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GaBI Scientific Meetings

ROUNDTABLE ON BIOSIMILARS
with participation by European Regulators and Medical Societies

12 January 2016, Sheraton Brussels Airport Hotel, Belgium

Analytical comparability covering product manufacturing, characterisation, purity and stability of biosimilars

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Analytical comparability covering product manufacturing, characterisation, purity and stability of biosimilars

European Medical Societies Roundtable on Biosimilars
Brussels, Belgium
12 January 2016

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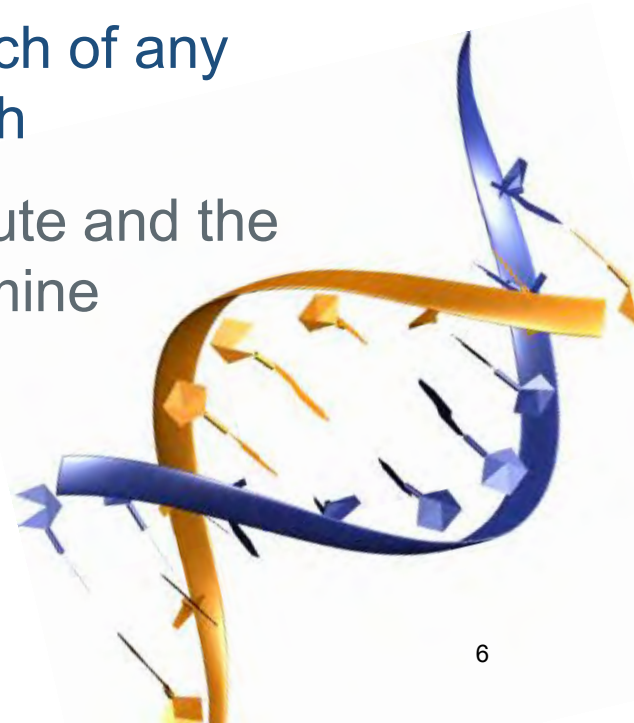
Content

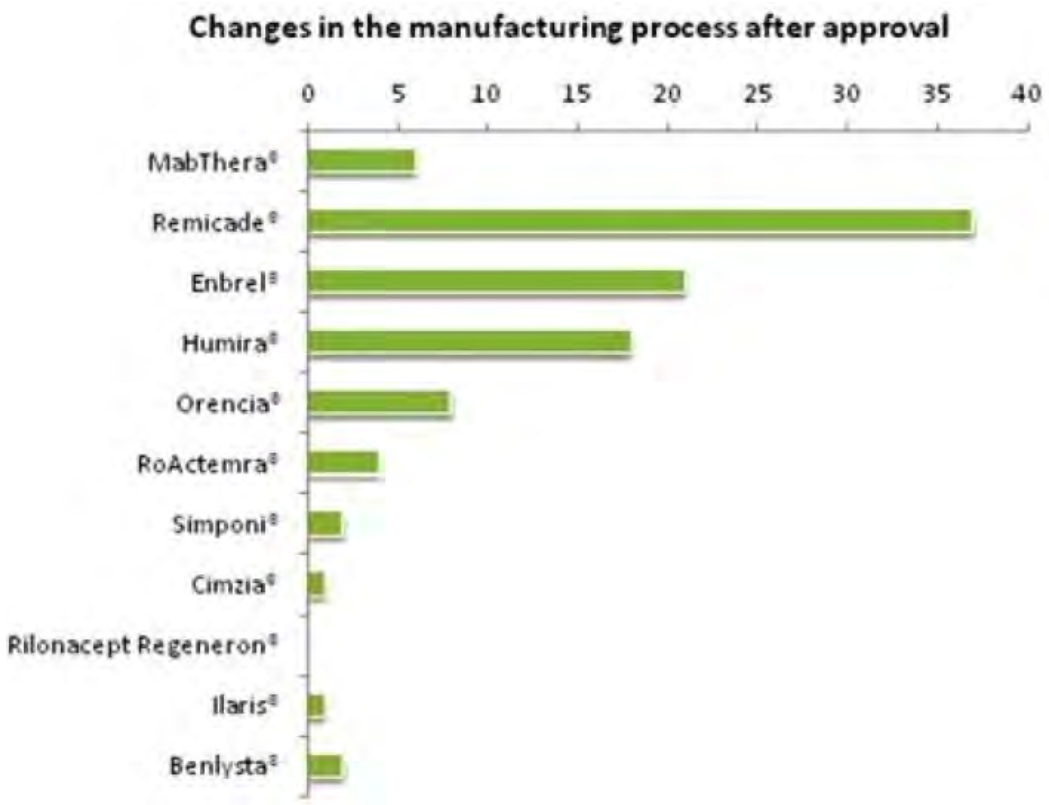
1. The concept of analytical similarity – from comparability to biosimilarity
2. Manufacturing process development
3. The pivotal evidence for analytical biosimilarity

What makes biotech products special

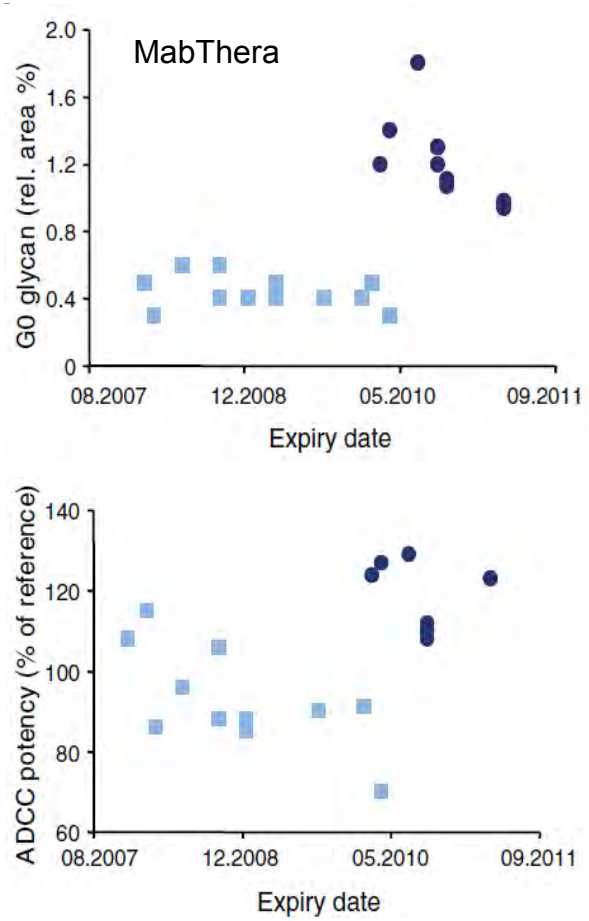
- Biotech products are large and complex molecules which can be difficult to fully characterise
- The use of living cells for production will always introduce heterogeneity into the drug substance
- Variability is inherent in biologics, no batch of any biologics is fully identical to another batch
- The link between a specific quality attribute and the clinical outcome can be difficult to determine
- “The product is the process” has been postulated - what about real life experience?



Manufacturing process changes are common for all biologics



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



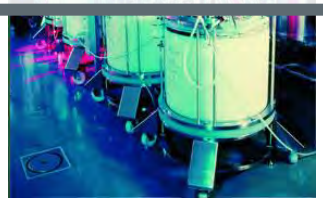
Schiestl M. et al, Nat Biotech, April 2011

Changes in the production process during development or post marketing

ICH Topic Q5E

Comparability of Biotechnological/Biological Products

Formulation and filling



Purification

Fermentation

ICH Topic Q 5 E
Comparability of Biotechnological/Biological Products

"..where the relationship between specific quality attributes and safety and efficacy has not been established, and *differences between quality attributes of the pre- and post-change product are observed, it might be appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise*."

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*

Biosimilar vs Reference Medicinal Product - How close is close enough?

- Must be the same
  - The amino acid sequence
  - Posology and the route of administration
- Must be similar
  - The active substance in terms of molecular and biological characteristics
- Need to be justified
  - Differences in strength, pharmaceutical form, formulation, excipients or presentation
- Not allowed
  - Intended changes to improve efficacy (”biobetters”)

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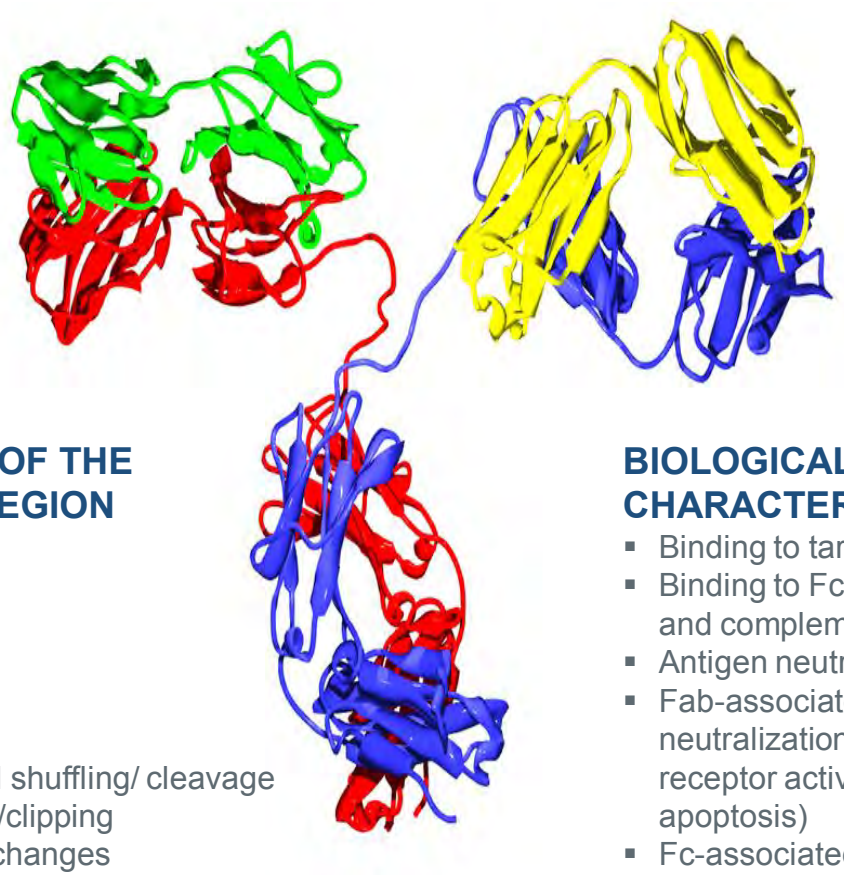
Analytical and functional characterisation of a typical monoclonal antibody

ATTRIBUTES OF THE VARIABLE REGION

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



PHYSICOCHEMICAL CHARACTERITICS

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

ATTRIBUTES OF THE CONSTANT REGION

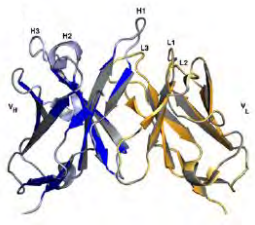
- Deamidation
- Oxidation
- Acetylation
- Glycation
- Glycosylation
- C-term Lys
- Di-sulfide bond shuffling/ cleavage
- Fragmentation/clipping
- Conformation changes

Figure from Wikipedia

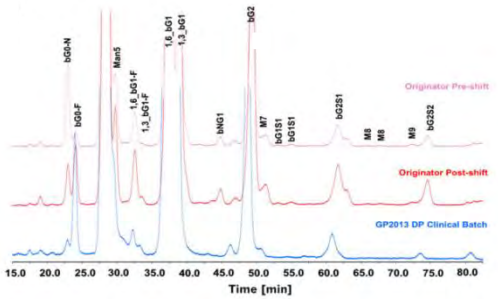
BIOLOGICAL/ FUNCTIONAL CHARACTERISTICS

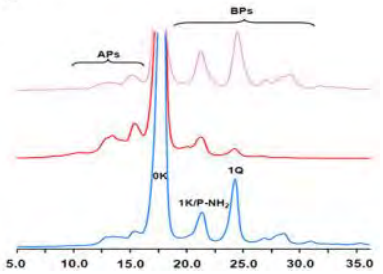
- Binding to target antigen(s)
- Binding to Fc γ receptors, FcRn and complement
- Antigen neutralisation (if relevant)
- Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation, induction of apoptosis)
- Fc-associated functions (ADCC and CDC)

Analytical tools commonly used in protein characterisation



- Amino acid sequence and modifications
  - MS, LC-MS, peptide mapping, N- and C-terminal sequencing, AA content
- 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, CD-SDS, SDS-PAGE
- Heterogeneity of charge and hydrophobicity
  - IEF, cIEF, IEX, 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



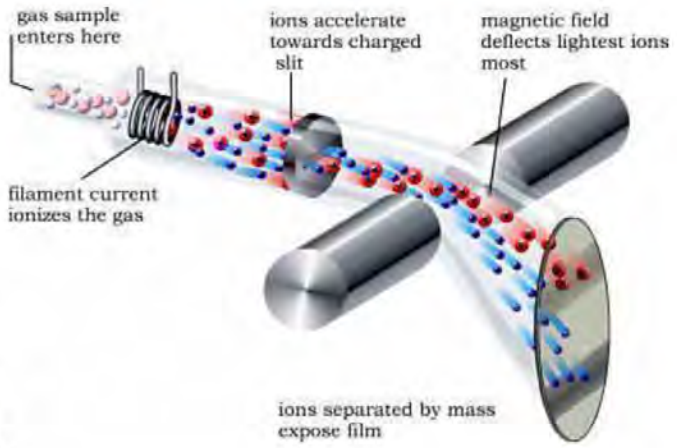


Figures from Visser J. et al. BioDrugs. 2013 Oct;27(5):495-507

Rapid advances in analytical sciences

The Sensitivity of Mass Spectrometer (MS) Methods is Progressing Rapidly

Year	Detection limit of peptide (pmol)
1990	100
1993	10
1997	1
2000	0.1
2003	0.01
2005	0.001
2008	0.0001
2011	0.00001



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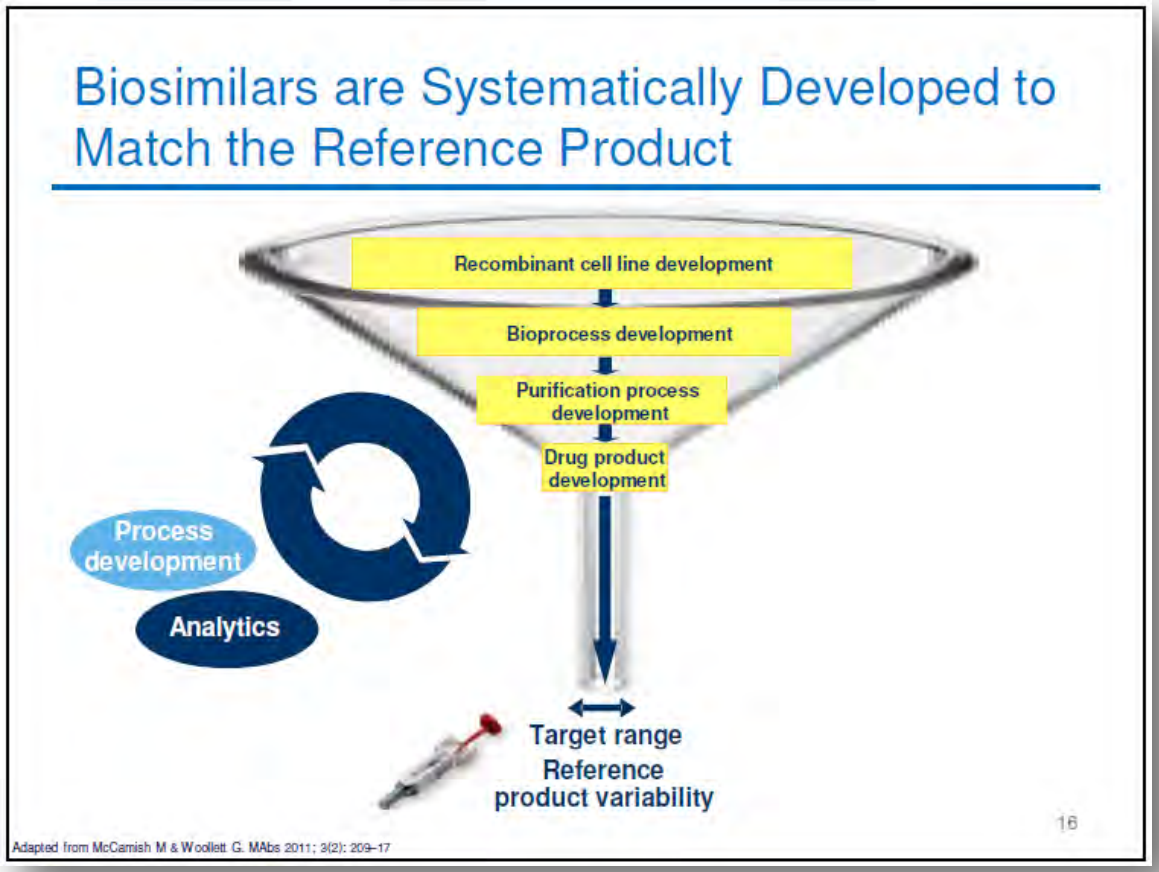
AMGEN

- 10 000 fold increase in sensitivity in 10 years
- 10 million fold increase in sensitivity in 20 years!

Slide presented by Tony Mire-Sluis (Amgen) at CASSS Mass Spec 2012

Biosimilar manufacturing process development



- Variability is inherent to biologics
- For each (critical) quality attribute, the variability is controlled within an acceptable range
- Attribute variability as measured from the reference product, forms the target range (QTPP) for biosimilar development

Slide presented by Sandoz at Oncologic Drugs Advisory Committee meeting on Zarxio (filgrastim), January 7, 2015

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; publicly available information and data obtained from extensive characterisation
- 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?
- Detailed at an early stage of development and forms the basis for the development of the biosimilar product and its manufacturing process

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

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The ”pivotal” evidence for analytical similarity

- An extensive, side-by-side (whenever feasible) comparability exercise is required to demonstrate high similarity
  - Composition, physical properties, primary and higher order structures, purity, product-related isoforms and impurities, and biological activity
  - Orthogonal methods should be used whenever possible
  - The aim is to show high similarity on the drug product level using material produced with the final (commercial) manufacturing process using sensitive analytical methods
- Quantitative comparability ranges should be established
  - Ranges should be based primarily on the measured quality attribute ranges of reference product and should not be wider than the range of variability of the representative reference product batches

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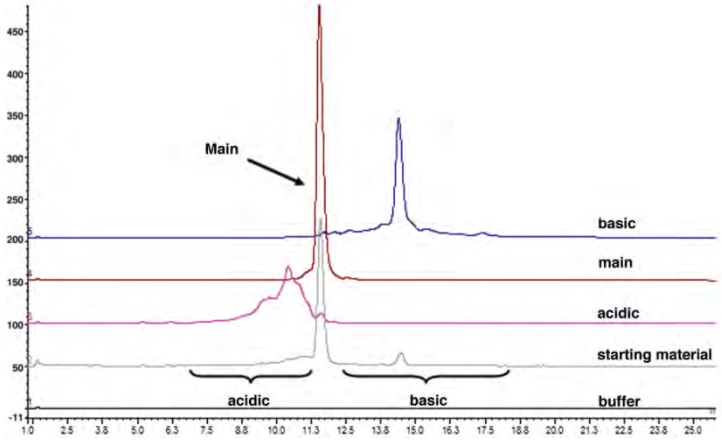
The ”pivotal” evidence for analytical similarity

- Any differences detected in quality attributes must be justified in relation to safety and efficacy
  - It may be challenging to claim biosimilarity if relevant quality differences are confirmed, clinical data cannot be used to justify substantial differences in quality attributes
  - For justifying differences in low criticality attributes, previous knowledge might be sufficient
  - For medium to high criticality attributes, Structure Activity Relationship (SAR) studies are usually required
- Additional comparative stability studies under accelerated conditions can be useful to compare degradation pathways, i.e. to reveal “hidden” differences

Example of a SAR study – The biological impact of IgG1 charge variants

Khawli L. et al mAbs 2:6, 613-624; November/December 2010

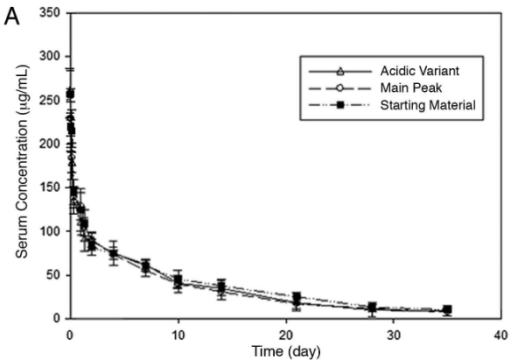
1. Separation and isolation of IEC fractions



2. Identification of variants present in isolated fractions

Table 3. Analytical characterization of isolated charge variant fractions

Method	Percentage of variants detected[a]
Acidic Variant Fraction	
IEC +/- Sialidase treatment	29% Sialylated
Reduced CE-SDS	7% Incompletely reduced
Non-reduced CE-SDS	29% Reduced disulfide
Boronate chromatography	17% Glycated
Peptide Map with Mass Spectrometry	18% Deamidated[b]
Basic Variant Fraction	
Peptide Map with Mass Spectrometry (for identification) and IEC (for quantification)	85% C-terminal heavy chain variants 15% N-terminal Val-His-Ser light chain variants



3. Rat PK study on isolated fractions

Summary - Marketing Authorisation Application

Originator