Niklas Ekman, PhD, Finland

- Senior Researcher, Finnish Medicines Agency (Fimea), Finland

GaBI Educational Workshops

First ASEAN Educational Workshop on Regulation and Approval of **Biosimilars/Similar Biotherapeutic Products**

23 July 2017, Amari Watergate, Bangkok, Thailand

Principles and challenges related to manufacturing process development and demonstration of analytical comparability for biosimilars – the infliximab case as an example

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23 July 2017

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Principles and challenges related to manufacturing process development and demonstration of analytical comparability for biosimilars – the infliximab case as an example

First ASEAN Educational Workshop on Regulation and Approval of Similar Biotherapeutic Products/ Biosimilars

23 July 2017, Bangkok, Thailand

Niklas Ekman, Ph.D., Senior Researcher
Quality assessor for biological medicinal products
Member of the Biosimilar Working Party (BMWP), EMA

Finnish Medicine Agency (FIMEA), Helsinki, Finland

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Outline

1. EU Biosimilars - The concept of analytical similarity
2. Manufacturing process development
3. The pivotal evidence for analytical biosimilarity
4. Product experience – reflections from assessment of a marketing authorisation application

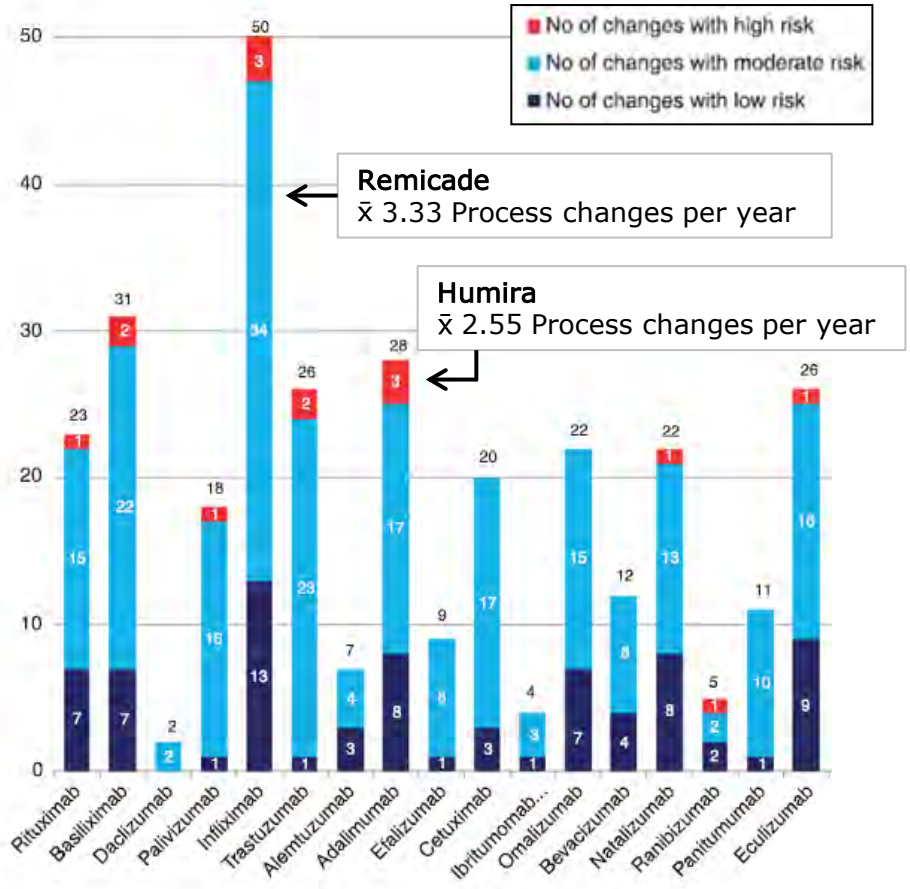
Source: Annie Spratt, https://unsplash.com

Disclaimer: The views expressed are those of the presenter and should not be understood or quoted as being made on behalf of the European Medicines Agency or its scientific Committees

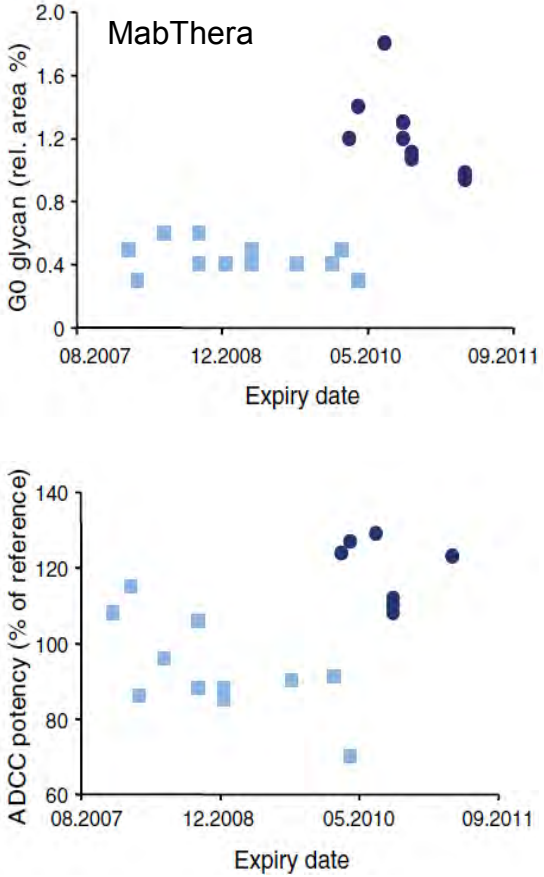
What makes biotech products special

- Biotech products are large and complex molecules
- 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



Modified from Vezér B et al. Curr Med Res Opin. 2016 May;32(5):829-34



Schiestl M. et al, Nat Biotech, April 2011

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*

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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”)

Assessment experience in the EU

July 2017

- 29 biosimilar medicinal products currently hold a valid marketing authorisation
  - 2 adalimumab, 2 enoxaparin, 5 epoetin (two different active substances), 2 etanercept (2 AS), 7 filgrastim (5 AS), 2 follitropin alfa (two AS), 3 infliximab (two AS), 2 insulin glargin (2 AS), rituximab, somatropin, 2 teriparatide (2 AS)
- 7 awaiting EC decision
  - Adalimumab, *insulin lispro*, 5 rituximab (2 AS)
- 14 biosimilar MA applications under review
  - 3 adalimumab, *2 bevacizumab*, infliximab, insulin glargine, *3 pegfilgrastim, 4 trastuzumab*

Over the last 10 years, the EU monitoring system for safety concerns has not identified any relevant difference in the nature, severity or frequency of adverse effects between biosimilar medicines and their reference medicines

1. EU Biosimilars - The concept of analytical similarity
2. **Manufacturing process development**
3. The pivotal evidence for analytical biosimilarity
4. Product experience – reflections from a recent marketing authorisation application

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; publicly available information and data obtained from extensive characterisation studies
- 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

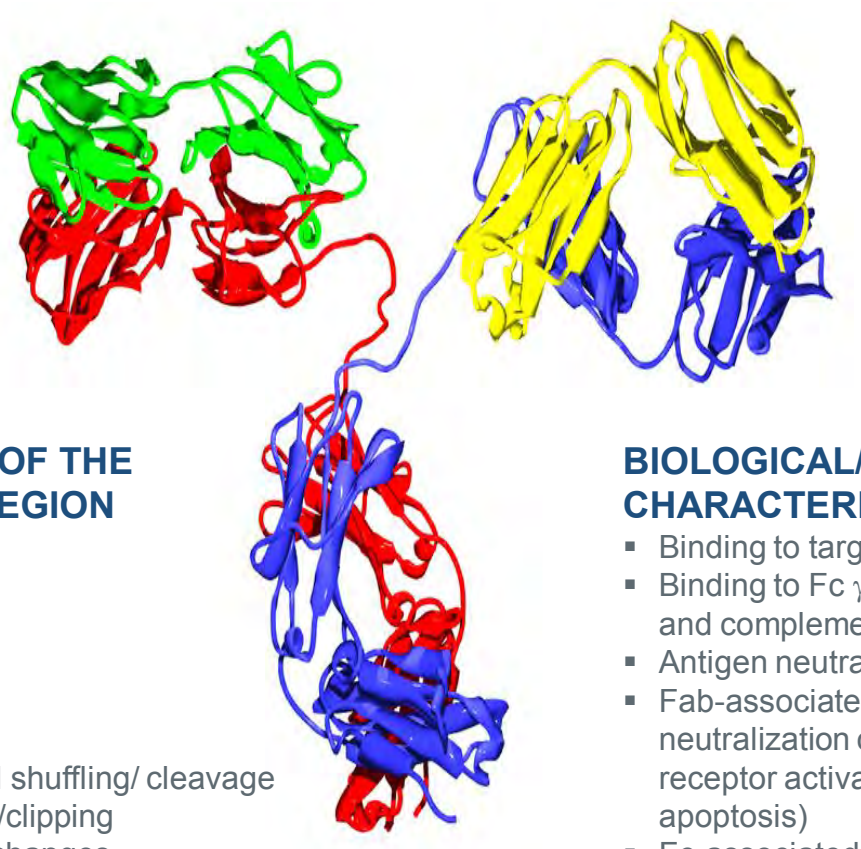
- Expression system development
  - Needs to be carefully considered taking into account expression system differences that may result in undesired consequences; atypical glycosylation, higher variability or a different impurity profile
- Upstream process development
  - To match product attributes; 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

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

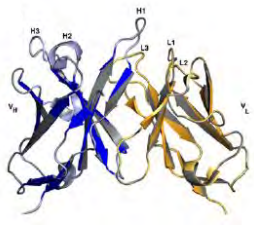
- Deamidation
- Oxidation
- Acetylation
- Glycation
- Glycosylation
- C-term Lys
- Di-sulfide bond shuffling/ cleavage
- Fragmentation/clipping
- Conformation changes

Figure from Wikipedia

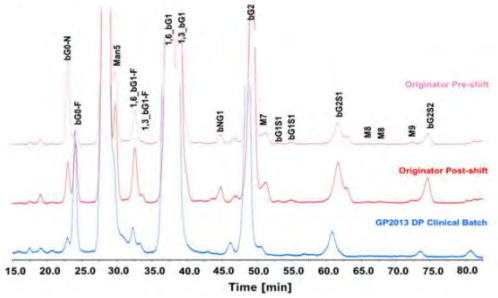
BIOLOGICAL/ FUNCTIONAL CHARACTERISTICS

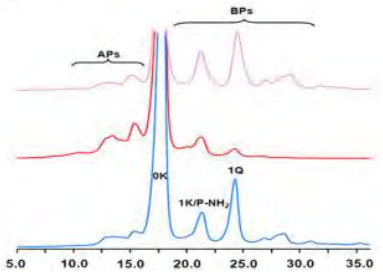
- Binding to target antigen(s)
- Binding to Fc γ receptors, FcRn and complement
- Antigen neutralisation (if relevant)
- Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation, induction of apoptosis)
- Fc-associated functions (ADCC and CDC)

Some analytical tools commonly used for mAb 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



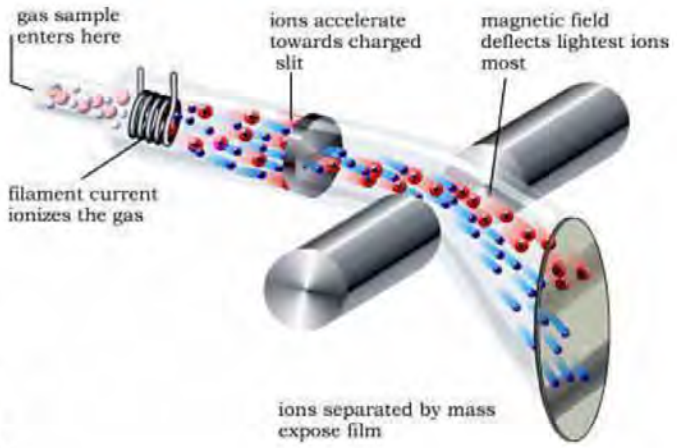


Figures from Visser J. et al. BioDrugs. 2013 Oct;27(5):495-507

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

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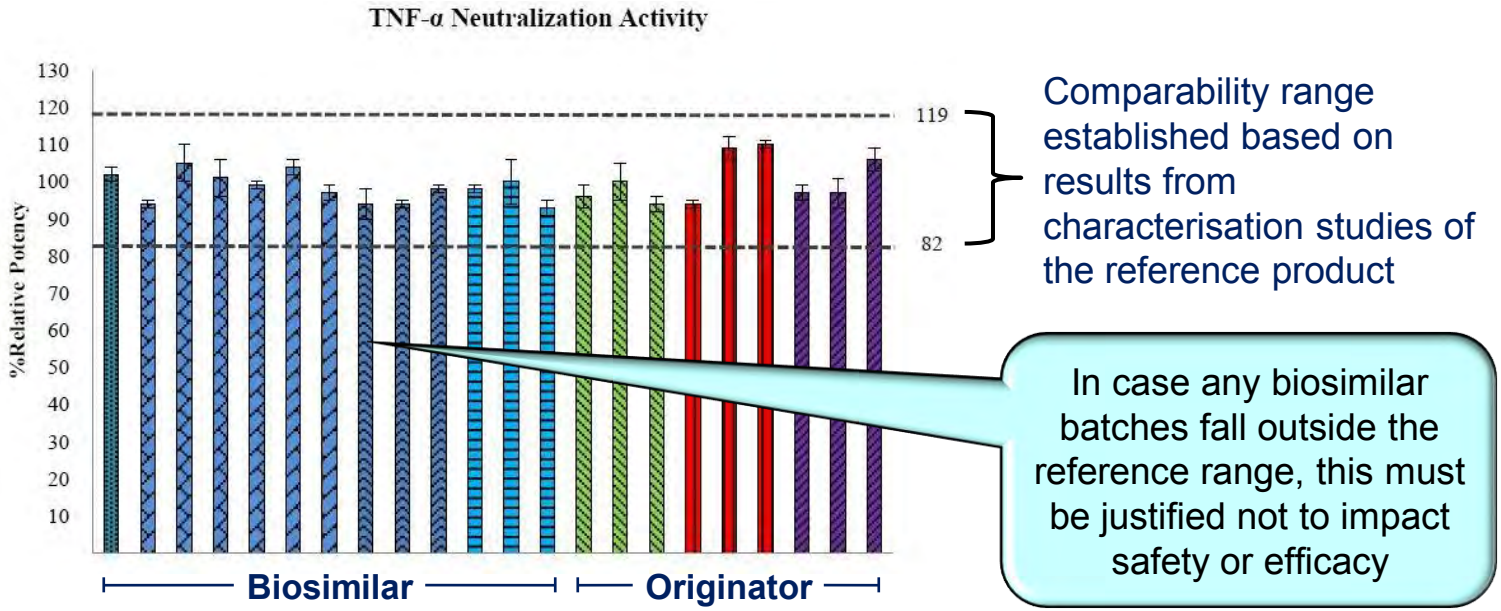
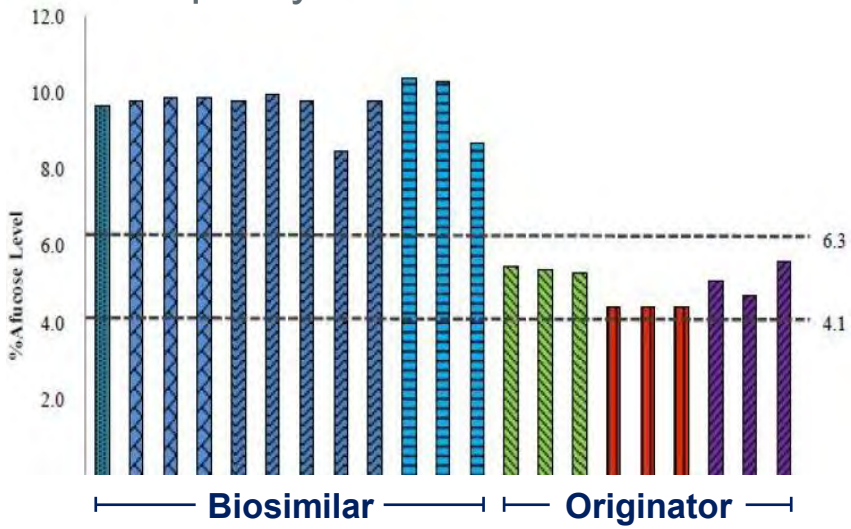
The ”pivotal” evidence for analytical similarity

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

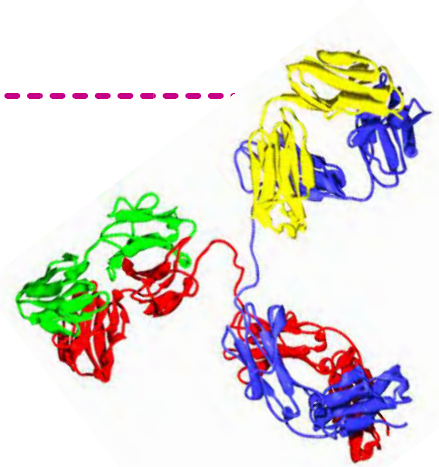


What to do when the biosimilar falls outside the comparability range?

- 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



Assessment experience from addressing the impact of glycosylation differences observed for a biosimilar monoclonal antibody

Marketing Authorisation Application for Remsima/ Inflectra[1]

[1] European public assessment report (EPAR) available at www.ema.europa.eu

Analytical Methods in Structural and Physicochemical Biosimilarity Studies

Primary Structure

- Peptide Mapping (HPLC)
- Peptide Mapping (LC-MS)
  - Deamidation – HC Asn57, HC Asn318, HC Asn364, HC Asn387, LC Asn41
  - Oxidation – HC Met255
  - C-terminal variant – HC Lys450
- Intact Mass (LC-MS)
  - Light chain
  - Heavy chain K0 – G0, G0F, G1F, G2F
  - Heavy chain K1 – G0F, G1F, G2F
- Amino Acid Analysis/Molar Absorptivity
  - Aspartic acid, Glutamic acid, Serine, Histidine, Glycine, Threonine, Arginine, Alanine, Tyrosine, Valine, Methionine, Phenylalanine, Isoleucine, Leucine, Lysine, Proline, Molar Absorptivity, Extinction Coefficient
- N-terminal Sequencing
  - Heavy chain
  - Light chain
- C-terminal Sequencing
  - Heavy chain
  - Light chain

Higher Order Structure

- FTIR
  - Amide I
  - Amide II
  - A
  - B
  - C
- DSC
  - Transition 1
  - Transition 2
  - Transition 3
- CD
- Free Thiol Analysis
- Disulfide Bond
  - H3-H12: 22-98
  - H15-H16: 147-203
  - H20-L19: 223-214
  - H21-H21: 229-229/232-232
  - H23-H29: 264-324
  - H37-H42: 370-428
  - L2-L7: 23-88
  - L10-L17: 134-194
- Antibody Array

Content

- Protein Concentration (UV_{280})

Glycosylation

- HPAEC-PAD
  - G0F, Man5, G0, G1F, G2F, SA1, SA2
- NP-UPLC
  - G0F-GN, G0, G0F, MAN5, G1F-GN, G1, G1F, G1F', G2, G2F, G1-GN+NGNA, G1F-GN+NGNA, G1F+NGNA, G1F'+NGNA, G2+NGNA, G2F+NGNA, G2F+2NGNA, Unknown species
- N-linked Glycan Analysis
  - Man5, G0F-GlcNAc. G0, G0F, G1F, G2F, G1F1NeuGc, G2F1NeuGc
- Sialic Acid Analysis
- Monosaccharide Analysis
  - Fuc, GlcNAc, Gal, Man
- Glycation (LC-ES-MS)
  - Light chain
  - Heavy chain

Purity/Impurity

- SEC-HPLC
  - Monomer
  - Dimer
- SEC-MALS
  - Monomer
  - Dimer
  - Monomer (MW)
  - Dimer (MW)
- AUC
  - Monomer
  - Higher species
- Non-reduced/Reduced CE-SDS
  - Intact IgG (NR)
  - H+L (R)
  - Non-glycosylated HC (R)
- Sub-visible particles (MFI & HIAC)

Charge Variants

- IEF
- IEC-HPLC
  - Peak 1, Peak 2, Peak 3, Peak 4, Peak 5, Peak 6

Excipients

- pH
- Polysorbate 80
- Sucrose

Slide presented at FDA Arthritis Advisory Committee Meeting for CT-P13 Feb 09, 2016; http://www.fda.gov/AdvisoryCommittees/Calendar/ucm481969.htm

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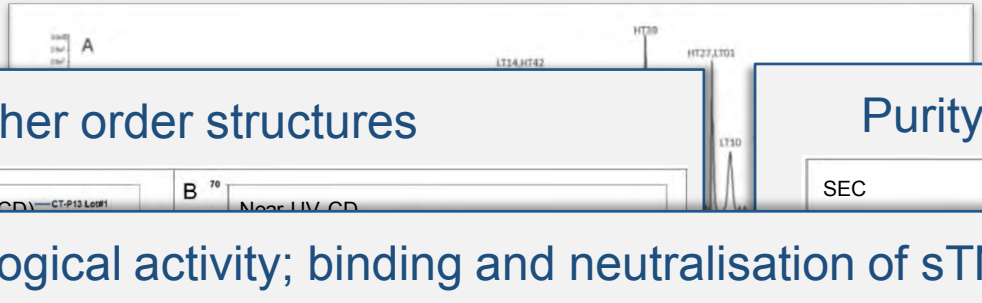
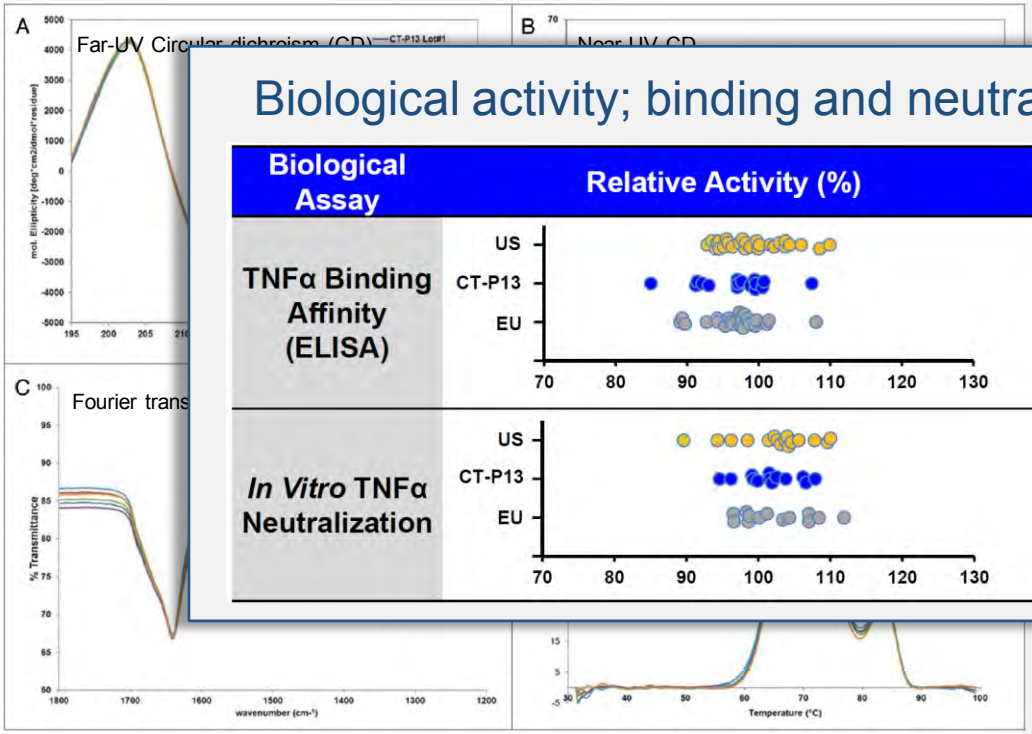
Primary structure; LC-MS peptide mapping



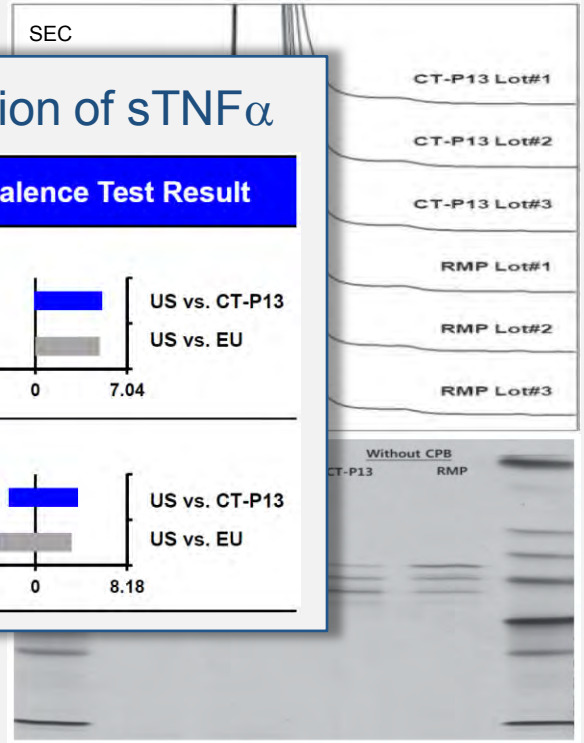
Higher order structures



Biological activity; binding and neutralisation of sTNFα

Biological Assay	Relative Activity (%)	Equivalence Test Result
TNFα Binding Affinity (ELISA)	US, CT-P13, EU; 70 80 90 100 110 120 130	US vs. CT-P13, US vs. EU; -7.04 0 7.04
In Vitro TNFα Neutralization	US, CT-P13, EU; 70 80 90 100 110 120 130	US vs. CT-P13, US vs. EU; -8.18 0 8.18

Purity/impurity profile



Pictures from Jung et al., mAb 6:5, 1163-1177, 2014 and FDA Arthritis Advisory Committee Meeting for CT-P13, Feb 09, 2016; http://www.fda.gov/AdvisoryCommittees/Calendar/ucm481969.htm

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Summary of the results from the analytical similarity assessment

- High similarity between the biosimilar and the reference demonstrated for
  - Primary, secondary and tertiary structures
  - *In vitro* TNFα neutralisation, binding affinity (soluble and transmembrane TNFα, TNFβ, FcγRIa, FcγRIIa, FcRn, C1q), in vitro functional tests (apoptosis, CDC, ADCC using PBMNC effector cells from healthy volunteers)
- Minor differences reported for
  - C-terminal lysine content, aggregates, intact IgG level, charged molecular variants, glycosylation pattern
  - Binding to FcγRIIIa

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

Glycan species	Safety/ immunogenicity	Biologic activity/ efficacy	Clearance (PK/PD)
Galactose	Unknown		Unknown
α1,3-galactose	−−	Unknown	Unknown
Fucose	(−)	++	
Bisecting GlcNac	(−)	+	Unknown
High mannose	Unknown	+	−−
NANA	Unknown	(−)	+
NGNA	−−	(−)	+
β1,2-Xylose/ α1,3-Fucose	−−	Unknown	Unknown
NGHC	Unknown	−	(−)

+ Positive impact; − negati… …pact; −− high negative impact; (+/−) potentia…

Gal(α1-3)Gal is a non-human glycan structure produced by e.g. many rodent cell lines. Immunogenic in human

Afucosylated structures show increased binding to FcγRIII leading to increased ADCC activity

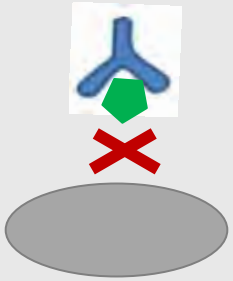
Mannose structures bind to mannose receptors which results in increased protein clearance

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

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

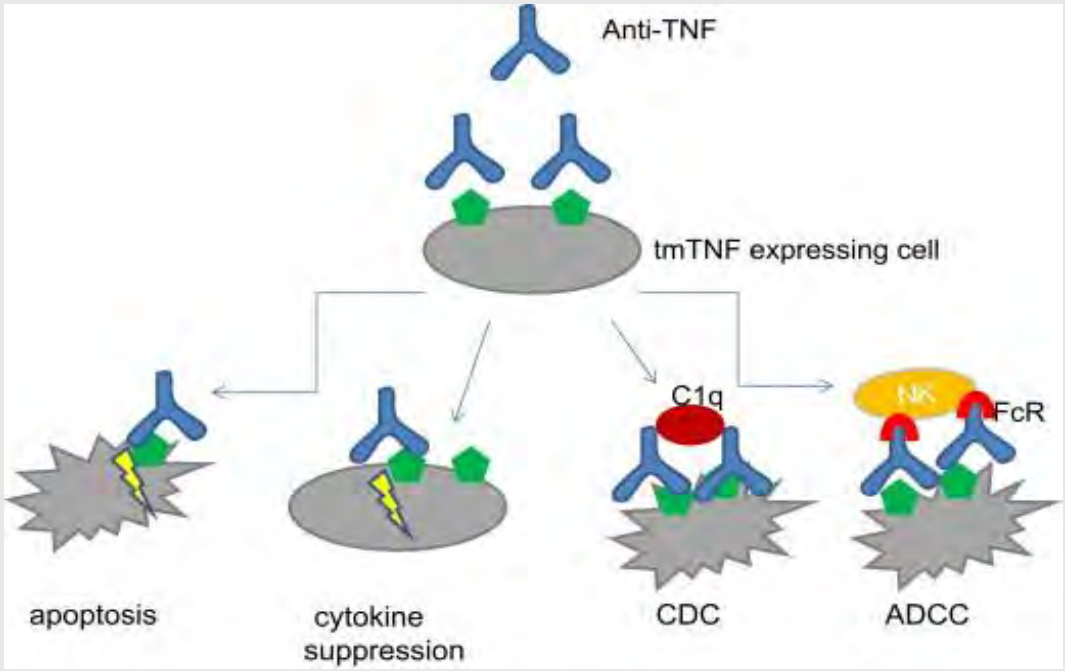
Potential mechanisms of action for anti-TNFs

Primary MoA



Neutralisation of soluble TNFα

Additional potential MoAs, especially in inflammatory bowel diseases (IBD)



Modified from Thalayasingam N. et al., Best Pract Res Clin Rheumatol. 2011 Aug;25(4):549-67

Can efficacy and safety data be extrapolated to all Remsima/Inflectra indications applied for?

- Remsima was shown to contain a lower level of afucosylated glycans than Remicade, resulting in a lower binding affinity to FcγRIIIa and FcγRIIIb

➢ As Fc-mediated functions could potentially be involved in the mode of action of infliximab, the Company was asked to provide further experimental data, in combination with an overall discussion, confirming that the differences detected do not affect efficacy or safety in any of the applied indications

- Directive 2001/83, part II, Annex 1

> *In case the originally authorised medicinal product has more than one indication, the efficacy and safety of the medicinal product claimed to be similar has to be justified or, if necessary, demonstrated separately for each of the claimed indications*

Test Method		Key Findings
F(ab')2 related		
Comparative apoptosis of Remsima and Remicade		The apoptotic effects by reverse signalling through tmhTNFα for Remsima and Remicade were comparable. No statistically significant differences were detected at any time point.
Comparative Reverse signalling		Blockade of pro-inflammatory cytokine production by reverse signalling through tmhTNFα for Remsima and Remicade were comparable, using PBMC from either healthy donors or CD patients.
Effect of blocking soluble TNFα in *in vitro* IBD model	Suppression of cytokine secretion in epithelial cell line by blocking soluble TNFα	Suppression of pro-inflammatory cytokine (IL-6 and IL-8) secretion from co-stimulated epithelial cell line was shown to be comparable and dose dependent for Remsima and Remicade; no statistical difference in pro-inflammatory cytokines suppression was found.
	Suppression of apoptosis in epithelial cell line cells by blocking soluble TNFα	Suppression of epithelial cell line apoptosis was shown to be comparable for Remsima and Remicade.
Fc-F(ab')2 related		
Comparative complement-dependent cytotoxicity (CDC) of Remsima and Remicade		CDC effects of Remsima and Remicade against tmhTNFα-Jurkat cells by lysis were comparable. No statistically significant differences were detected in relative CDC activity.
Comparative antibody-dependent cell-mediated cytotoxicity (ADCC) of Remsima and Remicade using tmhTNFα-Jurkat cells as target cells and human PBMC as effector cells		Remsima and Remicade had comparable ADCC activity and no statistically significant differences were detected.
Comparative ADCC of Remsima and Remicade using tmhTNFα-Jurkat cells as target cells and NK cells from healthy donor as effector cells		Comparable ADCC for Remsima and Remicade when NK cells from a healthy donor (genotype V/F) were used as effector cells.
Comparison of ADCC activity between Remsima and Remicade using transfected Jurkat cells as target cells and either PBMCs or NK cells from CD patients as effector cells		No differences in ADCC activity were detected using PBMC from CD patients (V/F or F/F genotype). Differences in ADCC with Remsima and Remicade were seen when NK cells from CD patients were used as effector cells. Effect was FcγRIIIa genotype specific; differences were observed with V/V and V/F, but not F/F genotypes.
Comparison of ADCC effect between Remsima and Remicade using transfected Jurkat cells as target cells and whole blood from healthy donor or CD patients as effector cells		No differences in ADCC were seen between various batches of Remsima and Remicade.
Comparison of ADCC between Remsima and Remicade using LPS-stimulated monocytes from healthy donor or CD patient as target cells and PBMC as effector cells		No ADCC activity was seen with Remsima and Remicade when PBMCs from a healthy donor (V/F) or a CD patient (V/F) were used as effector cells and LPS-stimulated monocytes were used as target cells.
Evaluation of Regulatory Macrophage Function	Suppression of T cell proliferation by induced regulatory macrophages in mixed lymphocyte reaction (MLR) assay	Inhibition of T cell proliferation of PBMCs from healthy donors and CD patients was shown to be comparable and dose dependent for Remsima and Remicade.
	Quantitation of the induced regulatory macrophages by FACS analysis	Induction of regulatory macrophages in a 2-way allogenenic MLR using FcγRIIIa genotype matched PBMCs, from either healthy donors or CD patients, was shown to be comparable for Remsima and Remicade.
	Induced regulatory macrophage-mediated wound healing of colorectal epithelium cells	Promotion of *in vitro* wound healing of colorectal epithelial cells by regulatory macrophages from healthy donors and CD patients (induced by Remsima or Remicade) in the MLR assay was comparable.

Only for illustration, complete list available in the EPAR

- No difference in reverse signaling through tmTNFα
  - Induction of apoptosis
  - Blockade of pro-inflammatory cytokine production
- No difference in blocking soluble hTNFα in an *in vitro* IBD model
  - Suppression of pro-inflammatory cytokine (IL-6 and IL-8) secretion from co-stimulated epithelial cell line
  - Suppression of epithelial cell line apoptosis
- No difference in Complement-dependent cytotoxicity (CDC) activation

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Clinical impact of the difference in FcγRIIIa binding?

- No difference in Regulatory Macrophage function (regMø)
  - Quantity of induced regulatory macrophages, suppression of T cell proliferation, *in vitro* wound healing
- No difference in Antibody-dependent cell-mediated cytotoxicity (ADCC) using
  - tmhTNFα-Jurkat cells as target cells and PBMCs (from healthy donors or CD patients), NK cells (from healthy donors) whole blood (from healthy donors) as effector cells
  - LPS-stimulated monocytes (from healthy donors or CD patients) as target cells and PBMC as effector cells

❖ Difference in ADCC functional assay detected using

- tmhTNFα-Jurkat cells as target cells and NK cells from CD patient donors (158V/V or 158V/F genotypes, but not 158F/F) as effector cells

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Conclusion from the Remsima assessment

The CHMP concluded that the differences detected were not clinically meaningful;

- Functional difference was seen only in an ADCC assay employing artificially high tmTNFα expressing Jurkat target cells in combination with highly purified NK effector cells
- No differences in experimental models regarded as more relevant to the pathophysiological conditions in CD patients
- No published reports describing the induction of ADCC by TNF antagonists in CD patients
- No firm evidence that the FcγRIIIa polymorphism has an impact on the clinical course of CD

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Biosimilars – prerequisites for extrapolation

- Similar physicochemical, structural characteristics and biological functions in *in vitro* models
- Similar human pharmacokinetics (exposure)
- Similar pharmacodynamics, efficacy, safety, and immunogenicity usually at least in one therapeutic indication
- Sound scientific justification
  - Clinical experience and available literature data
  - Mechanism of action of the active substance in each indications
  - Evidence that the lead indication is representative for the other therapeutic indications, both with regard to safety and efficacy

➢Thorough physicochemical and biological characterisation is a prerequisite in biosimilar develppment and a foremost important enabler for succesful extrapolation of similarity data from one indication to another

Thank you for your attention!

EMA Website
http://www.ema.europa.eu/ema/

Biosimilar guidelines
http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000408.jsp&mid=WC0b01ac058002958c

Remsima EPAR (European public assessment report)
http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002576/human_med_001682.jsp&mid=WC0b01ac058001d124

Niklas Ekman, Ph.D.

Senior Researcher, Quality/CMC Assessor

Finnish Medicine Agency (FIMEA), Helsinki, Finland

niklas.ekman@fimea.fi