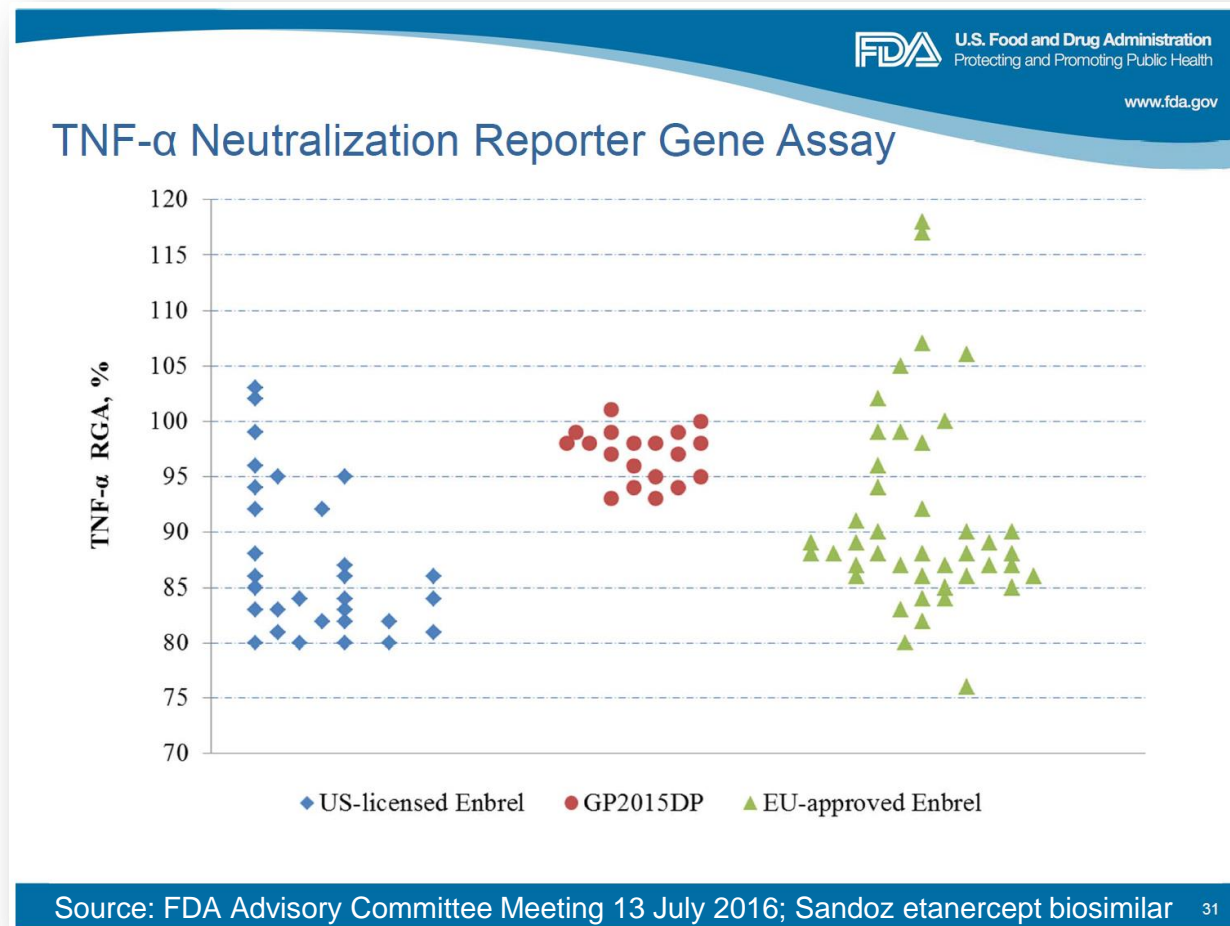
1. The concept of demonstrating comparability/ similarity for biological medicinal products
2. Manufacturing process development - Quality Target Product Profile (QTPP)
3. Assessment of physicochemical and biological similarity
4. What if/ when differences are present?
5. Setting specifications for biosimilars



Source: Annie Spratt, <https://unsplash.com>

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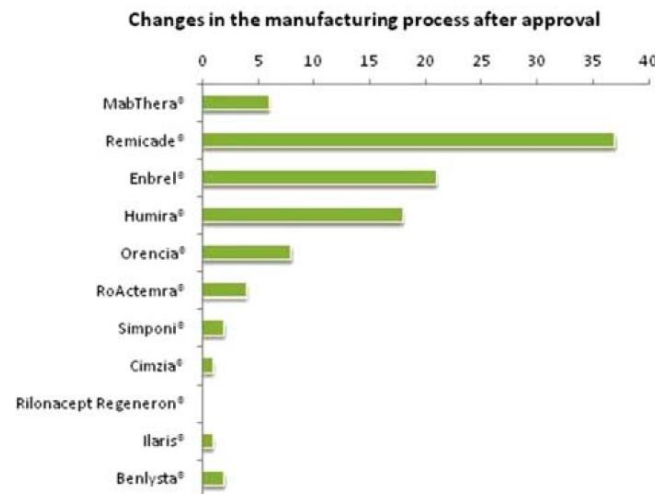
Batch to batch variability in biological medicinal products



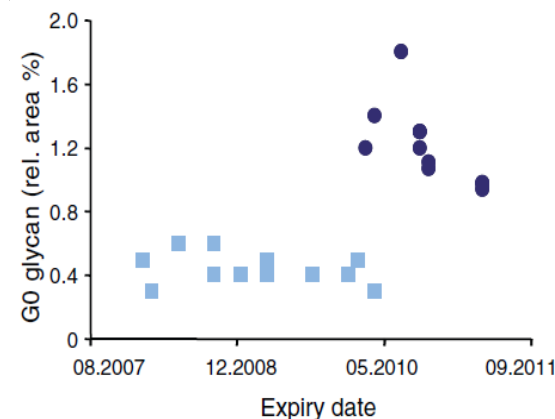
Manufacturing process changes are common for all biologics

Application number	Scope	Opinion/ Notification ¹ issued on
II/0111	B.I.a.1.e - Change in the manufacturer of AS or of a starting material/reagent/intermediate for AS - The change relates to a biological AS or a starting material [-] used in the manufacture of a biological/immunological product	23/06/2016
IB/0120	B.II.b.3.z - Change in the manufacturing process of the finished or intermediate product - Other variation	22/06/2016
IB/0119/G	This was an application for a group of variations. B.I.b.1.c - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Addition of a new specification parameter to the specification with its corresponding test method B.I.b.2.e - Change in test procedure for AS or starting material/reagent/intermediate - Other changes to a test procedure (including replacement or addition) for the AS or a starting material/intermediate	21/06/2016

MabThera assessment history available at EMA website



Schneider C., Ann Rheum Dis March 2013 Vol 72 No 3



Schiestl M. et al, Nat Biotech, April 2011

Comparability assessment for biologics

- Batch-to-batch variability is inherent for biologics, no batch is fully identical to another
- Manufacturing process changes with the potential to alter the quality profile are frequently implemented
- The pre- and post-change version of the medicinal product needs to be demonstrated to be comparable through a comparability exercise in line with the recommendations given in the ICH Q5E guideline
- Manufacturers and regulators are used to assess the impact of process changes – also in the case of complex biologics

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5E/Step4/Q5E_Guideline.pdf



What is a biosimilar?

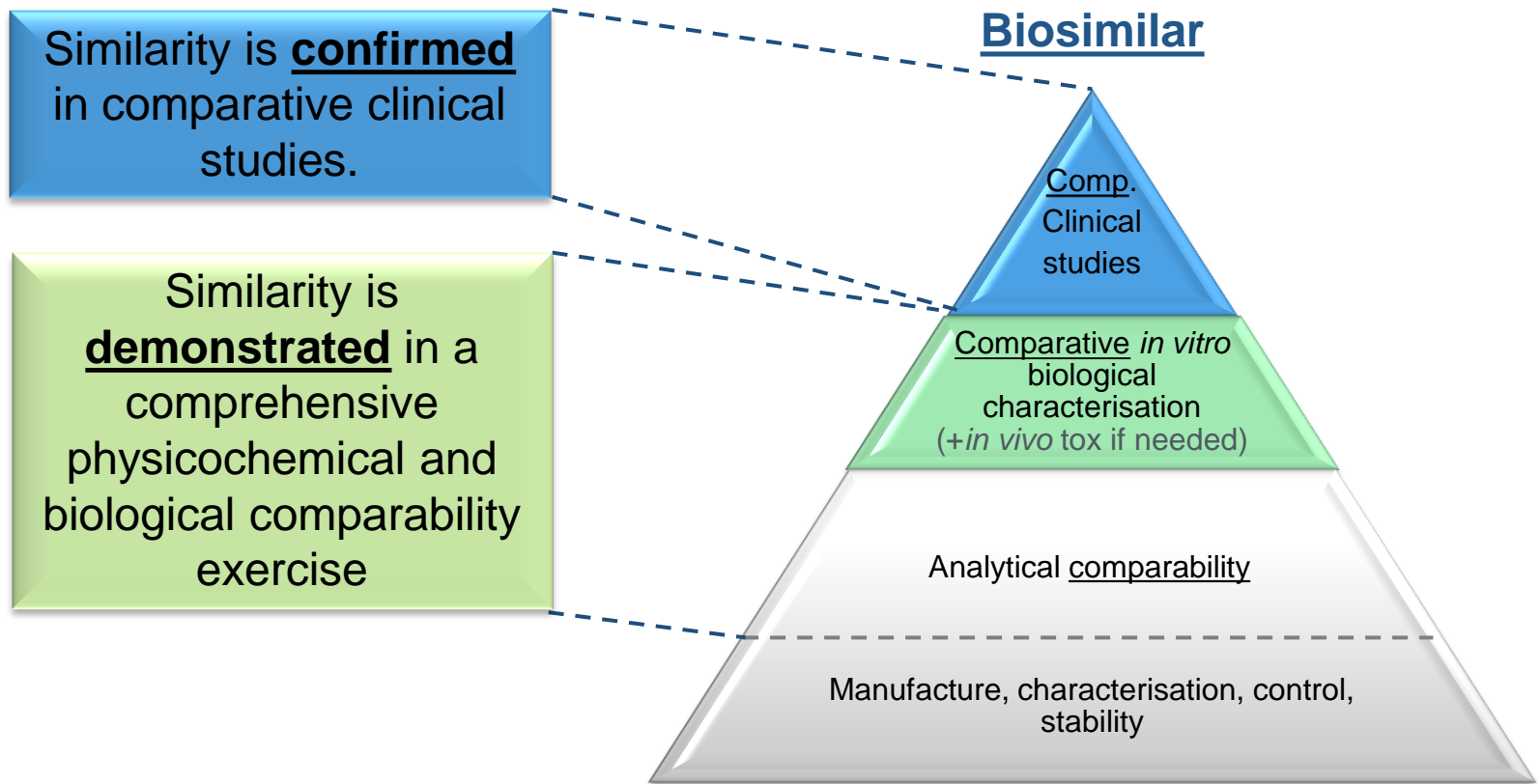
Current EU regulatory definition of biosimilars

A biosimilar is a biological medicinal product that *contains a version of the active substance* of an already authorised original biological medicinal product (reference medicinal product).

A biosimilar demonstrates similarity to the reference medicinal product in terms of quality characteristics, biological activity, safety and efficacy based on a *comprehensive comparability exercise*

- *The scientific principles of a biosimilar comparability exercise are based on those applied for evaluation of the impact on changes in the manufacturing process of a biological medicinal product*

How to demonstrate biosimilarity?



Successful biosimilar development critically depend on the manufacturers ability to;

1. **Consistently** produce a close copy version of the reference
2. **Demonstrate** high similarity through an extensive physicochemical and *in vitro* biological comparability exercise
3. **Understand** the impact of any differences detected
4. **Confirm** similarity with regard to PK, safety and efficacy



Source: Tim Marshall, <https://unsplash.com>

Manufacturing process development - Quality Target Product Profile (QTPP)

- A prospective summary of the quality characteristics of a drug product that ideally will be achieved
- Based on data collected on the reference medicinal product
- Detailed at an early stage of development
- The importance of the quality attributes/ characteristics for the biological function of the protein need to be understood
 - Single or multiple mode of action?
 - Impact of post-translational modifications?
- **Attribute variability** as measured from the reference product, forms the basis for biosimilar development



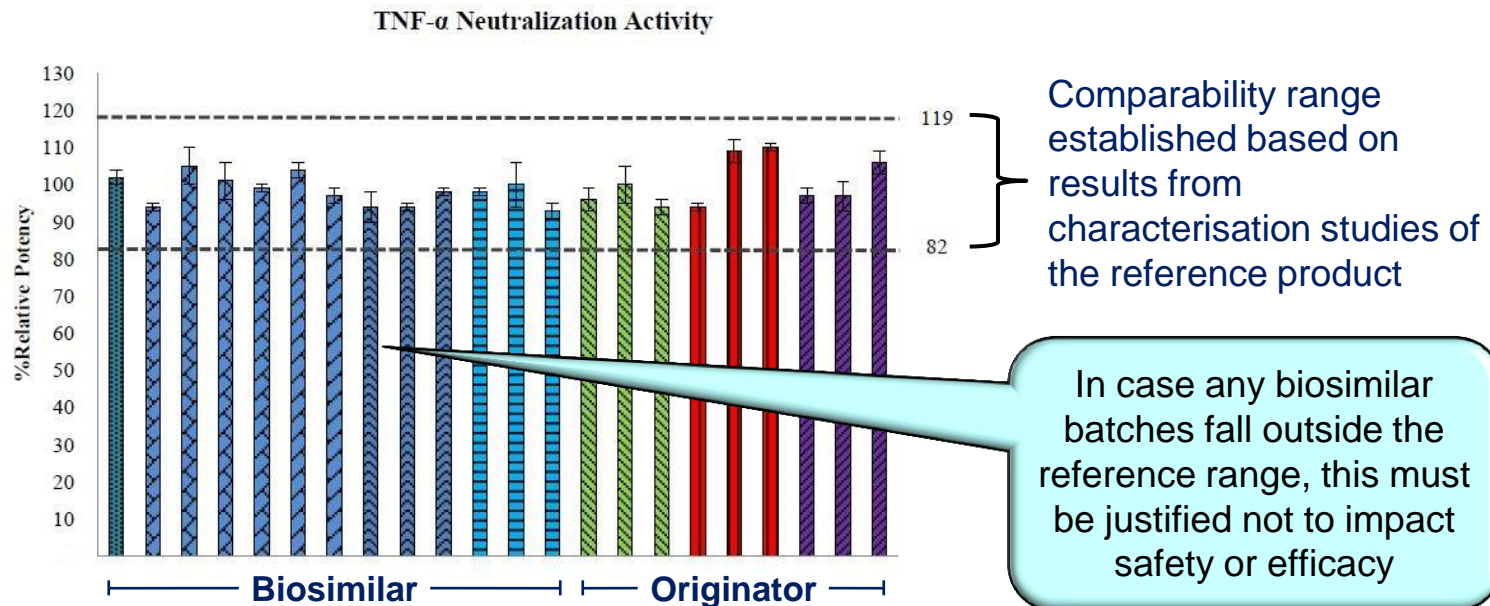
Reverse Engineering Approach

- Expression system development
 - Needs to be carefully considered taking into account expression system differences that may result in undesired consequences; atypical glycosylation, higher variability or a different impurity profile
- Upstream process development
 - To match product attributes; Media composition, fermentation parameters, growth characteristics etc.
- Downstream process development
 - To match product variants; Purification principles and chromatographic parameters used

The goal is to design a manufacturing process that consistently produces a high quality biosimilar product fulfilling the established Quality Target Product Profile

The "pivotal" evidence for analytical similarity

- Biosimilarity should be demonstrated in an extensive, side-by-side (whenever feasible) comparability exercise
- Quantitative comparability ranges should primarily be based on the measured reference product ranges (QTPP)



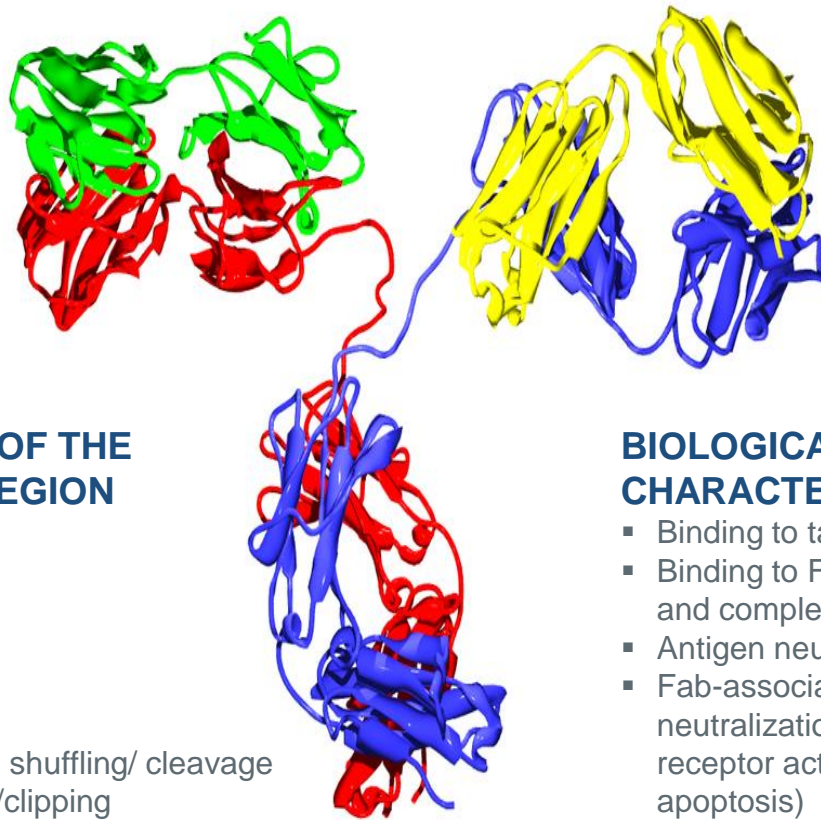
Typical quality attributes and characteristics to be considered in the similarity assessment of a mAb

ATTRIBUTES OF THE VARIABLE REGION

- Deamidation
- Oxidation
- N-term Pyro-Glu
- Glycosylation
- Glycation
- Conformation changes

ATTRIBUTES OF THE CONSTANT REGION

- Deamidation
- Oxidation
- Acetylation
- Glycation
- Glycosylation
- C-term Lys
- Di-sulfide bond shuffling/ cleavage
- Fragmentation/clipping
- Conformation changes



PHYSICOCHEMICAL CHARACTERISTICS

- Structure (primary, higher order structures)
- Molecular mass
- Purity/ impurity profiles
- Charge profile
- Hydrophobicity
- O- and N-glycans

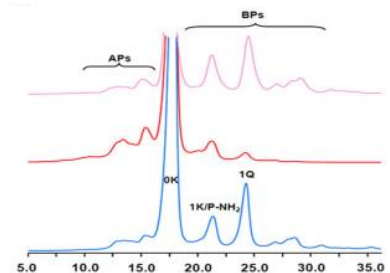
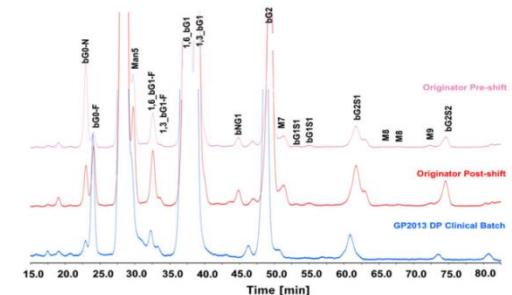
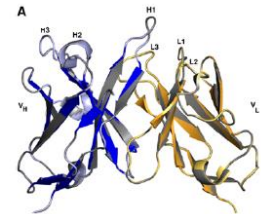
BIOLOGICAL/ FUNCTIONAL CHARACTERISTICS

- Binding to target antigen(s)
- Binding to Fc γ receptors, FcRn and complement
- Antigen neutralisation (if relevant)
- Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation, induction of apoptosis)
- Fc-associated functions (ADCC and CDC)

Figure from Wikipedia

Some analytical tools commonly used for mAb characterisation

- Amino acid sequence and modifications
 - MS, LC-MS, peptide mapping, N- and C-terminal sequencing, total AA analysis
- Disulphide bridging, protein folding and higher-order structures
 - Peptide mapping, Ellman's assay, CD, FTIR, HDX-MS, NMR, DSC, X-ray crystallography
- Glycosylation and glycation
 - Anion exchange, enzymatic digestion, peptide mapping, CE, MS, BAC
- Size heterogeneity
 - SEC, AUC, AF4, MALDI-TOF, CE-SDS, SDS-PAGE
- Heterogeneity of charge and hydrophobicity
 - IEF, cIEF, IEX, CZE, RP-HPLC
- Functional characterisation and bioassays
 - Target and/or receptor binding; SPR, ELISA, cell-based assays
 - Bioassays; Signal transduction, ADCC, CDC, other cell-based assays



Figures from Visser J. et al. BioDrugs. 2013 Oct;27(5):495-507

Primary structure

- Identical **AA sequence** is expected
- **Peptide map** should ideally provide 100% coverage
 - Also provides info on disulphide bridges, oxidation, deamidation, glycosylation
- The different glycan structures present should be taken into account when determining **molecular weights**
- **Oxidation** of conserved Met252 & Met428 decreases FcRn binding and reduces half life
- **Deamidation** may effect degradation and immunogenicity

- **Amino acid sequencing**
- **Peptide map (e.g. trypsin, Lys-C...)**
- **Molecular weight (MS)**
- **Disulfide bond analysis**
- **Free sulfhydryls**
- **N-term sequence**
- **C-term sequence**
- **Met oxidation**
- **Deamidation**



N- and C-terminal sequence

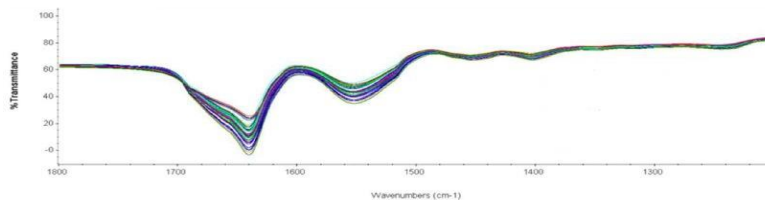
- C-terminal lysine variants can be clipped - 0K, 1K and 2K variants
 - Lysine is removed *in vivo* quickly after injection so difference in lysine variants aren't a concern for biosimilars
- Also e.g. N-terminal pyroglutamate (pE) occurs naturally *in vivo* and is generally not a safety concern

Protein content

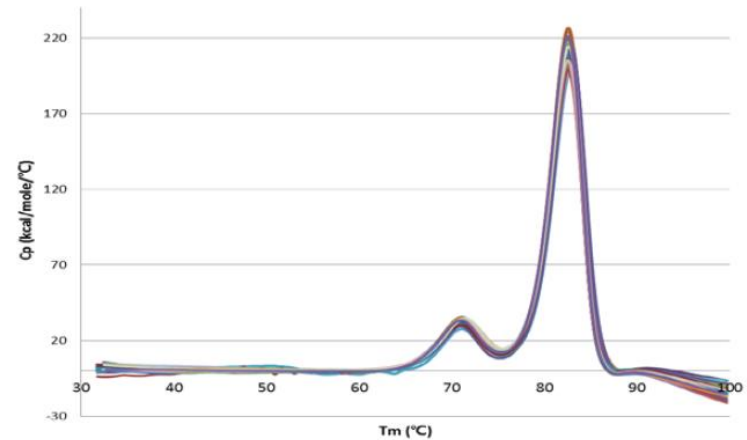
- In addition to amino acid sequence and potency, protein content is one of the most important aspects of biosimilarity
- Biosimilar must have the same strength as the reference
- Biopharmaceuticals are normally filled and labelled based on weight
 - Possibility for standardisation by providing extinction coefficient in product-specific monographs?

Higher order structures

- Far UV circular dichroism (CD) spectroscopy
- Fourier transform infrared spectroscopy (FTIR)
- Near UV CD spectroscopy
- Differential scanning calorimetry (DSC)
- nuclear magnetic resonance (NMR)
- Fluorescence spectroscopy
- Hydrogen/deuterium exchange (HDX)



Example of overlaid spectra from FTIR analysis



Example of overlaid DSC thermograms

- Mainly provides spectra and thermograms for visual comparisons, restricted amount of quantitative data
- Complementary data to e.g. disulphide bound analyses and bioactivity assessment

Purity/ impurity profile

- At least two orthogonal methods are required for measurement of aggregates
 - For HMW and LMW species, levels don't have to be equivalent to originator, demonstration of equal or lower levels of impurities is sufficient
 - Main peak or %HC + LC should be equal or greater to the reference product
 - Process-related impurities are expected to differ both qualitatively and quantitatively and do not usually need to be directly compared, but should be kept at minimum
- SEC
 - CE-SDS
 - SDS-PAGE
 - Analytical ultracentrifugation (AUC)
 - Multiangle light scattering (MALS)
 - Field flow fractionation (FFF)

Heterogeneity of charge and hydrophobicity

- Cation/ anion exchange chromatography (IEX)
- Isoelectric focusing (IEF)
- Capillary IEF (cIEF)
- Imaged capillary IEF (icIEF)
- Capillary Zone Electrophoresis (CZE)
- Reverse Phase Chromatography (RPC)

Acidic

- Deamidation
- Sialylated glycans
- Fragments
- Glycation
- Cyclized glutamine

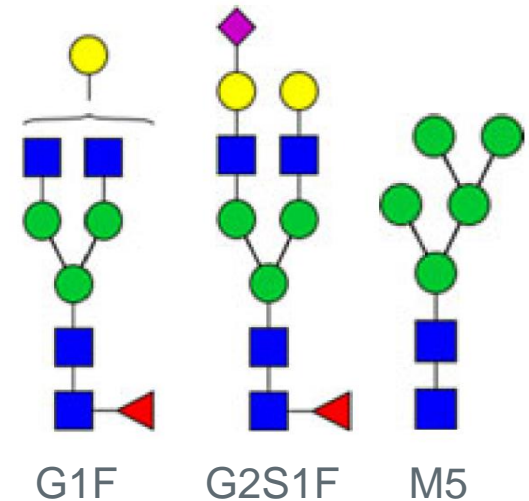
Basic

- C-term. Lys
- Met Oxidation
- N-term. glu
- Asp isomerisation
- Pro amidation
- Aggregates
- Fragments

- Common to see differences → source of charge variation should be identified and justified e.g. isolate each peak by preparative CEX-HPLC and perform SAR studies
- Difference between biosimilar and reference product often related to age of batches e.g. increase in deamidation, oxidation, fragmentation etc. and/or differences in C/N-terminal sequences

N-linked Glycosylation

Saccharides	Type
Oligosaccharides	% afucose (G0, G1, G2), G0F, G1F, G2F
High mannose	%Man5, Man6, Man7, Man8
Monosaccharides	%Fuc, GlcN, Gal, Man
Sialic acids	%Neu5Ac (NANA), Neu5Gc (NGNA)
Other	O-glycosylation, α -gal



- Recombinant mAbs contain complex glycan structures that require detailed characterisation and comparison
 - Oligosaccharide profiling (e.g. using PNGase F released, 2-AB labeled and UPLC analysed glycans), site specific analysis (if needed)
 - Sialic acid content, high-mannose variants, afuc%, gal%
 - Non-human structures, e.g. Gal(α 1-3)Gal, Neu5Gc (NGNA)
 - O-linked glycans (when/ if relevant)

Specific glycan structures may affect safety/ immunogenicity, activity and/or clearance

Glycan species	Safety/ immunogenicity	Biologic activity/ efficacy	Clearance (PK/PD)
Galactose	Unknown	Unknown	Unknown
<u>α1,3-galactose</u>	--	Unknown	Unknown
<u>Fucose</u>	(-)	++	Unknown
Bisecting GlcNAc	(-)	+	Unknown
<u>High mannose</u>	Unknown	+	--
NANA	Unknown	(-)	+
<u>NGNA</u>	--	(-)	+
β 1,2-Xylose/ α 1,3-Fucose	--	Unknown	Unknown
NGHC	Unknown	-	(-)

Gal(α 1-3)Gal is a non-human glycan structure produced by e.g. many rodent cell lines. Immunogenic in human

Afucosylated structures show increased binding to Fc γ RIII leading to increased ADCC activity

Mannose structures bind to mannose receptors which results in increased protein clearance

Neu5Gc (NGNA) is a sialic acid not present in humans; immunogenic.

+ Positive impact; - negative impact; (+/-) potential impact; -- high negative impact

Reusch and Tejada Glycobiology, 2015 Dec; 25(12): 1325–1334.

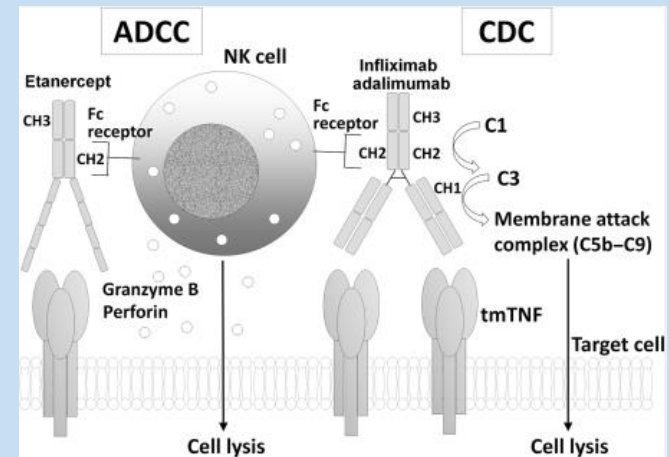
Binding and Functional assays

Binding assays (e.g. ELISA, FRET, SPR, cell-based)

- Target binding
- Binding to all relevant Fc receptors and complement protein
 - FcγRIa (CD64A)
 - FcγRIIa (CD32A)
 - FcγRIIb (CD32B)
 - FcγRIIc (CD32C)
 - FcγRIIIa (CD16A)
 - FcγRIIIb (CD16B)
 - FcRn
 - C1q

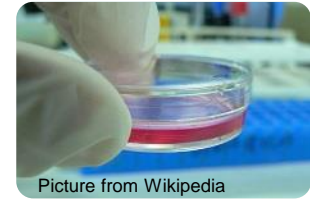
Functional assays

- Cell based assay potency
- ADCC
- CDC
- Apoptosis...



Picture: Horiuchi et al, Rheumatology (Oxford). 2010 Jul;49(7):1215-28

Some considerations on biological assays

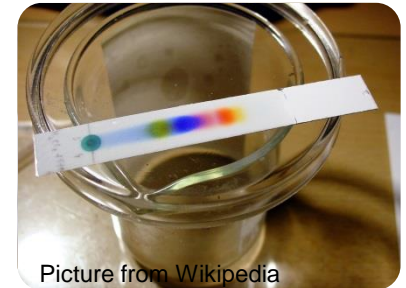


Picture from Wikipedia

- **FcγRIIIA** is the most important Fc receptor in terms of effector function
 - Compared to the low affinity allotype 158F, the high affinity 158V allotype is likely more sensitive to detect small binding differences
- Where effector function is important (and/or there are differences in Fc binding), a **larger amount of functional assays** might be needed to demonstrate similarity
- **Functional cell-based assays** often suffer from relatively high assay variability. Implications for the sensitivity of the assay to detect differences
- **“Indication-specific” assays** often applied to strengthen the claim for indication extrapolation

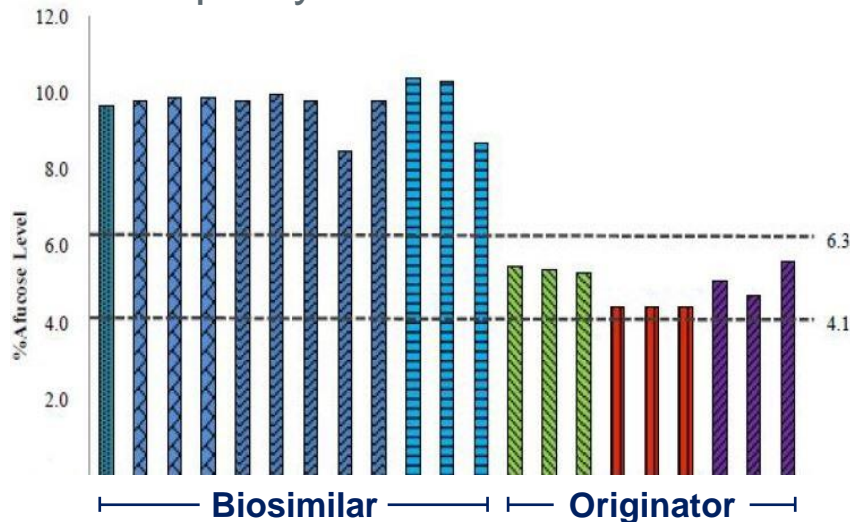
Analytical methods

- The methods have to be properly qualified for the purpose of comparability
 - Needs to be shown that the methods are capable of detecting subtle differences which might exist between the biosimilar and reference product
 - If applicable, publicly available reference standards (e.g. Ph. Eur.) plays an important role in method development, qualification and standardisation
- Analytical methods used only in the comparability exercise do not have to be fully validated
- Biosimilarity should be demonstrated at the level of drug product unless formulation interferes with the assay
- Orthogonal methods should be used where possible



What to do when the biosimilar falls outside the comparability range?

- The biosimilar is not expected to be analytical identical to the reference product
 - Any differences detected in quality attributes must be justified in relation to safety and efficacy
 - Clinical data cannot be used to justify substantial differences in quality attributes

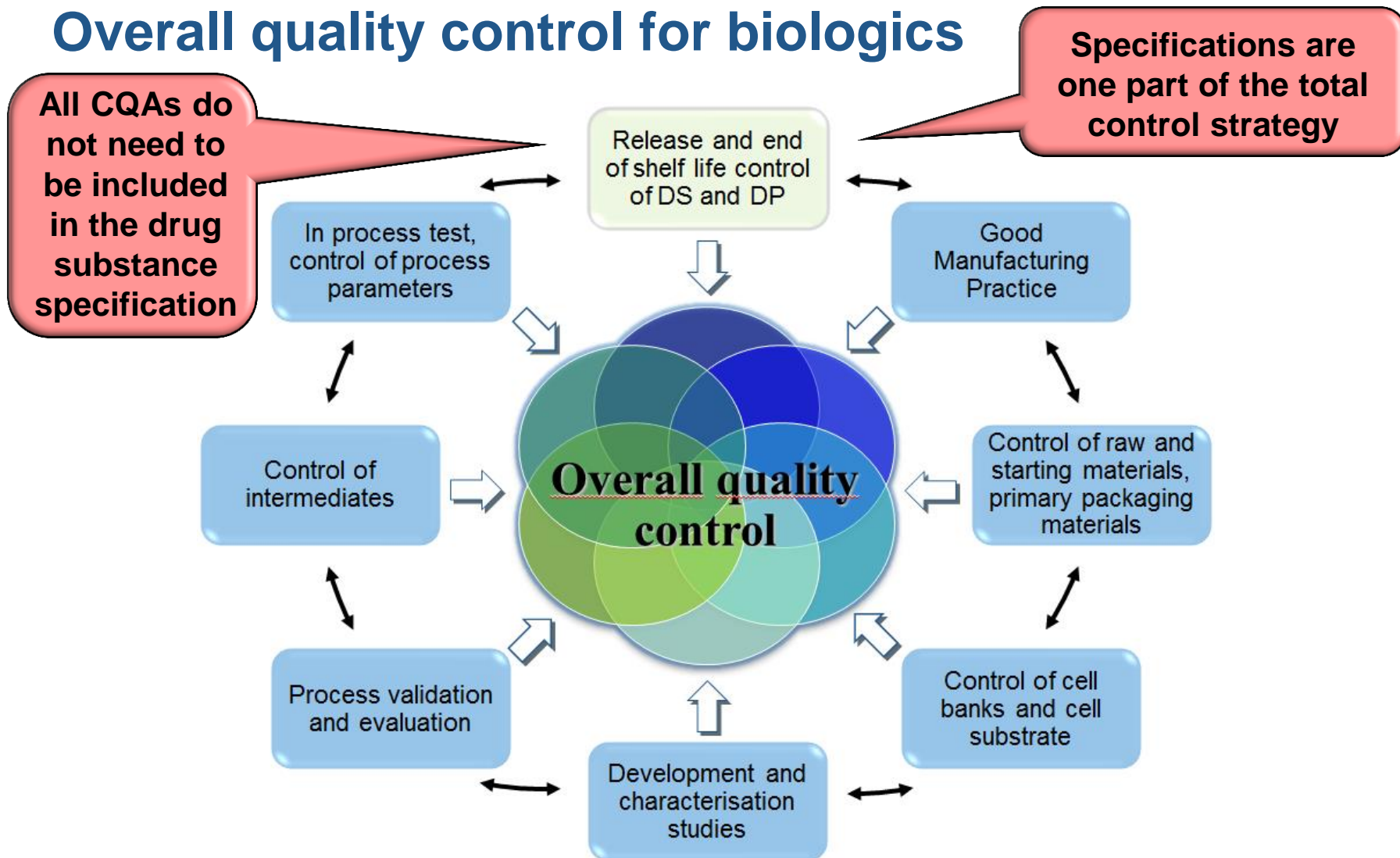


- Previous knowledge might be sufficient for justifying differences in **low criticality attributes**
- For **medium to high criticality attributes** the impact of the difference need to be addressed, primarily using suitable *in vitro* functional assays

Specifications for biosimilars

- Specifications are chosen to confirm the quality of the drug substance and the drug product
- The selection of tests to be included in the specifications for biosimilars should be defined as described in ICH Q6B
- Acceptance criteria should be established and justified based on data obtained from;
 - Biosimilar batches used in clinical studies
 - Biosimilar batches used for demonstration of manufacturing consistency and biosimilarity, other relevant development data
 - Characterisation results from the reference product can be used as supporting data for the justification of specification acceptance limits for the biosimilar

Overall quality control for biologics



Ph. Eur. monographs and other texts are central in ensuring the quality of all medicinal products, including biosimilars

- ✓ Compliance with available monographs **is mandatory**, but all tests listed in a monograph do not have to be performed at release
- ✓ When agreed by the competent authority, alternative (validated) methods may be used for control purpose

- ✓ Comparison of the biosimilar to a pharmacopoeial monograph is not sufficient for the purpose of demonstrating biosimilarity
- ✓ A pharmacopoeial standard preparation can not be used as the reference medicinal product

Ph. Eur. monographs and other texts are central in ensuring the quality of all medicinal products, including biosimilars

- ✓ 2. Methods of analysis
 - ❖ Appearance, pH, Sterility, Endotoxin, Microbial enumeration, Host-cell proteins...
- ✓ 3. Materials and Containers
 - ❖ Glass containers, Plastic containers, Silicon oil...
- ✓ 5. General Text
 - ❖ Viral safety, Statistical analysis...
- ✓ 6. General Monographs
 - ❖ mAbs, rDNA technology products...
- ✓ 7. Dosage forms
 - ❖ Parenteral preparations...
- ✓ Monographs
 - ❖ Water for injections...

Ph. Eur. monographs and other texts are central in ensuring the quality of all medicinal products, including biosimilars

Product-specific monographs

- ✓ From an assessor's point of view, product-specific monographs do not play a major role in the the assessment and approval of biosimilar MAAs
- ✓ Provides methods suitable for evaluating only a portion of the critical quality attributes, usually against broad limits
- ❖ Harmonized testing makes the activities of e.g. independent laboratories (OMCLs) a little bit easier
- ❖ Enables direct comparison between two or more products, e.g. originator and biosimilar (but only for those quality attribute included in the monograph)

Thank you for your attention!



Source: Ruth Caron, <https://unsplash.com>

Acknowledgment

Sean Barry (HPRA, IE)

**More information on
biosimilars is found on the
EMA website**

<http://www.ema.europa.eu/>

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