Biosimilars and the role of analytical technologies

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Analytical Technologies Europe, 15 – 18 March 2016

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The role of analytics in biopharmaceutical industry

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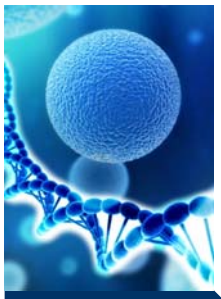
Biopharmaceutical manufacturing



Modify host cells

(e.g. bacteria, yeast, mammalian) to produce recombinant proteins

Grow cells

under controlled conditions (fermentation, upstream process)

Extract, refold, purify

to generate drug substance (downstream process)

Formulate to stable finished drug product

vial, syringe, cartridge

Adapted from EGA Handbook on biosimilar medicines; available from http://www.egagenerics.com/index.php/publications/

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The role of analytics in biosimilar development

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What is a biosimilar?

Biosimilarity means

- that the biological product is **highly similar** to the reference product notwithstanding minor differences in clinically inactive components; and that
- there are **no clinically meaningful differences** between the biological product and the reference product in terms of safety, purity, and potency of the product.[1]

An approved biosimilar medicine and its reference medicine contain **essentially the same** active ingredient and are expected to have the **same** safety and efficacy profile[2]

[1] Section 7002(b)(3) of the Affordable Care Act, adding section 351(i)(2) of the PHS Act
[2] European Comission Consensus Information Document "What you need to know about Biosimilar Medicinal Products" http://ec.europa.eu/DocsRoom/documents/8242/attachments/1/translations/en/renditions/native

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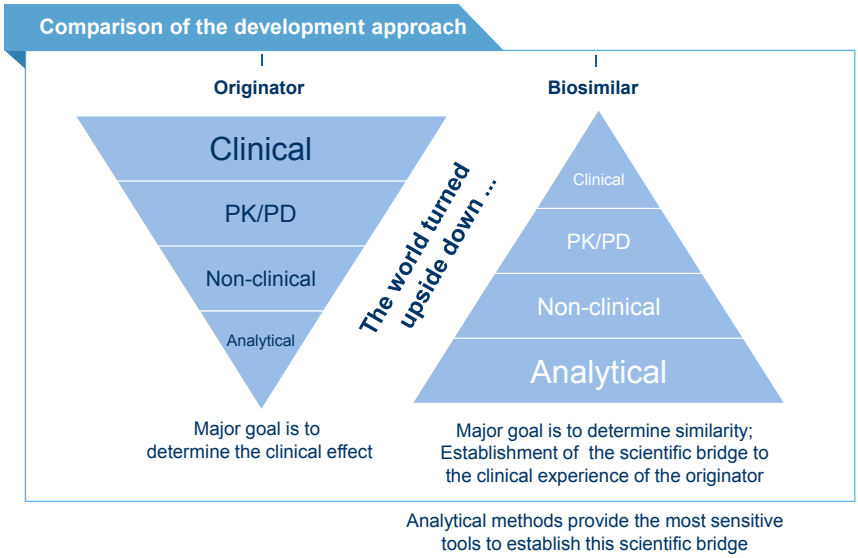
Different focus between originator and biosimilar development



Analytical methods provide the most sensitive tools to establish this scientific bridge

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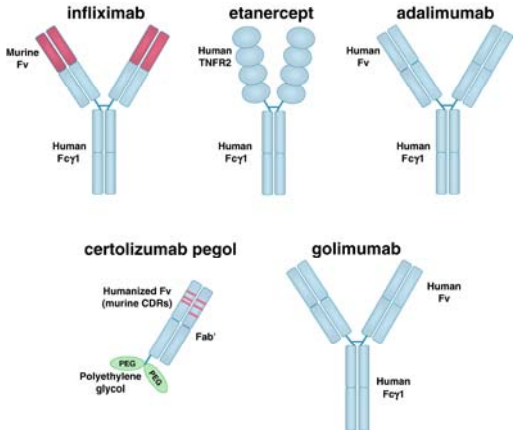
Molecular structures of 5 therapeutic anti-TNFs



Tracey et al (2008) Pharmacol & Therap, 117:224-279

- Those molecules are easy to differentiate using physico-chemical and functional testing
- Different scaffolds and origin can impact several pharmacological properties that have an impact on binding, activity, PK and immunogenicity
- Correspondingly, clinical pharmacology can vary
- However, traditional clinical end-points based on efficacy may not be sensitive enough to resolve any potential pharmacological differences that may exist

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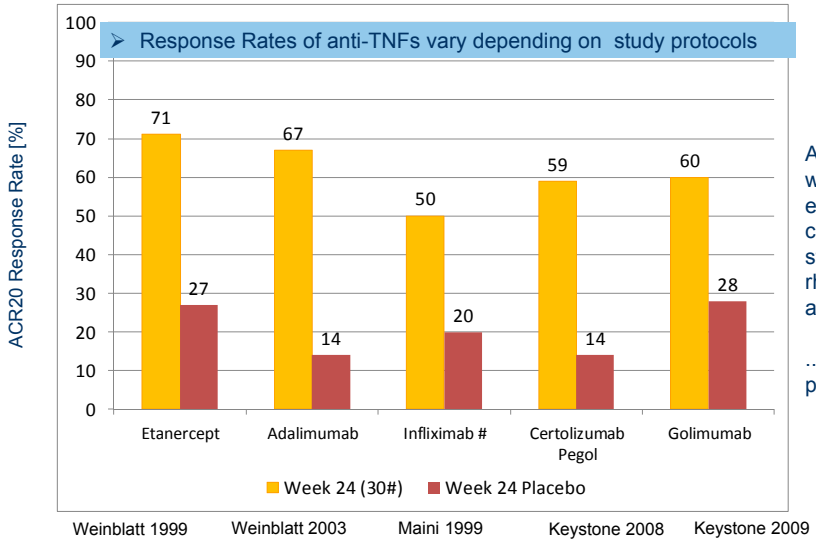
Clinical trials are not sensitive enough to differentiate different Anti-TNF biologics



ACR20 is a well accepted endpoint for clinical studies in rheumatoid arthritis

... for new products

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Targeted development of a biosimilar

Target definition - Analyzing numerous batches of the reference product

Iterative optimization of all process steps to match the reference product

1. Cell line development
2. Bioprocess development
3. Protein purification
4. Drug product development

Knowledge of relevance of quality attributes for efficacy and safety

Demonstration of similarity

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Variability is in the nature of glycoproteins

Batch-to-batch

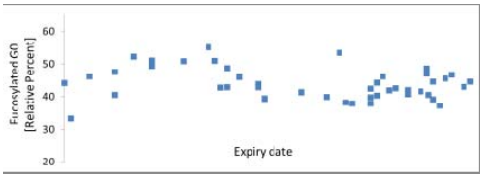
- Non-identicality is a normal principle in glycosylated proteins
- No batch of any biologic is "identical" to the other batches
- Variability is natural even in the human body and usually not problematic

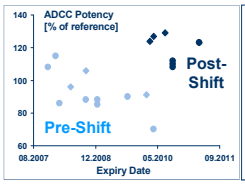
Manufacturing changes

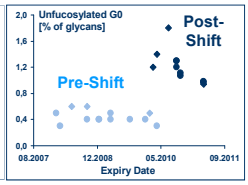
- Manufacturing changes are made frequently
- Differences in attributes often larger than batch-to-batch variability
- Such changes are stringently controlled by regulators and approved only if they do NOT lead to clinically meaningful differences

Safety/efficacy demonstrated

Variability of major glycan variant in commercial mAB







Schiestl M, et al. Nat Biotechnol 2011;29(4):310–2; Sandoz data

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Variability is in the nature of glycoproteins

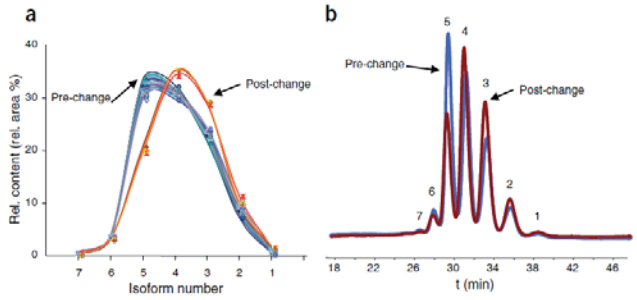

Figure 1 Comparison of the pre- and post-change Aranesp batches measured by capillary zone electrophoresis. (**a**) Relative content of the individual isoforms of the pre-change ($n = 18$) and the post-change ($n = 4$) batches. (**b**) Representative electropherograms; peaks are labeled with the isoform number.

Manufacturing change of darbepoetin alfa was supported by comparative clinical phase III study

Ref: EPAR Aranesp-H-332-X-42, EMA website

Schiestl M, et al. Nat Biotechnol 2011;29(4):310–2; Sandoz data

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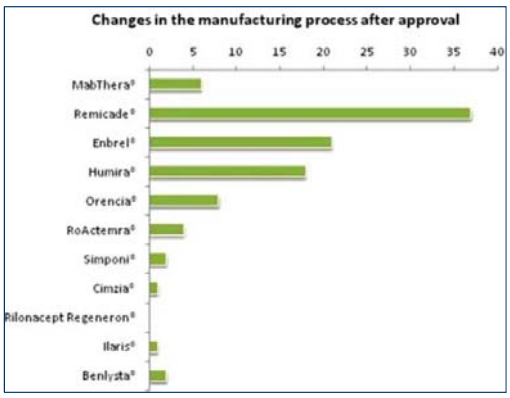
Biosimilar regulation is based on experience with manufacturing process changes of originator products

Originators are changing biologics manufacturing processes multiple times after approval



Changes include e.g.

- Change in the supplier of a cell culture media
- New purification methods
- New manufacturing sites

Such changes are well understood today and tightly controlled by regulators (ICH Q5E)

Source: C Schneider, Ann Rheum Dis March 2013 Vol 72 No 3
Number of changes in the manufacturing process after approval for monoclonal antibodies/cepts authorized in rheumatological indications. Products in order of date of approval in Europe (from MabThera, authorised on 2 June 1998 for the initial authorization in oncology, to Benlysta, licensed on 13 July 2011)

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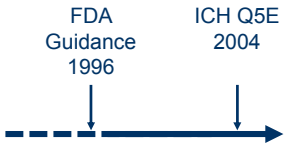
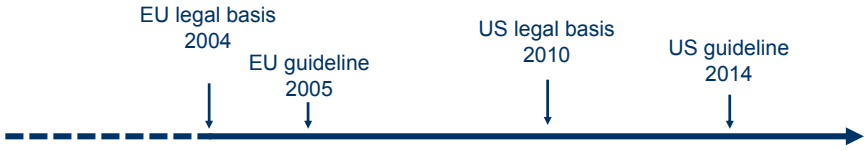
Biosimilar concept evolved from experience with manufacturing changes

- Regulation of manufacturing changes



- Biosimilar regulation



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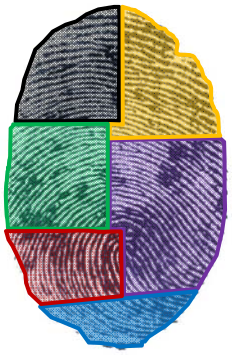
The biosimilar must match the reference product in all relevant structural and functional attributes (mAb example)

Primary structure e.g.:
- LC-MS intact mass
- LC-MS subunits
- Peptide mapping

Impurities and related substances e.g.:
- CEX, cIEF acidic/basic variants
- LC glycation
- Peptide mapping deamidation,
- oxidation, mutation, glycation
- SEC/FFF/AUC aggregation

Biological activity e.g.:
- Binding assay
- ADCC assay
- CDC assay



Higher order structure e.g.:
- NMR
- CD spectroscopy
- FT-IR

Glycosylation:
- NP-HPLC-(MS) N-glycans
- AEX N-glycans
- MALDI-TOF N-glycans
- HPAEC-PAD N-glycans
- MALDI-TOF O-glycans
- HPAEC-PAD sialic acids
- RP-HPLC sialic acids

Combination of attributes:
- Evaluated using MVDA, mathematical algorithms
- Checks redundant data for consistency
- Takes additive or subtractive effects of combinations of attributes into account

- Combined data from **~60 different attributes**
- Attributes are ideally measured by more than one method (redundancy)

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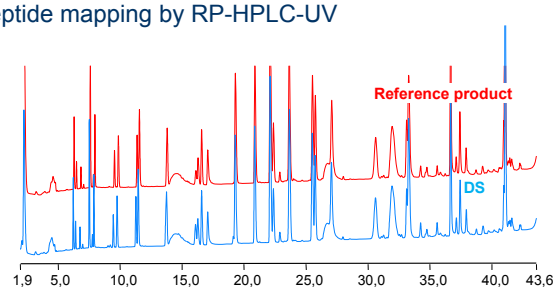
Structural Characterization of GP2013
Primary Sequence – Biosimilar Rituximab candidate

- ✓ Primary structure
- Intact mAb Mass and HC & LC by RP-HPLC-ESI-MS
- Amino acid sequence by RP-HPLC-ESI-MS/MS
- Peptide mapping by RP-HPLC-UV



Peptide map of biosimilar mAb and originator mAb

- Identical amino acid sequence for biosimilar candidate and reference product

Visser J, et al. BioDrugs 2013;27(5):495–507

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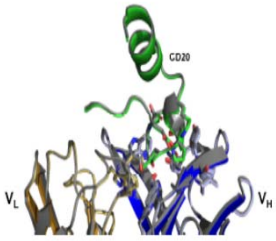
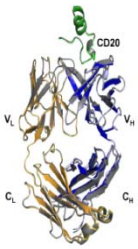
Structural Characterization of GP2013
Higher-Order Structure – Biosimilar Rituximab candidate

- ✓ Higher order structure
- X-ray crystallography of Fab-fragment and binding region



Close up of binding region Grey: Originator, Colour: Biosimilar



Superposition of biosimilar and originator product

- Identical higher order structure, fully superimposable structures

Visser J, et al. BioDrugs 2013;27(5):495–507

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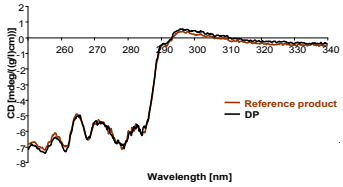
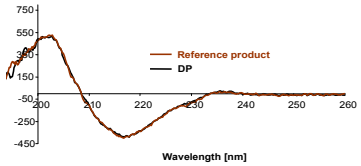
Structural characterization: GP2013

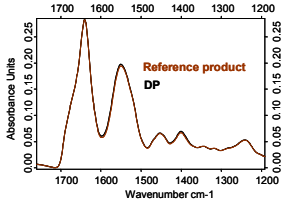
Higher Order Structure - CD, FTIR, HDX - Biosimilar Rituximab candidate

- Circular Dichroism Spec. (near & far UV) - comparable





- FTIR Spec. – comparable



- H/D Exchange – comparable

Visser J, et al. BioDrugs 2013;27(5):495–507

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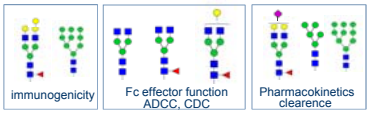
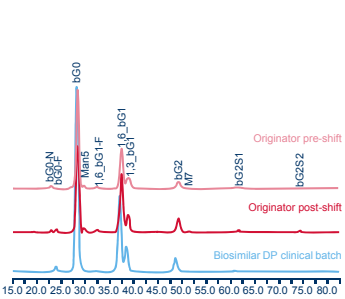
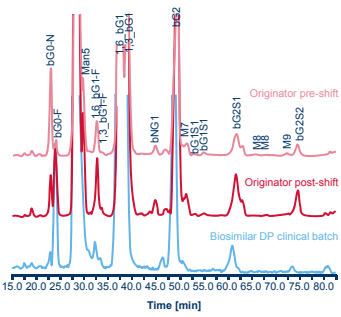
Structural Characterization of GP2013

Glycan Variants – Biosimilar Rituximab candidate

- ✓ Glycosylation heterogeneity
- NP-HPLC-FL(MS)







- Glycan pattern of biosimilar candidate is comparable to reference product

Visser J, et al. BioDrugs 2013;27(5):495–507

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Functional characterisation GP2013 Biosimilar Rituximab candidate

	Target binding	ADCC	CDC	Apoptosis	C1q
GP2013	99 - 119 %	85 - 108 %	92 - 111 %	88 - 103 %	94-114
Originator	96 – 110 %	70 – 132 %	95 – 127 %	88 – 112 %	101-108

- Using a comprehensive set of bioassays and binding assays, which covered rituximab's possible mechanisms of action, GP2013 could not be functionally distinguished from the reference product

	Affinity constants (K_D), µM	
Receptor	Rituximab	GP2013
FcRn	0.55–0.58	0.54–0.58
FcγRIa	10.4–11.8 nM	10.9–12.4 nM
FcγRIIa	2.4–2.7	2.4–2.7
FcγRIIb	11.4–12.8	11.0–12.7
FcγRIIIa F158	7.4–10.3	8.5–10.9
FcγRIIIa V158	3.5–4.9	4.2–5.0
FcγRIIIb	9.2–11.7	9.9–12.4

Visser J, et al. BioDrugs 2013;27(5):495–507

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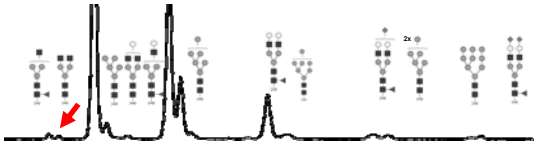
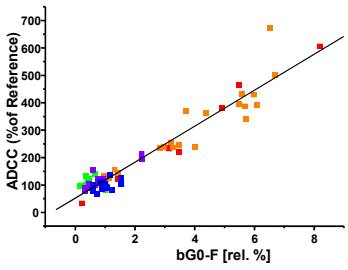
Elucidating structure function relationships

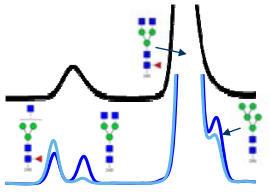
Characterization of mAb glycosylation heterogeneity

High resolution identification and quantification of major and minor glycan structures



ADCC activity and fucosylation





Column

HILIC Amide 80 5 µm

HILIC Amide 80 3 µm (1)

(1) Melmer et al., Anal.Bioanal.Chem., 398 (2010) 905-914

Data on File, Sandoz Inc.

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Which Quality Attributes Matter Clinically? Criticality Assessment

Quality attributes related to

- Active molecule
- Process materials
- Excipients

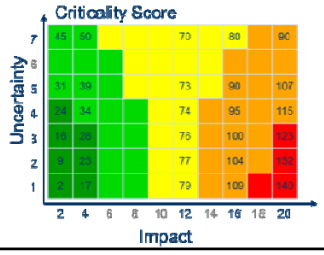
Assessment on clinical relevance

- Immunogenicity
- Safety/Toxicity
- Pharmacokinetics
- Efficacy

Existing product knowledge

- Literature
- In-house studies
- Related molecules

Criticality	Criticality score
Very high	121 - 140
High	86 – 120
Moderate	56 – 85
Low	31 – 55
Very low	2 – 30



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The clinical relevance of molecular attributes is well understood: efficacy example for an antibody

Attribute (examples)	Potential effect	Analytical methods (examples)
Amino acid sequence	Same as the reference product (AAS must be identical)	Orthogonal peptide maps with high resolution MS and MS/MS sequencing
Folding, disulphide bridges	Misfolding can decrease target binding/effector functions	CD spectroscopy, H-D-Exchange, FT-IR, X-ray, 1D and 2 D NMR, peptide mapping
C-terminal Lysine	Generally no effect	AEX, CZE
Deamidation, IsoAsp, oxidation	Reduces target binding in some cases	CEX, Papain-IEX, RP-HPLC, Papain-HIC, peptide map, MS
Glycation	Reduces target binding in some cases	Boronate affinity, LCMS, peptide map
Glycosylation: Fucosylation	Afucosylated variants lead to higher ADCC	2AB-NP-HPLC, ESI-MS, exoglycosidase digestion, MALDI TOF/TOF, CGE, peptide map
Glycosylation: Mannose X	Mannose X variants lead to higher ADCC	2AB-NP-HPLC, ESI-MS, exoglycosidase digestion, MALDI TOF/TOF, CGE, peptide map
Glycosylation: Sialylation	Higher sialylation leads to lower ADCC	NP-HPLC, WAX, HPAEC, RP-HPLC after DMB-labeling, mass spectrometry
Glycosylation: Galactose	Higher galactosylation reported to increase CDC in some cases	2AB-NP-HPLC, ESI-MS, exoglycosidase digestion, MALDI TOF/TOF, CGE, peptide map

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Safety/Toxicity: Biologics are highly specific

- **Pharmacological toxicity**
  - Caused by biological activities of molecule
  - Activities of biosimilar designed to match reference product
- **Off-target toxicity**
  - Biologics highly specific to their targets
  - Consequently, off-target toxicities are highly unlikely

This means: Comparable biological funactions/efficaccy result in comparable safety

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The clinical relevance of molecular attributes is well understood: immunogenicity example

Attribute (example)	Potential effect	Analytical methods (examples)
Amino acid sequence	Defines the product must be identical	Orthogonal peptide maps with high resolution MS and MS/MS sequencing
Aggregates	Certain types potentially immunogenic	SEC, FFF, MALLS, DLS, AUC, imaging methods, particle characterization
Folding, disulphide bridges	Misfolded variants potentially immunogenic	CD spectroscopy, H-D-Exchange, FT-IR, X-ray, 1D and 2 D NMR, peptide mapping
Degradation	Potentially immunogenic if not natural to body	RP-HPLC, CEX, Papain-HIC, Papain-IEX, peptide map, MS
Host cell proteins	Adjuvant effect or complex formation	ELISA, mass spectrometry
Leachables/extractables	Adjuvant effect or effect on folding/aggregation	HPLC with highly sensitive detectors, mass spectrometry
Glycosylation: Galactose-α1,3-Galactose	Anaphylaxis reported for cetuximab patients pre-sensitized by tick bites only	NP-HPLC of 2AB-labeled glycans coupled to ESI-MS, exoglycosidase digestion, MALDI TOF/TOF, CGE, peptide map
Glycosylation: N-glycolyl-neuraminic acid (NGNA)	Potentially immunogenic	NP-HPLC, WAX, HPAEC, RP-HPLC after DMB-labeling, mass spectrometry

Immunogenicity confirmation remains the main reason for clinical studies

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Different focus between originator and biosimilar development