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Principles and challenges related to manufacturing process development and demonstration of analytical comparability for biosimilars

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Lääkealan turvallisuus- ja kehittämiskeskus | Säkerhets- och utvecklingscentret för läkemedelsområdet | Finnish Medicines Agency

Principles and challenges related to manufacturing process development and demonstration of analytical comparability for biosimilars

2nd MENA Stakeholder Meeting on Regulatory Approval, Clinical Settings, Interchangeability and Pharmacovigilance of Biosimilars

10 October 2018, Dubai, United Arab Emirates

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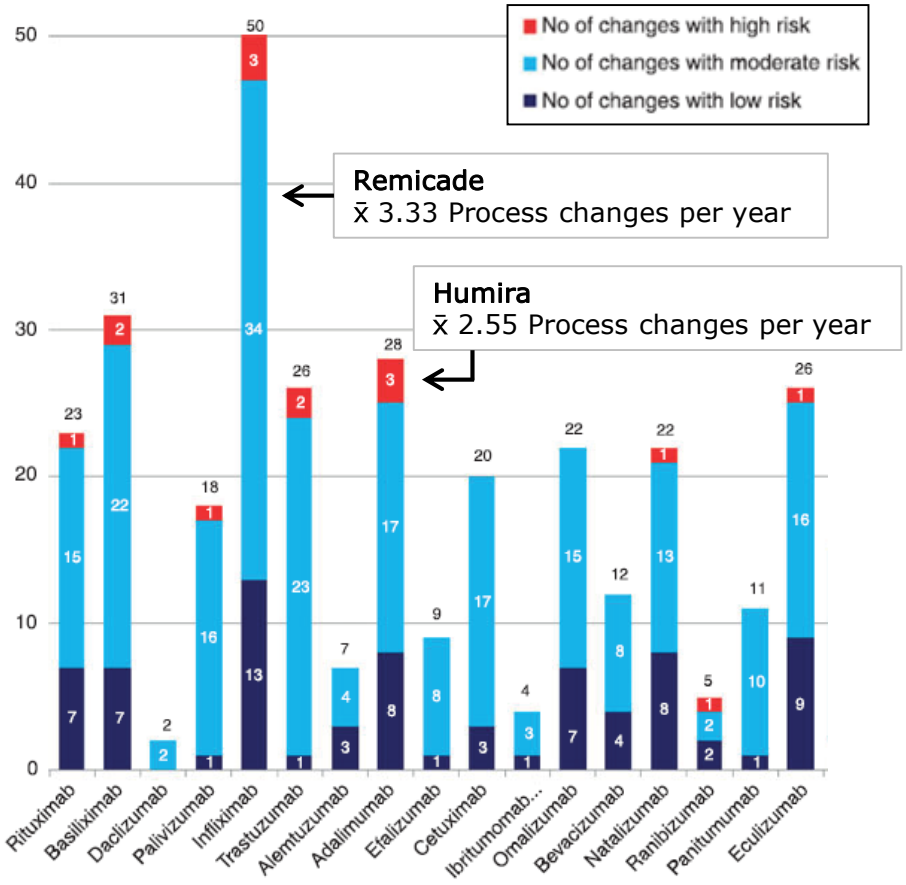
Outline

1. EU Biosimilars - The concept of analytical similarity
2. From manufacturing process development to pivotal demonstration of analytical biosimilarity
3. Product experience – reflections from two recent marketing authorisation application assessments

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

Manufacturing process changes are common for all biologics



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

- Comparability between **pre- and post-change versions** need to be demonstrated (ICH Q5E)
- Manufacturers and regulators are **used to assess the impact of process changes** – also in the case of complex biologics

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The 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*

1. EU Biosimilars - the concept of analytical similarity
2. **From manufacturing process development to pivotal demonstration of analytical biosimilarity**
3. Product experience – reflections from two recent marketing authorisation application assessments

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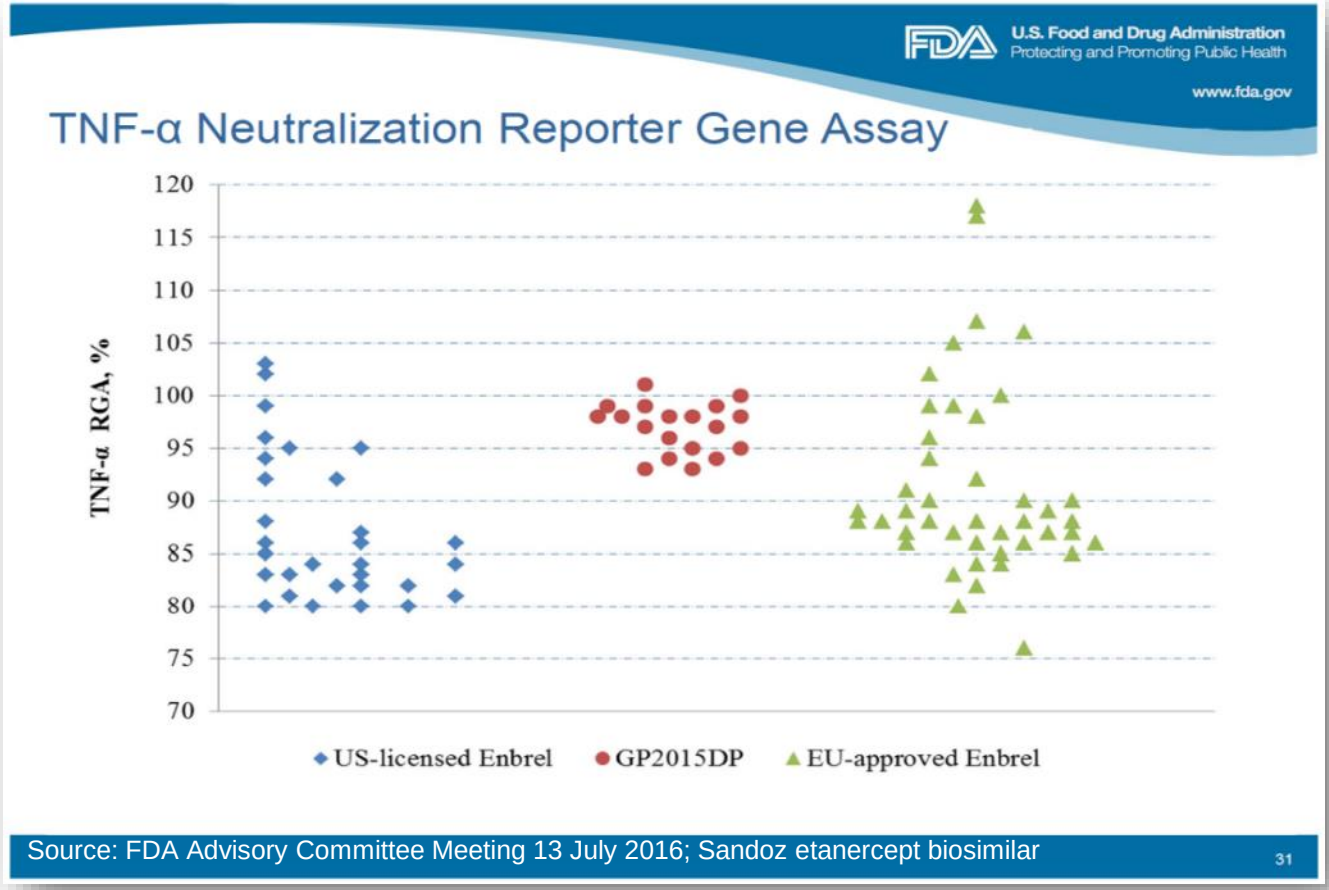
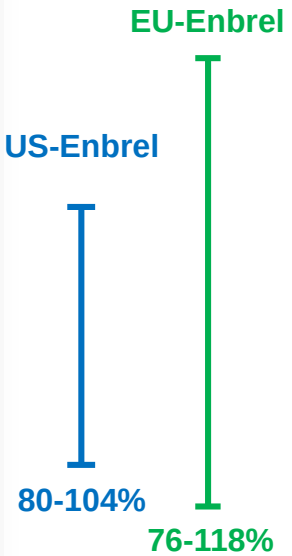
Biosimilar vs Reference Medicinal Product - How close is close enough?

- **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”)

Batch to batch variability of biologics





Measured potency ranges

Manufacturing process development - Quality Target Product Profile (QTPP)

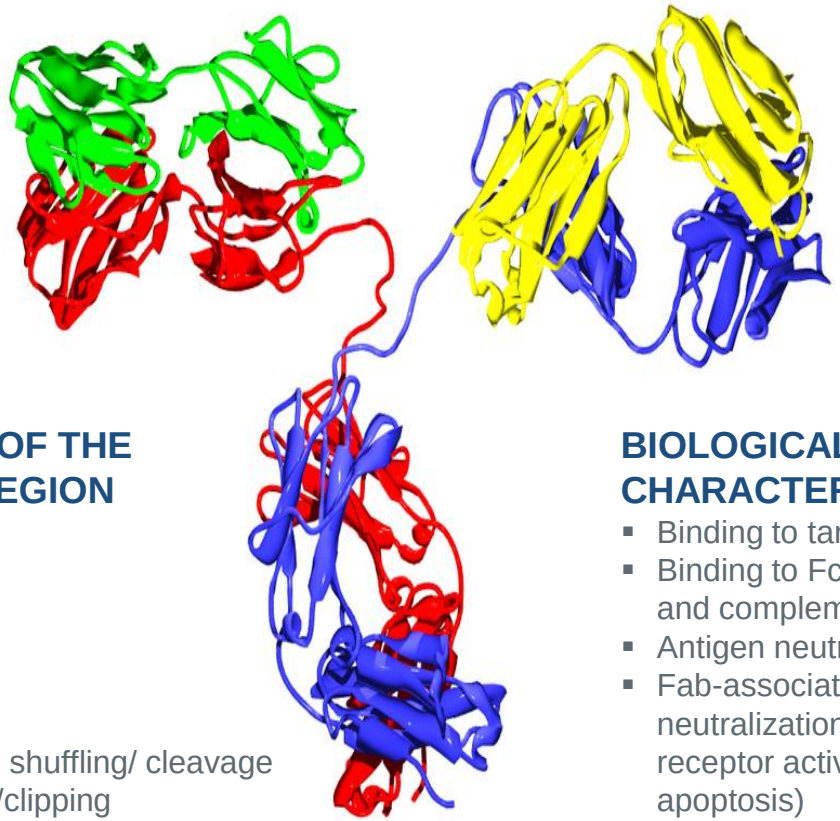
- A **prospective summary** of the quality characteristics of a drug product that ideally will be achieved
- **Based on data collected on the reference medicinal product**; publicly available information and data obtained from extensive characterisation studies
- The **importance of the quality attributes/ 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

Picture: Freepik.com

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

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

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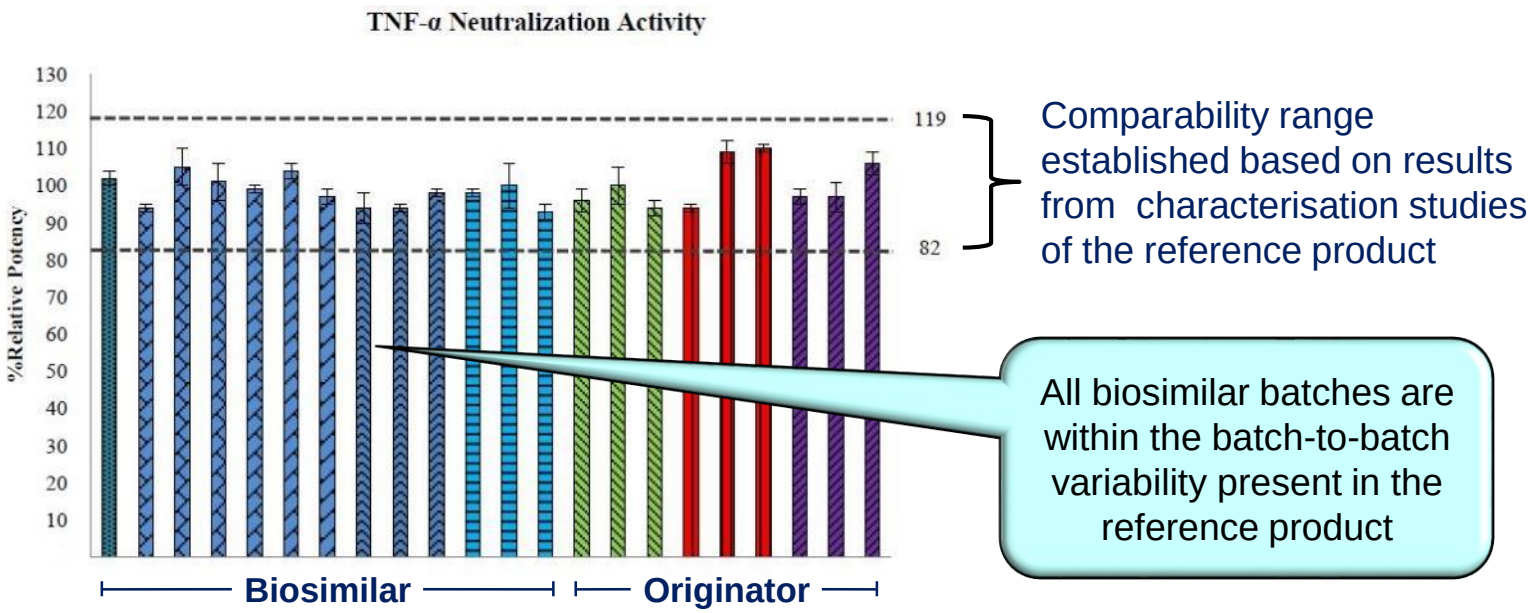
Reverse Engineering Approach

- **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

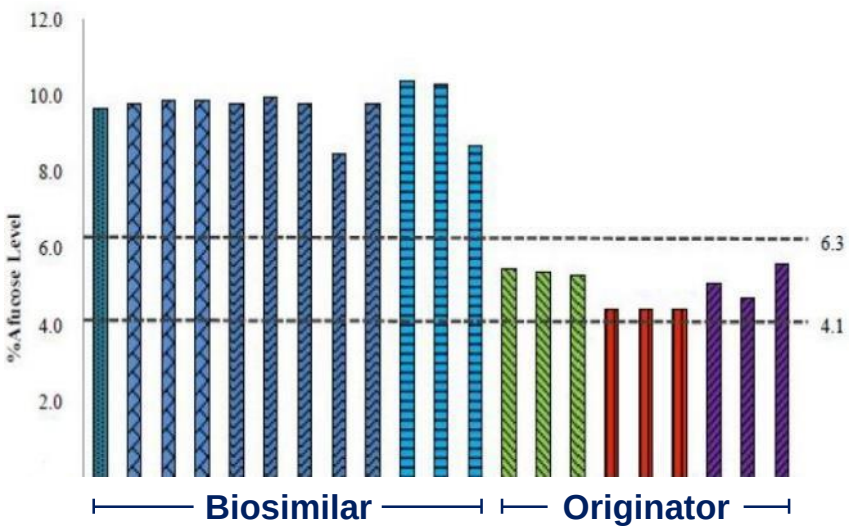
The ”pivotal” evidence for analytical similarity

- Biosimilarity **should be demonstrated** in an extensive, side-by-side (whenever feasible) comparability exercise
- **Quantitative comparability ranges** 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**



- 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

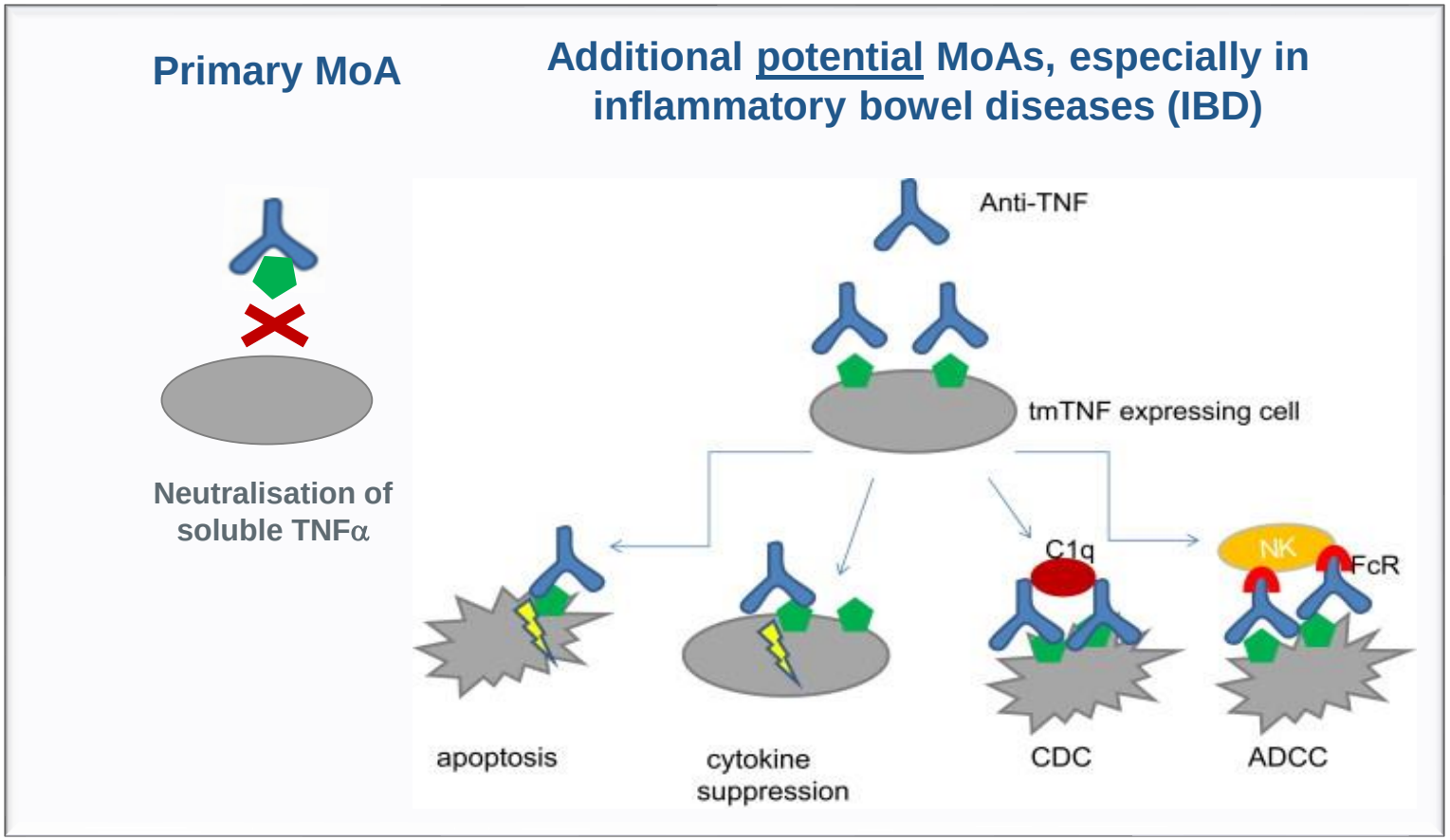
1. EU Biosimilars - The concept of analytical similarity
2. From manufacturing process development to pivotal demonstration of analytical biosimilarity
3. **Product experience – reflections from two recent marketing authorisation application assessments**

Case 1: Addressing the impact of glycosylation differences observed for a biosimilar monoclonal antibody

Marketing Authorisation Application for Remsima/Inflectra infliximab[1]

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

Potential mechanisms of action for anti-TNFs



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

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Tabular overview of clinical studies

Protocol	Design	Objectives	Treatment	Study population
CT-P13 1.2 (pilot study)	Prospective Phase 1, randomised double-blind, parallel-group, multiple single-dose intravenous (i.v.) infusion, multicentre	Primary: To determine C_{max}, PK profiles of CT-P13 and Remicade at Weeks 0, 2 and 6 Secondary: PK profile, PD, efficacy, and safety of CT-P13 in comparison to Remicade up to Week 102.	CT-P13 plus MTX or Remicade plus MTX	RA patients with active disease while receiving MTX Planned: 20 Randomised: 19 CT-P13: 9 Remicade: 10
CT-P13 1.1 PK equivalence (Study name: PLANET AS)	Prospective Phase 1, randomised, double-blind, multicentre, multiple single-dose i.v. infusion, parallel-group	Primary: To demonstrate comparable PK at steady state in terms AUC_{τ}, $C_{max,ss}$ between CT-P13 and Remicade determined between Weeks 22 and 30. Secondary: long-term efficacy, PK and overall safety up to Week 54	CT-P13 or Remicade	AS patients with active disease Planned: 246 (ratio: 1:1) Randomised: 250 CT-P13: 125 Remicade: 125
CT-P13 3.1 Therapeutic equivalence (Study name: PLANET RA)	Prospective Phase 3, randomised, double-blind, multicentre, multiple single-dose i.v. infusion, parallel-group	Primary: To demonstrate that CT-P13 is equivalent to Remicade, in terms of efficacy as determined by clinical response according to ACR20 at Week 30. Secondary: long-term efficacy, PK, PD, and overall safety up to Week 54	CT-P13 plus MTX or Remicade plus MTX	RA patients with active disease while receiving MTX Planned: 584 (ratio: 1:1) Randomised: 606 CT-P13: 302 Remicade: 304

ACR20=20% improvement according to the ACR criteria; AS=Ankylosing spondylitis; AUC_{τ}=Area under the concentration-time curve over the dosing interval; Cmax=maximum serum concentration; i.v.=intravenous; MTX=methotrexate; PK=Pharmacokinetics; PD=Pharmacodynamics; RA=Rheumatoid arthritis

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 clearance

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

+ Positive impact; − negati[illegible] [illegible]act; −− high negative impact; (+/−) potentia[illegible]

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

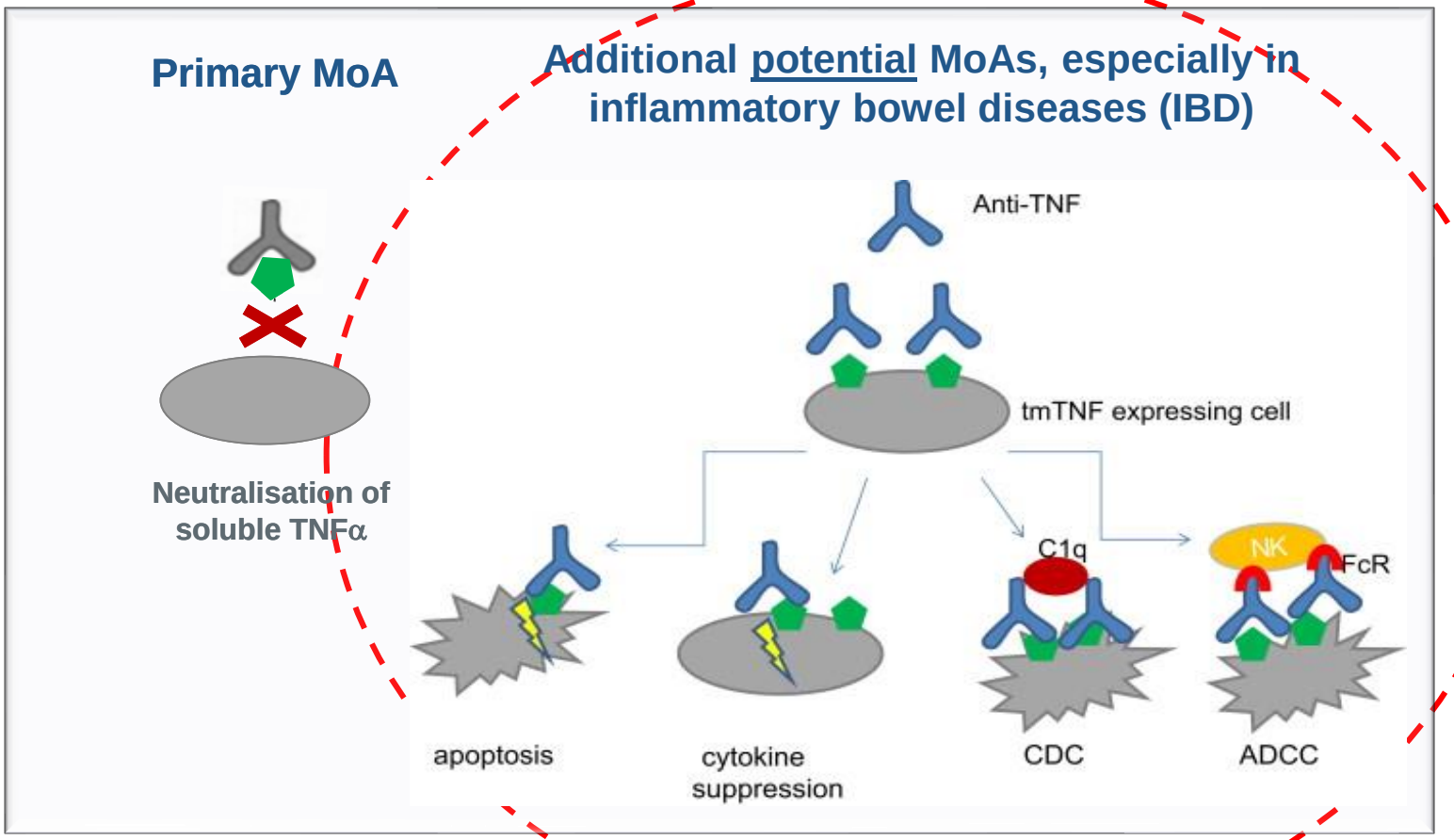
Afucosylated structures show increased binding to FcγRIII leading to increased ADCC activity

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



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

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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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Based on the totality of evidence, 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

Case 2: Challenges associated with a shift observed for a critical quality attributes of the reference product

Marketing Authorisation Application for Ontruzant trastuzumab[1]

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

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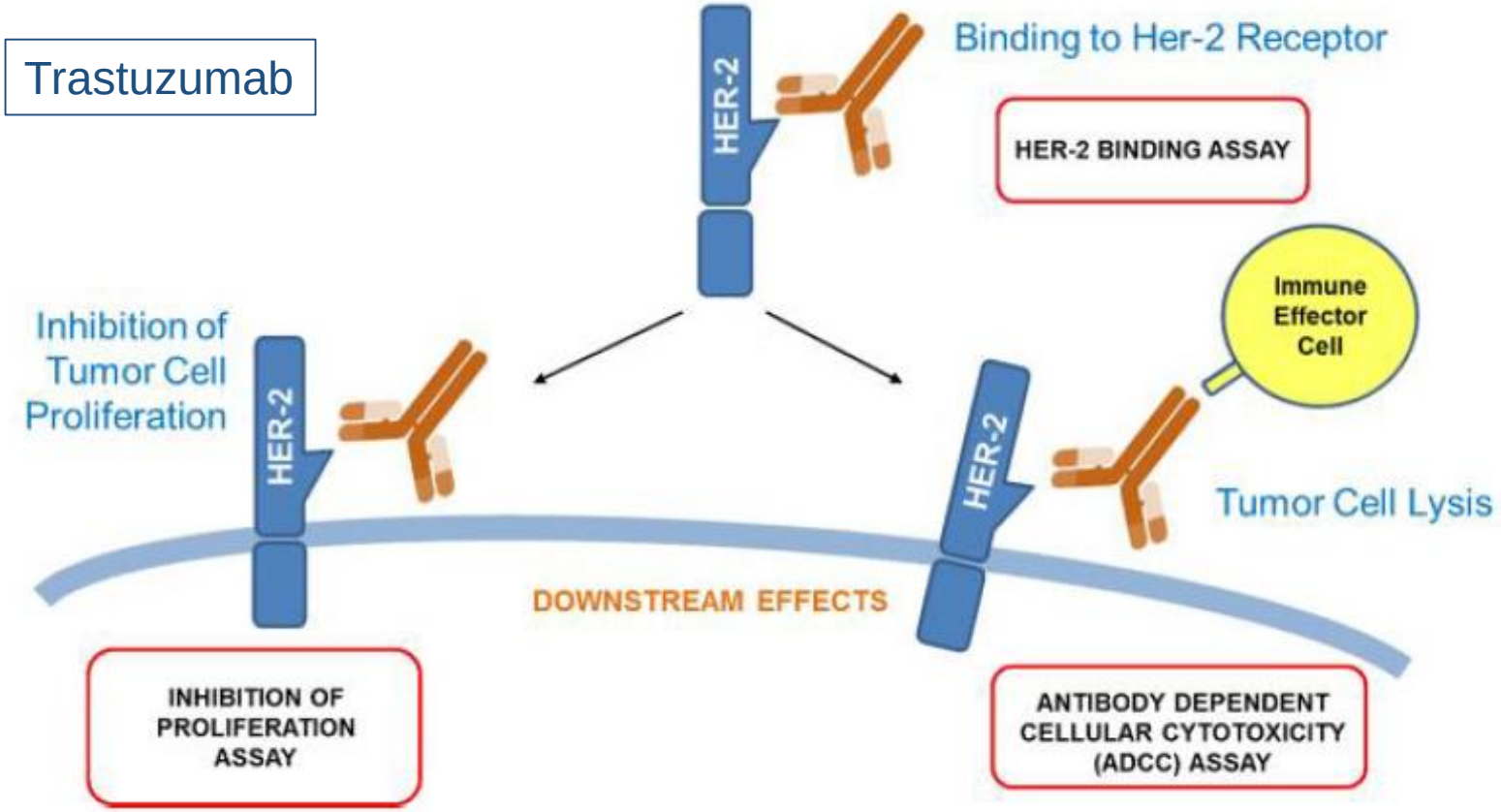
Clinically Relevant Functional Assays

Trastuzumab



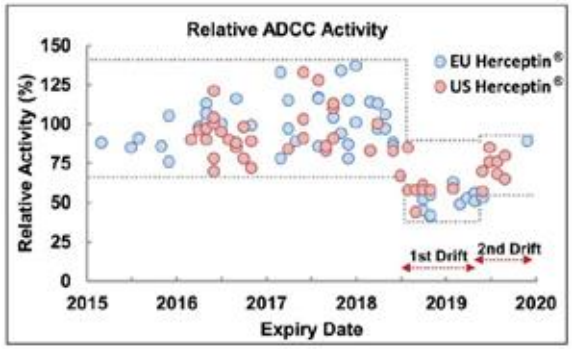
Slide presented at the FDA Oncologic Drugs Advisory Committee meeting on trastuzumab MYL-14010 , July 13, 2017

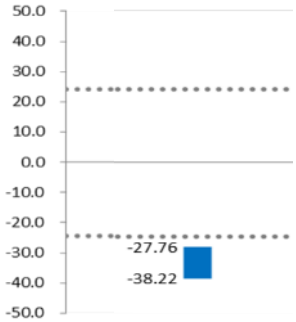
Ontruzant; analytical and functional similarity assessment

- The results from physicochemical and biological characterisation studies (including HER2 binding, anti-proliferation, ADCC, C1q and Fc receptor binding) were similar between Ontruzant and EU Herceptin

➢ **For the marketing authorisation application, analytical and functional similarity was adequately demonstrated**

However, in extended characterisation studies comparability **within the originator** (pre/post shift) could not be demonstrated using equivalence testing of means





From Hyungki Park presentation at the Workshop on the Use of Statistical Methodologies in the Comparability Assessment of Quality Attributes held at EMA, London on May 3, 2018

Kim et al., Mabs 2017, Vol. 9, No. 4, 704–714

Ontruzant; pharmacokinetic similarity assessment

Table 4: Statistical comparison of primary PK parameters between SB3 and EU sourced Herceptin (PK population)

PK Parameter	Treatment	N	n	Geo-LSMean	Ratio A/B	90% CI of Ratio
AUC_{inf} (µg·h/mL)	SB3	36	36	34331.4	0.969	0.908;1.034
	EU sourced Herceptin®	36	36	35426.8		
AUC_{last} (µg·h/mL)	SB3	36	36	33902.9	0.971	0.911;1.034
	EU sourced Herceptin®	36	36	34932.8		
C_{max} (µg/mL)	SB3	36	36	151.747	1.001	0.935;1.072
	EU sourced Herceptin®	36	36	151.520		

A: SB3, B: EU sourced Herceptin®.
LSMean = least squares mean; CI = confidence interval; N = number of subjects in PK population; n = number of subjects who contributed to analysis.

Randomised, double-blind, three-arm (Ontruzant, EU- and US-Herceptin), parallel group, single-dose PK study, in healthy volunteers, N=36 per arm

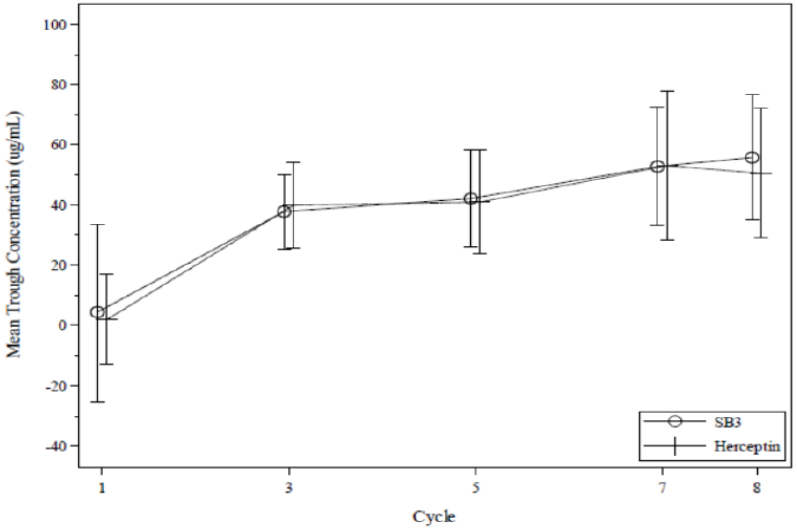


Figure 7: Mean Serum Trough (Pre-dose) Concentration-time Profiles from Cycle 1 to Cycle 8

Phase III study in patients with Her2+ advanced breast cancer

➢ **Similarity in PK profiles demonstrated**

Ontruzant; efficacy/safety similarity assessment

Table 23: Primary analysis of difference in bpCR Rate, **SB3-G31-BC**

Analysis Set	Treatment	n/n'	(%)	Adjusted Difference	95% CI
PPS	SB3 (N=402)	208/402	(51.7)	10.70%	[4.13%, 17.26%]
	Herceptin® (N=398)	167/398	(42.0)		
FAS	SB3 (N=437)	214/419	(51.1)	9.86%	[3.41%, 16.31%]
	Herceptin® (N=438)	174/415	(41.9)		

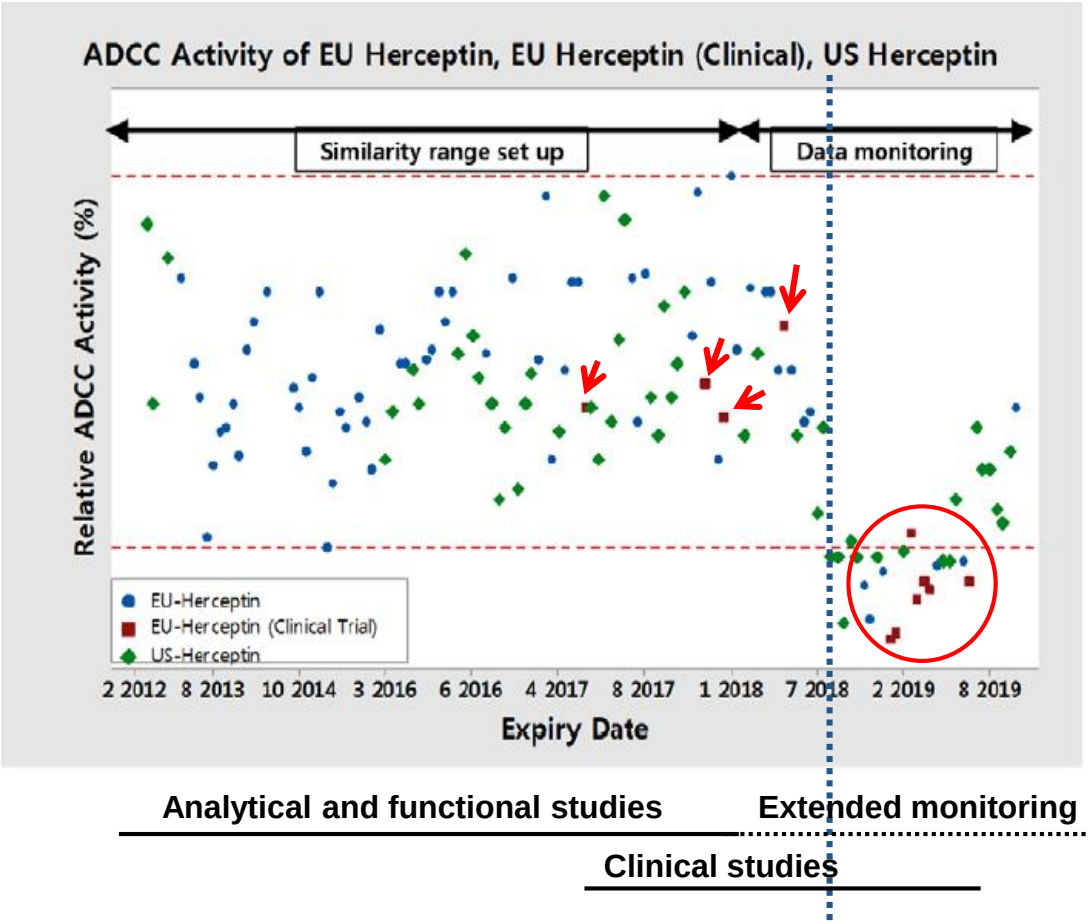
bpCR = breast pathological complete response; CI = confidence interval; FAS = Full Analysis Set; N = number of subjects in Analysis Set; n = number of responders; n' = number of subjects with available assessment results of pathological T category staging; PPS = Per-protocol Set.
Percentages were based on n'.
The adjusted difference and its 95% CI were analysed by a stratified Cochran-Mantel-Haenszel test with hormone receptor status, breast cancer type, and region as factors.
For the FAS, only available data were included in the analysis.

Pre-defined equivalence margin ±13%

- **Primary efficacy endpoint**: proportion of patients achieving breast pathological complete response (bpCR)
- **Secondary**: tpCR rate, overall response rate, event-free survival, overall survival, safety/ tolerability, PK and immunogenicity

➢**Similar safety profiles, but apparent difference in efficacy(!)**

Ontruzant; efficacy/safety similarity assessment



- For clinical studies both pre- and post-shift Herceptin batches were used
- The proportion of patients achieving bpCR in the subset of Herceptin group only **exposed to pre-shift Herceptin batches was 44.1%,** while in those exposed to **post-shift batches was 40.1%.**

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CHMP conclusion

- Similarity demonstrated for **physicochemical and biological parameters**
- Similar **PK and safety profiles**, however, **a difference was seen for efficacy**
- The **shift in ADCC activity** could have contributed to the apparent superiority of Ontruzant in terms of bpCR
  - The magnitude of the differences observed can, however, be in part attributed to other factors, the true difference is likely to fall within the equivalence margins
  - The overall contribution of ADCC activity on the therapeutic benefit of trastuzumab is not clear

➢ **Considering all the available information, Ontruzant was considered highly similar to Herceptin**

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Main learnings from the two cases

- A difference detected in a potentially critical quality attribute needs to be **carefully addressed** and the consequence of the difference on the clinical performance needs to be **convincingly understood**
- Comprehensive physicochemical and biological characterisation, thorough understanding of the mechanism of action, as well as sound scientific justifications are **prerequisites for successful extrapolation** of similarity data from one indication to another

- **Quality shifts** of the reference product do happen, these can be challenging to handle for the biosimilar developer
- Without reference product characterisation data spanning over a long period of time, quality **shifts might remain undetected**

➢ **A thorough understanding of both the candidate biosimilar and the reference product characteristics is critical for successful biosimilar development!**

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Assessment experience in the EU

September 2018

- **46 biosimilar medicinal products currently hold a valid marketing authorisation**
  - 6 adalimumab (5 different active substances), bevacizumab, 2 enoxaparin, 5 epoetin (two different active substances), 2 etanercept (2 AS), 7 filgrastim (5 AS), 2 follitropin alfa (two AS), 4 infliximab (3 AS), 3 insulin glargin (3 AS), insulin lispro, 6 rituximab (2 AS), somatropin, 2 teriparatide (2 AS), 4 trastuzumab (4 AS)
- **3 awaiting EC decision**
  - *3 pegfilgrastim (3 AS)*
- **12 biosimilar MA applications under review**
  - adalimumab, bevacizumab, etanercept, 6 pegfilgrastim, 2 rituximab, trastuzumab

Over the last 12 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

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Thank you for your attention!

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

Remsima (infliximab) EPAR (European public assessment report)
http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002576/WC500151486.pdf

Ontruzant (trastuzumab) EPAR
http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004323/WC500242488.pdf

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