Establishing Biosimilarity: Primary contribution of analytical comparability data to totality of evidence

3rd Joint Conference of Human Pharmacological Societies
Brussels, 22nd May 2015

Paul Chamberlain

Agenda

- Biosimilarity by design:
  - The quality profile of the RMP defines the biosimilar product & process
- Weight of evidence:
  - Analytic specificity / sensitivity & number of data points
- Understanding criticality of detected differences:
  - Structure *vs*. Activity *vs*. Immunogenicity
- Is a globally acceptable programme feasible?
  - Bridging data across different regions

Biosimilarity by design

Bisimilar product & process is defined by demonstrated structural and functional properties of RMP

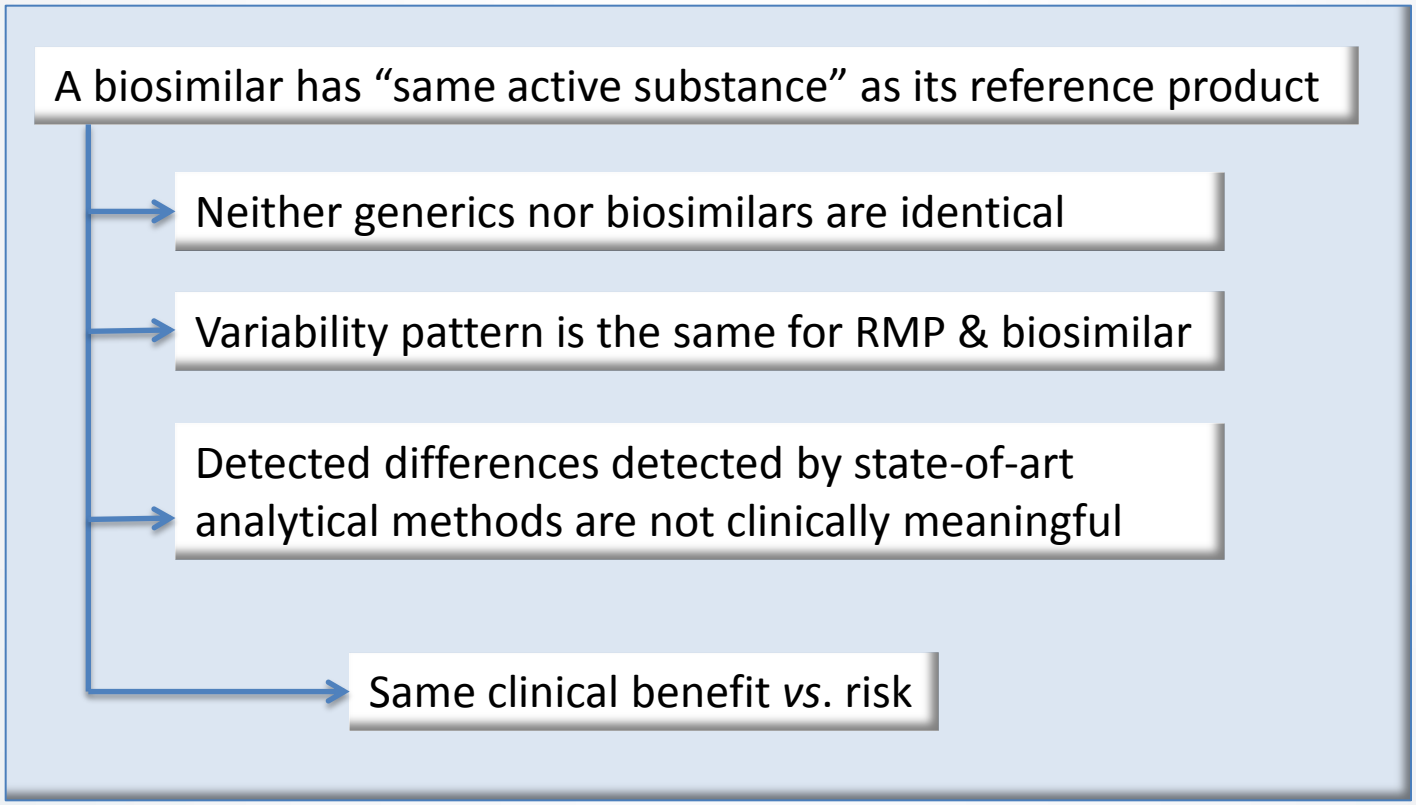
Statistically rigorous side-by-side testing using state-of-art methods

Known structure – function – immunogenicity relationships of RMP

Regulatory test of biosimilarity = differences detected at analytical level have no clinically meaningful impact

Regulator's perspective

The EU regulatory approach to generics and biosimilars is essentially similar
Van der Plas RM, van Zwieten-Boot, Hoefnagel M & Jongen PM; GaBI Journal, 2015, 4 (1)



Scientific Basis

Scientific considerations in demonstrating biosimilarity to a reference product: Guidance for Industry, FDA (CDER/CBER), April 2015

Demonstration of biosimilarity based on sameness of:

- ➔ Structure *vs.* *in vitro* activity
- ➔ Structure *vs.* *in vivo* activity (PK / PD)
- ➔ Structure *vs.* immunogenicity

Sensitive methodology & Adequate weight of evidence

Multi-dimensional relationships, i.e. "fingerprint"

- ➔ Orthogonal methods
- ➔ Data patterns

- ➔ Different patient populations
- ➔ Different disease status
- ➔ Different co-medications

Same clinical benefit *vs.* risk

Defining limits for variability

Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1); EMA/CHMP/BWP/247713/2012, 22 May 2014

Biosimilarity ranges for defining acceptance criteria for definitive comparability exercise should be based on data from testing of a sufficient number of RMP batches

Ranges should be set for each parameter / assay individually

- Based on Quality Target Product Profile (QTPP)
- Not wider than range of variability of representative RMP, unless otherwise justified

Number of batches depends on assay and batch variability
”Representative” = geographic source & age of batches

Weight of evidence

EMA/CHMP/BWP/247713/2012, 22 May 2014

Choice of cell line / expression system

The biosimilar *could* be manufactured using a different cell line / expression system, e.g. SP2/0 ➔ CHO

But this would increase weight of evidence to justify lack of clinical impact if more differences, e.g. in post-translational glycosylation, were to be detected

N-glycan profiling *vs*. Fc receptor binding *vs*. ADCC & CDC & PK

Inference: minimise weight of evidence by selection of cell line that most closely matches that used for RMP (as far is known / available)

Approach = Reverse engineering

- Define primary amino acid sequence by testing Reference Medicinal Product (RMP)
- Select cell line that has potential to yield similar post-translational modification
- Test multiple batches of the RMP to define an initial Quality Target Product Profile (QTPP) to guide manufacturing process design
- Repeat analytical side-by-testing as manufacturing process is refined / scaled for production of GMP batches for clinical trials
- Conduct definitive analytical comparison to establish biosimilarity relative to pre-defined acceptance criteria that are aligned to criticality
- Perform non-clinical and clinical studies as necessary to confirm absence of clinically-relevant differences

Extent of differences detected will determine the weight of non-clinical & clinical evidence required

EMA/CHMP/BWP/247713/2012, 22 May 2014

Stages of evaluation

EMA/CHMP/BWP/247713/2012, 22 May 2014

Stage 1 = define initial Quality Target Product Profile (QTPP)

Test ≥ 6 batches of Reference Medicinal product (RMP)

Stage 2 = directly comparative testing: Biosimilar *vs*. RMP

Include relevant *in vitro* & *in vivo* pharmacology
Multiple Biosimilar batches manufactured at different scales

Stage 3 = Justification of differences detected

Criticality Risk Assessment

Stage 4 = Definition of acceptance criteria for definitive demonstration of similarity

Statistical rigor defined by criticality

Stage 1: Initial QTPP

EMA/CHMP/BWP/247713/2012, 22 May 2014

QTPP

➔ Based on RMP testing & prior knowledge

➔ Development tool / refined as more data become available

Needs to reflect quality profile of RMP in the market for registration is sought

➔ Account for geographic and batch age-related differences

➔ Adequate number of batches to reflect true variability

No regulatory requirement for number of batches:
In practice, minimum of 6 RMP batches per region for initial QTPP

Stage 2: Comparative testing

Process development batches of biosimilar e.g.:

- 3 batches from 50 L scale
- 3 batches from 250 L scale
- 3 batches from 1000 L

Directly comparative analytical characterisation *vs*. RMP;

Scale-up / process refinement informed by QTPP

Descriptive comparison of biosimilar profile *vs*. RMP for progression into clinic

Minimise risk by performing extensive *in vitro* pharmacology to qualify functional impact of any detected differences

Minor process refinements could be made

Definitive analytical comparability exercise to support registration; e.g. n ≥ 20 to 30 batches of RMP & biosimilar

Stage 3: Assessing criticality

Zarxio™ AdCOM

Quality Attribute	Criticality	Relevant for	Methods Used
Amino acid sequence	Very High	Efficacy, Safety, Immunogenicity	Edman, peptide mapping, MS
Potency	Very High	Efficacy, Safety	Bioassay
Target binding	Very High	Efficacy, Safety	Surface plasmon resonance
Protein concentration	Very High	Efficacy	Content determination
Higher order structure	High	Efficacy, Immunogenicity	CD and NMR spectroscopy
High-molecular weight variants/aggregates	High	Immunogenicity	Size exclusion chromatography
Oxidized variants	High	Efficacy	Reversed phase chromatography
Subvisible particles	High	Immunogenicity	Light obscuration
Truncated variants	Low	None	RP-HPLC-MS
Norleucine	Very Low	None	Reversed phase chromatography
Deamidation	Very Low	None	Cation exchange chromatography

Slide prepared by Sandoz

Stage 4: Acceptance Criteria

Rigor of pre-defined acceptance criteria for definitive comparability exercise should be based on results for RMP and criticality / uncertainty of impact

RISK RANKING #	Data analysis
High criticality	Equivalence testing
Moderate	Range defined by mean ± x SD
Low	Descriptive (raw / graphical) data comparison
Not critical	None

No standard algorithm:

Risk ranking should take into account probability and severity impact (on efficacy, safety & immunogenicity) as well as the uncertainty associated with the evidence for the impact

EQUIVALENCE TESTING for high criticality

Zarxio™ AdCOM transcript

Bioactivity & Content

"For EP2006 bioactivity and content are two critical quality attributes for tier 1. Their analytical similarity was tested by statistical equivalence testing…

…the equivalence margin is defined as minus plus 1.5 times sigma C. Again, sigma C is the variability or the standard deviation of the comparator, which can be either U.S.-licensed Neupogen or EU-approved Neupogen, depending on the specific analysis being conducted. In addition, ***sigma C is estimated from Sandoz data on Neupogen products****."*

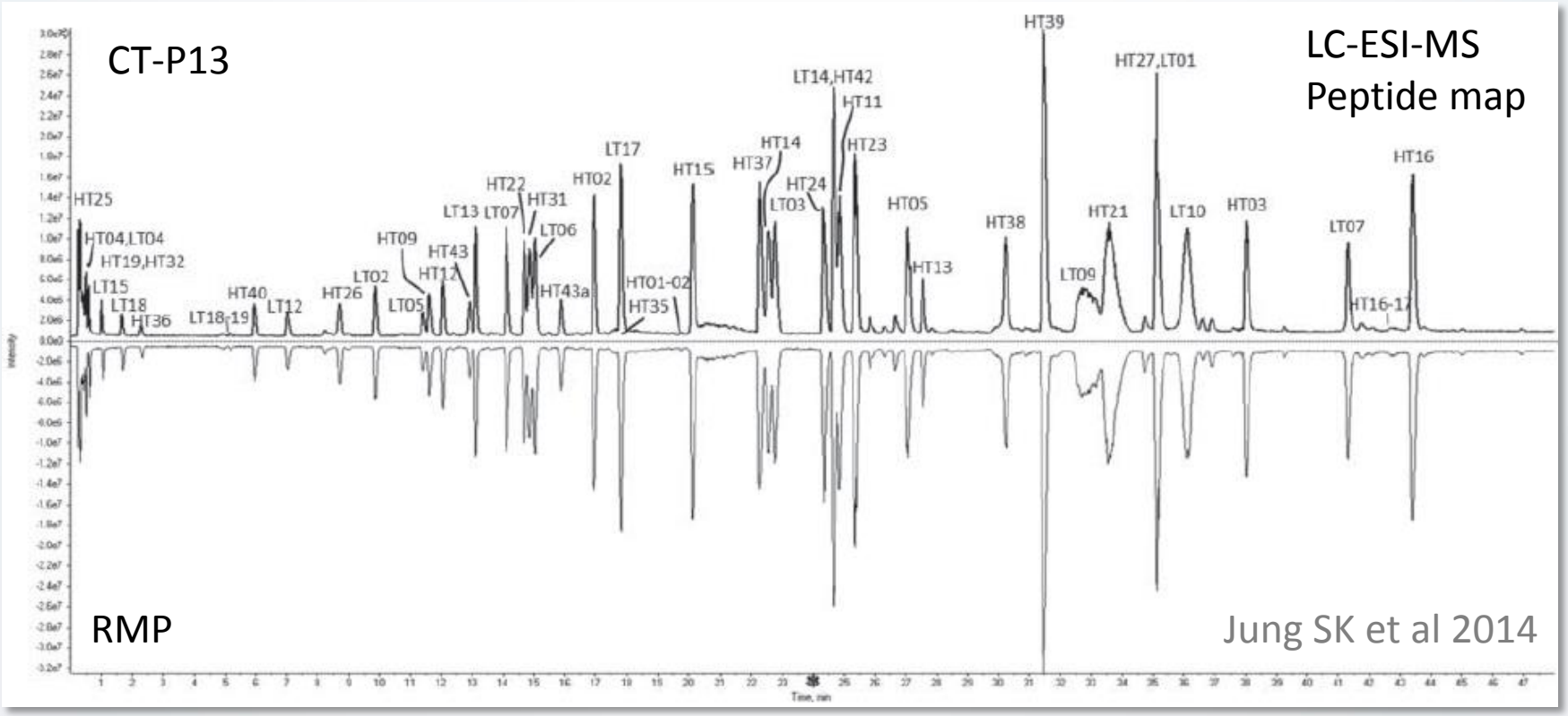
17 batches of Zarxio™ *vs*. **82** batches of RMP

Understanding criticality: N-glycan variability

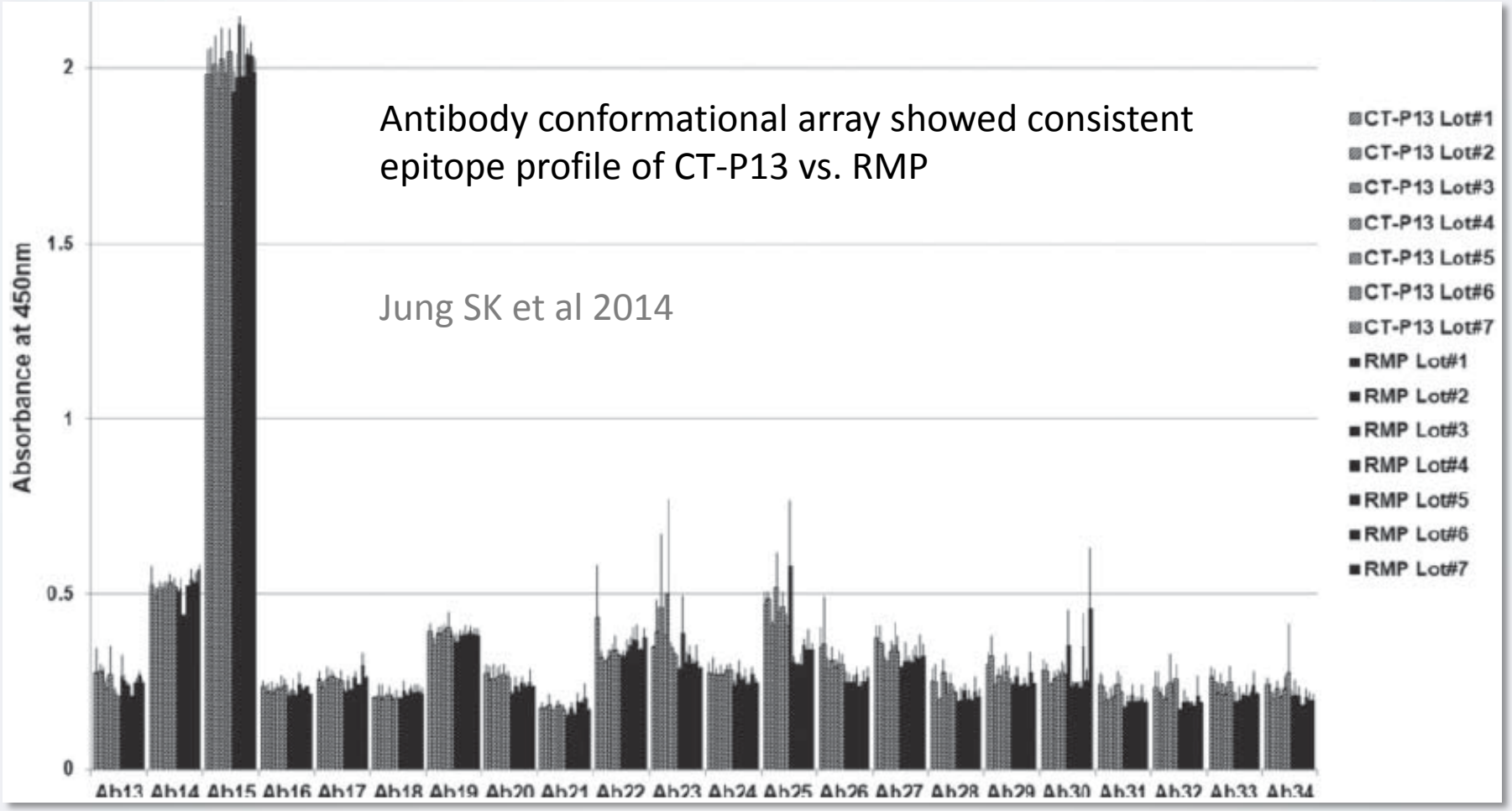
Property	Impact	Reference
Core fucose	ADCC	Shields RL et al 2002
Galactose	Fcγ RIIIa binding CDC CDC Uptake by mannose receptor	Kumpel BM et al 1994 Hodoniczky J et al 2005 Chen X et al 2009 Dong X et al 1999
Bisecting GlcNAc	ADCC ADCC	Umana P et al, 1999 Davies J et al 2001
High mannose	PK	Kanda Y et al 2007 Goetze AM et al 2011
Sialic acid	Fcγ RIIIa binding & ADCC	Scallon BJ et al 2007
Neu5Gc	Enhanced clearance	Ghaderi D et al 2010
Gal-α 1,3-Gal (Fab)	Type I hypersensitivity	van Beuren et al 2011

Primary structure

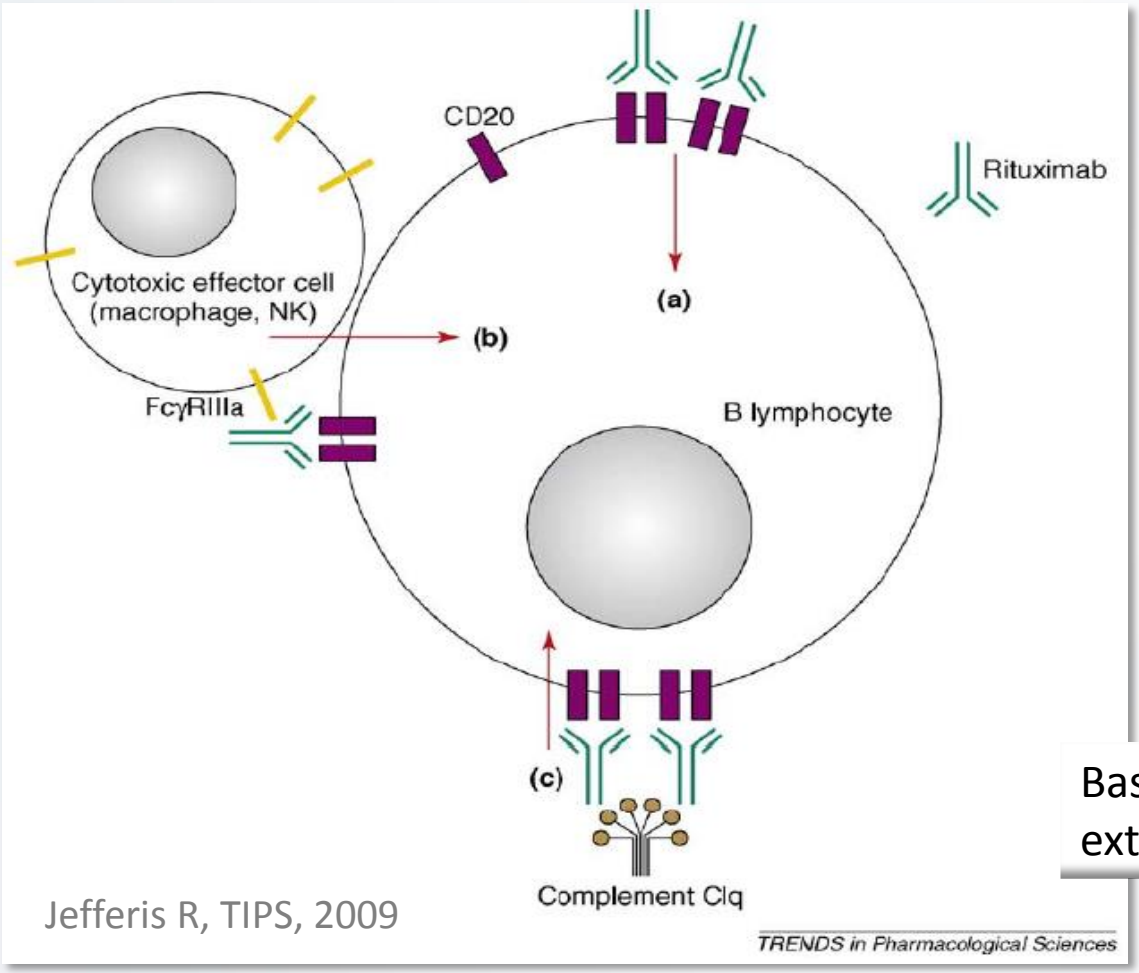
"The target amino acid sequence of the biosimilar should be confirmed and is expected to be the same as for the RMP" EMA/CHMP/BWP/247713/2012, 22 May 2014



3-Dimensional conformation



Potency: Evaluation of multi-mechanistic effect



Jefferis R, TIPS, 2009

- Antigen binding affinity
- CDC
- ADCC
- Apoptosis
- FcγR IIIa binding
- C1q binding
- FcRN binding

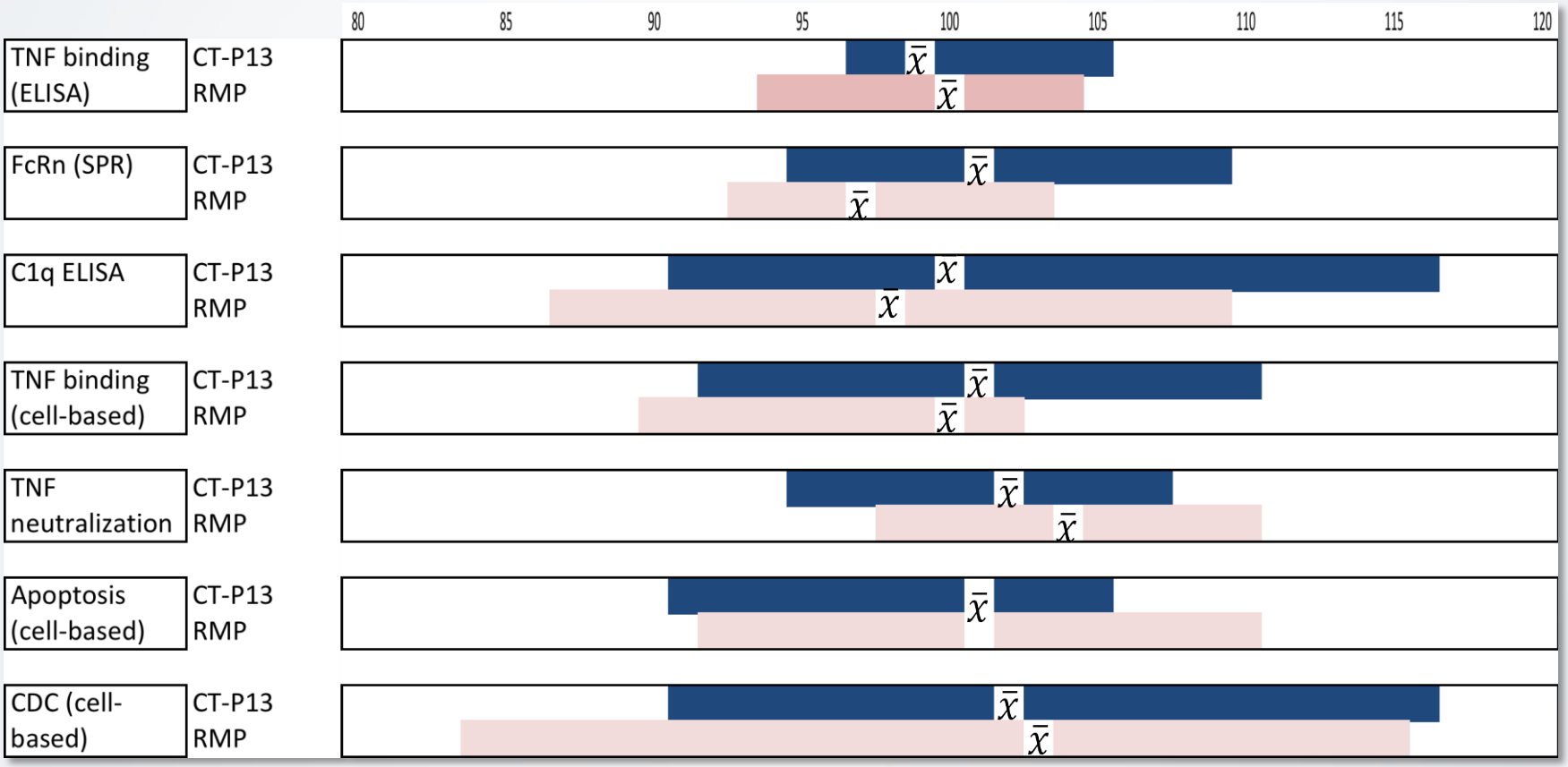
Statistically rigorous comparison of BS *vs*. RMP

- Multiple batches
- Side-by-side

Basis for equivalent posology & extrapolation across indications

Potency

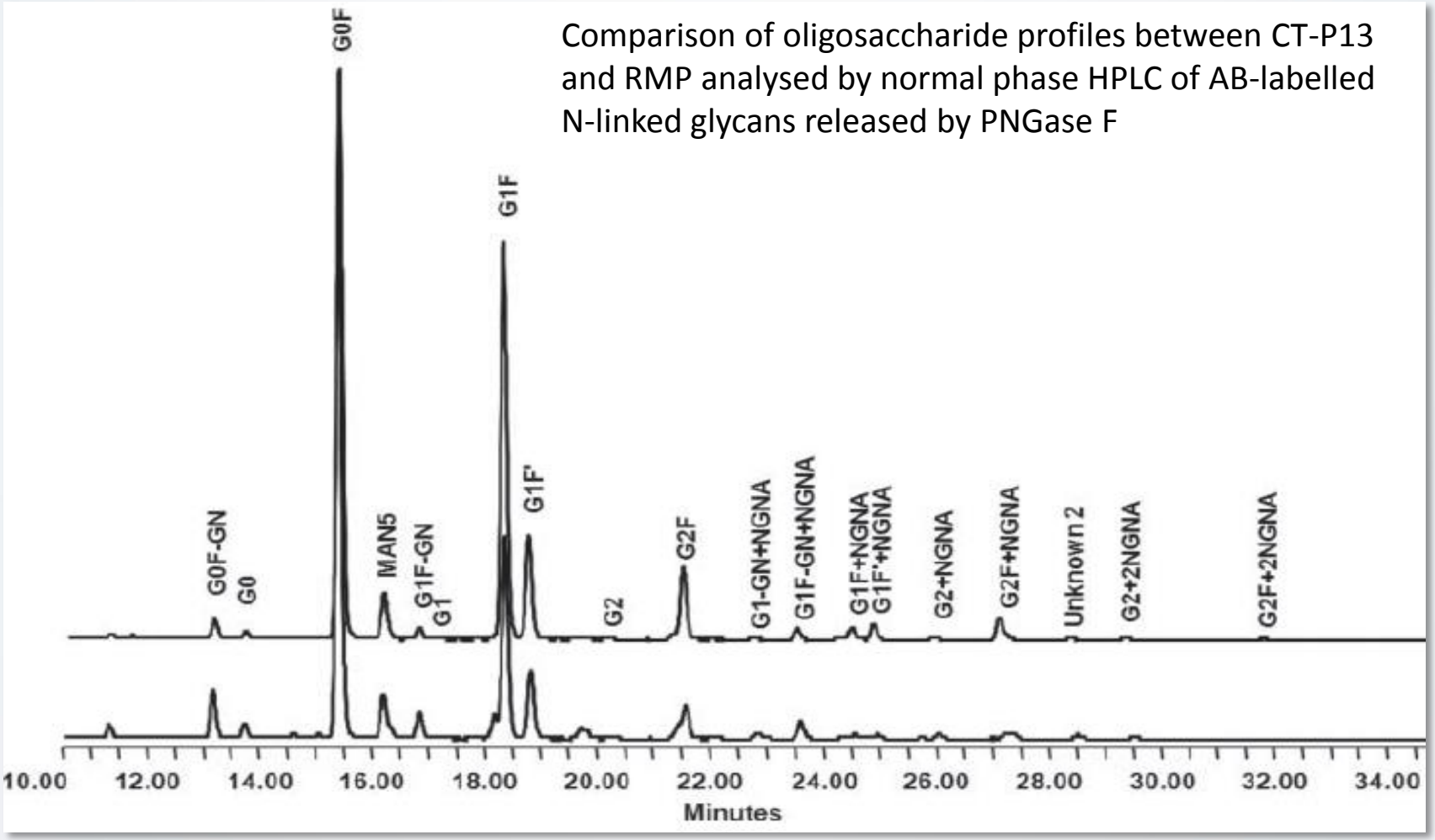
Jung SK et al 2014



90% CI of ratios between the two products within 80-125% for all parameters
p values < 0.05 on both sides of two one-sided t-test (CT-P13 = RMP)

N-glycan profile

Jung SK et al 2014



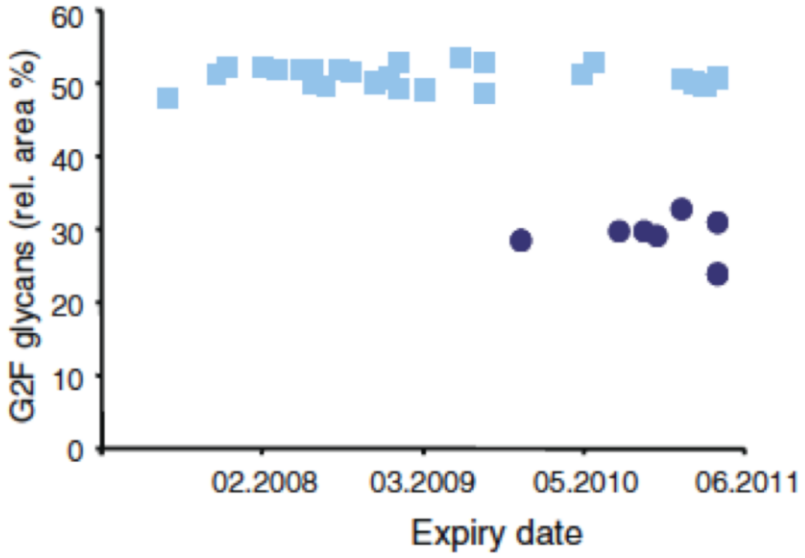
Comparison of oligosaccharide profiles between CT-P13 and RMP analysed by normal phase HPLC of AB-labelled N-linked glycans released by PNGase F

RMP variability: Enbrel®

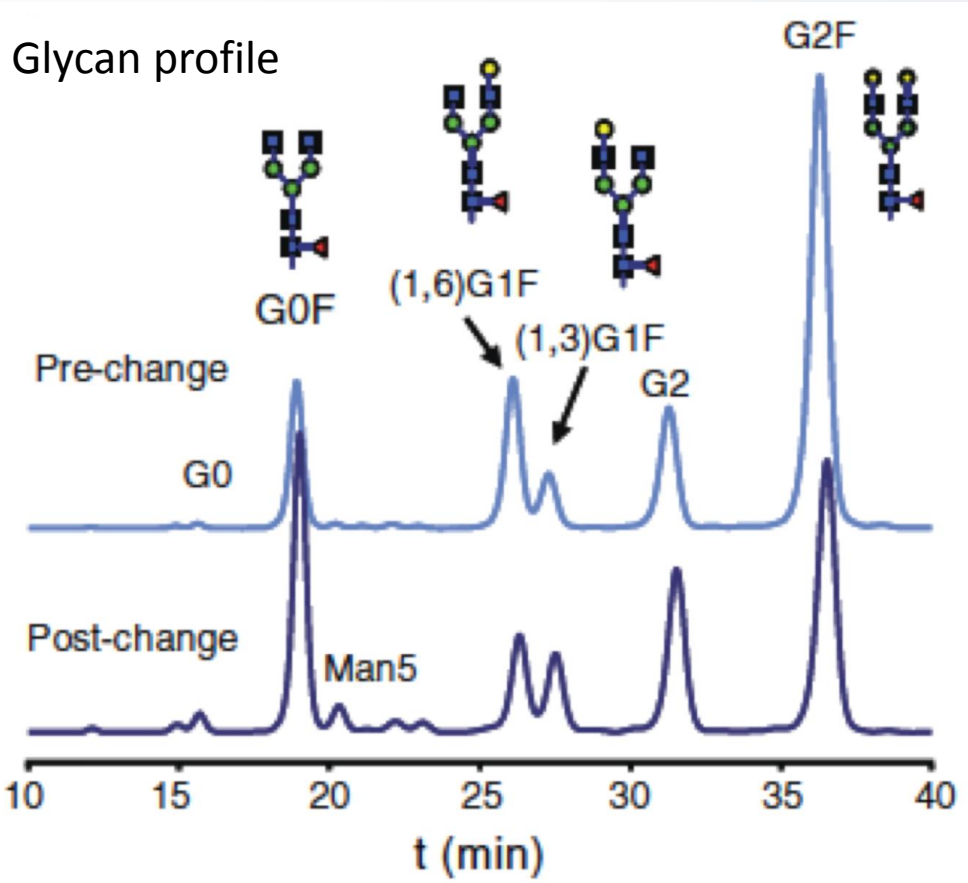
Schiestl et al NBC 2011

Pre- (n=25) vs. post (n=9)-change Enbrel

G2F glycan

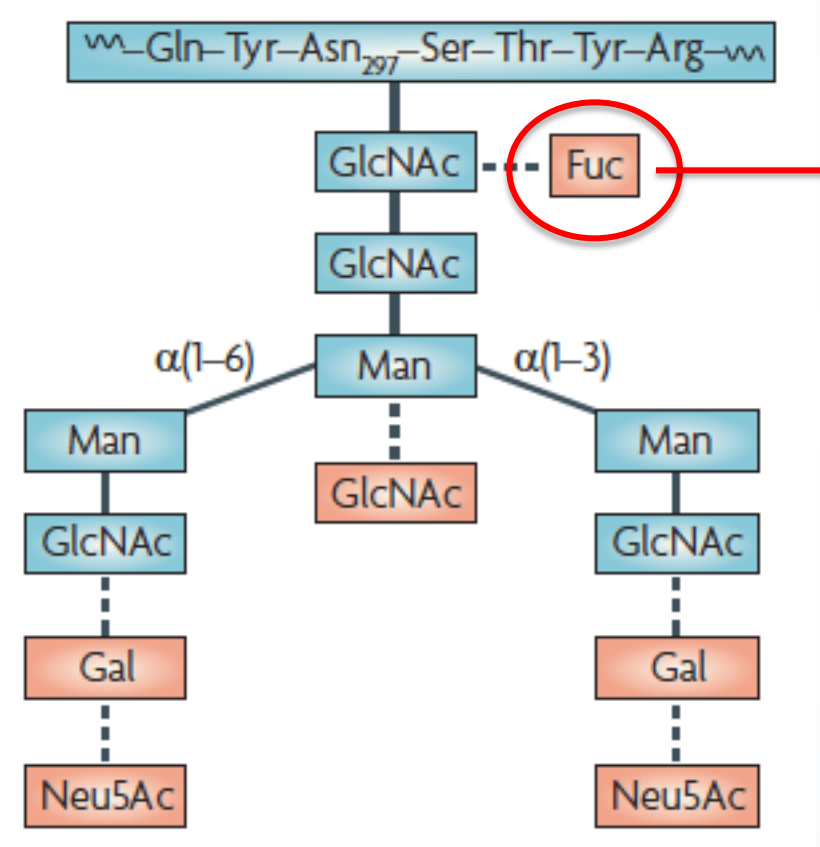


Glycan profile



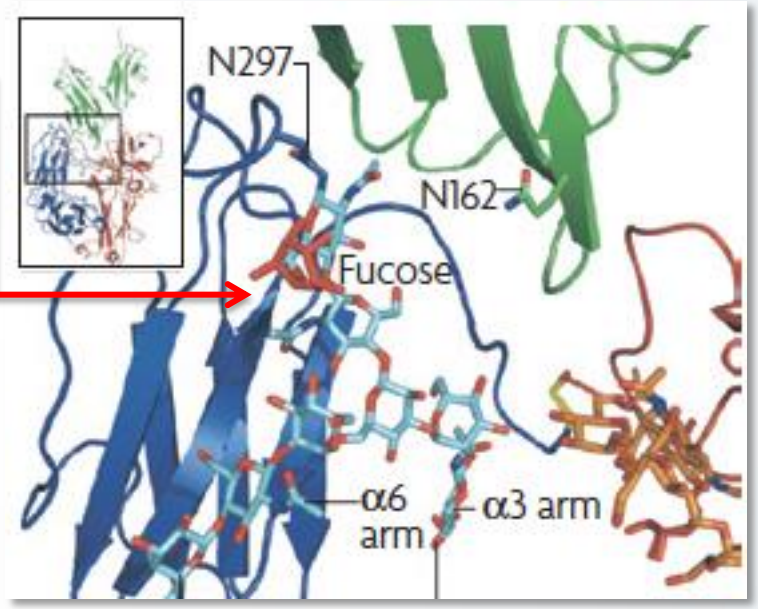
2 versions of Enbrel on market at same time

Core fucose as a functional variable



Jefferis R, NRDD 2009

Nimmerjahn & Ravetch, NRI 2008



- ↓ core fucose
- ↑ binding to Fcy RIIIa on NK cells
- ↑ ADCC

Structure *vs*. activity: ADCC *in vitro*

Optimise *in vitro* sensitivity / respect *in vivo* conditions:

- ➔ Test target cells expressing different levels of ligand / antigen
- ➔ Test different effector cells (genotyped PBMCs & NK cells)
- ➔ Test different effector-to-target cell ratios
- ➔ Test in presence and absence of autologous serum

- Shields RL *et al. J Biol Chem* 2002, 277 (30), 26733-26740
- Preithner S *et al. Mol Immunol* 2006, 43, 1183-1193

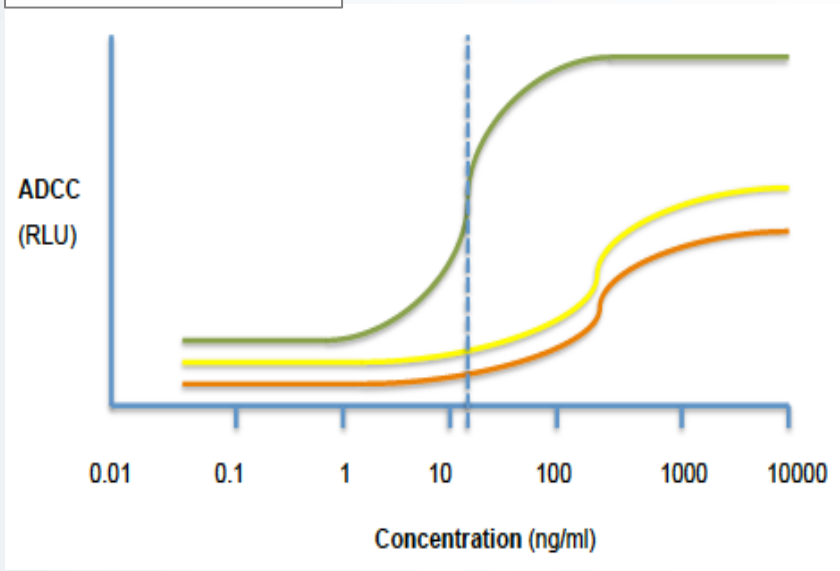
Endogenous levels of human IgG_1 substantially higher than the steady state circulating concentration of therapeutic protein IgG Fc:

➔ Out-competing the therapeutic protein IgG Fc for binding to the FcγRIIIa receptor on NK cells, which is the primary mediator of ADCC

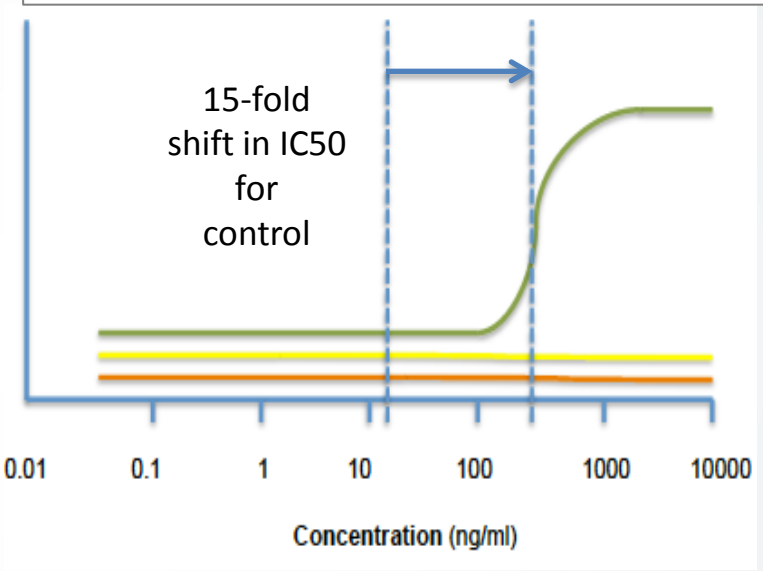
Understanding bias: ADCC *in vitro*

Unpublished data

Without serum



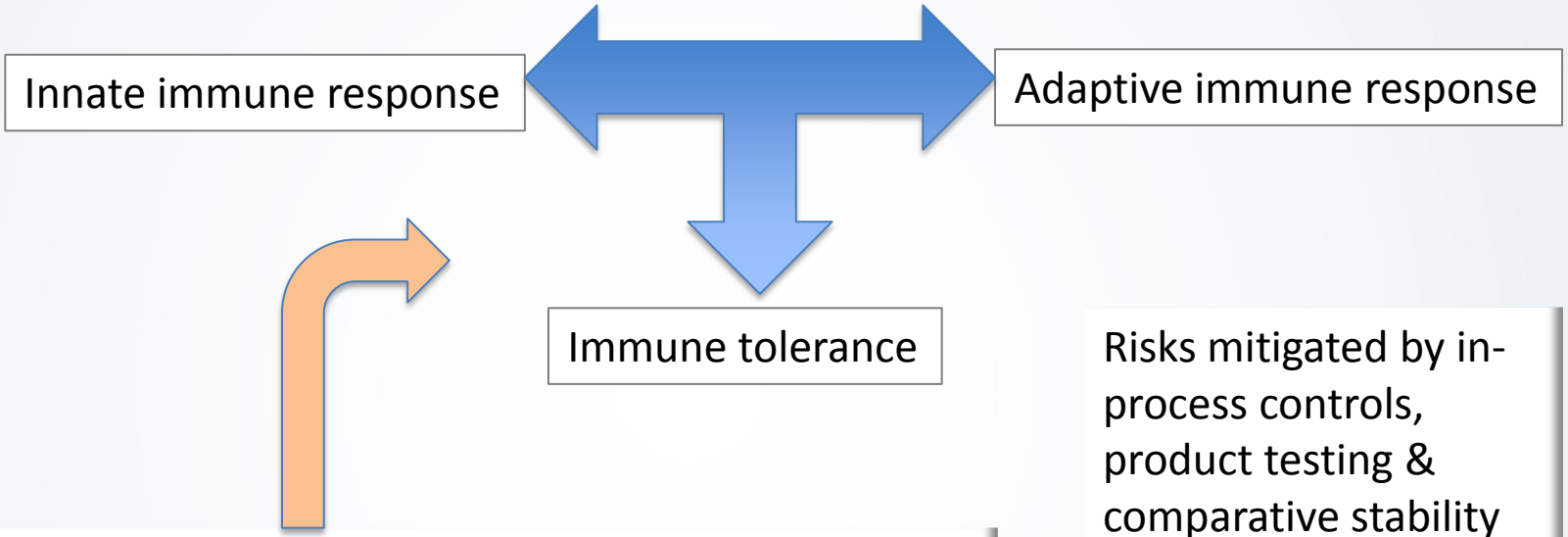
+ autologous human serum (10% v/v)



Control = Humira®
Biosimilar etanercept
RMP (Enbrel®)

Experimental system is "over-sensitive" for detection of physiologically relevant ADCC due to relatively high expression of tm-TNFα in target cells allied to under-representation of endogenous IgG

Structure *vs*. immunogenicity



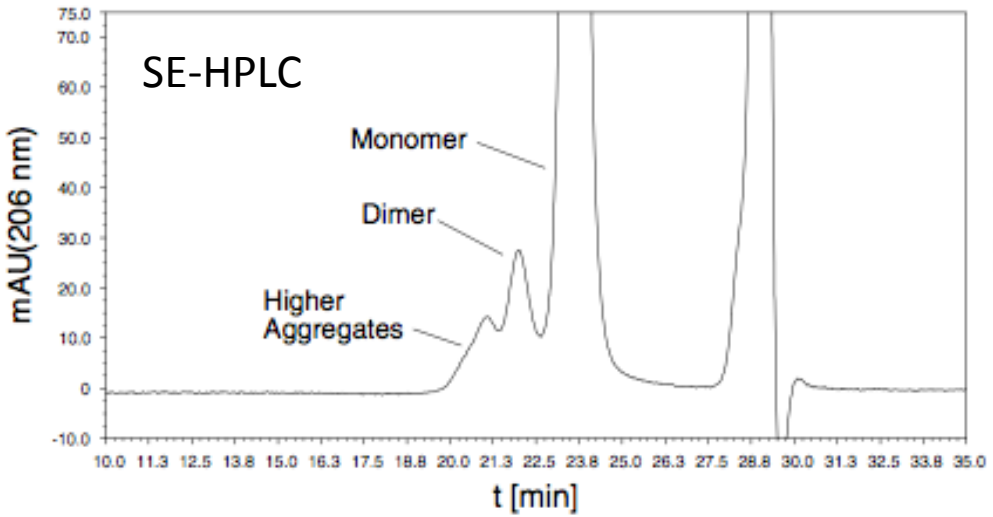
Product-related risk factors:

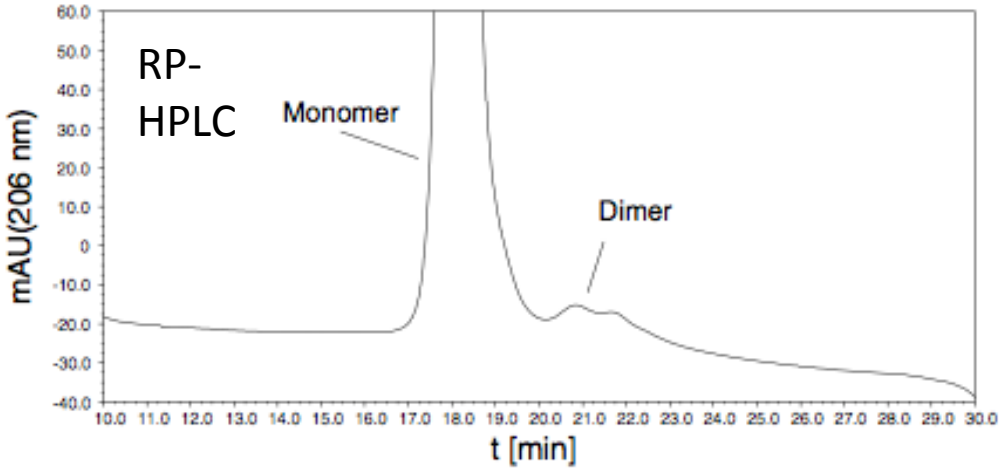
- Process-related impurities, e.g. HCP's, endotoxins
- Aggregates / sub-visible particles
- Non-human post-translational modifications

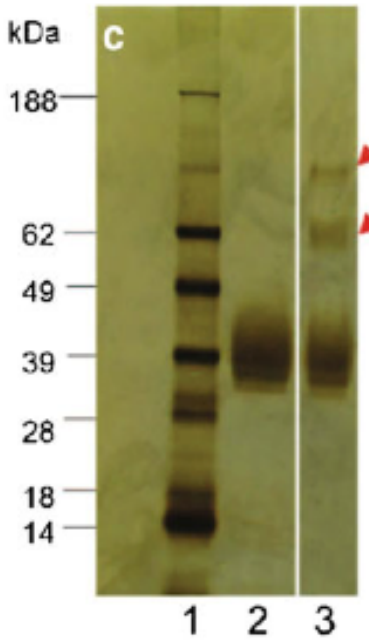
Risks mitigated by in-process controls, product testing & comparative stability testing (real-time, accelerated & forced degradation) in final formulation- container combination

Tungsten-induced denaturation/aggregation of rhEPO during primary packaging

Seidl A *et al*; Pharm Res 2011







HMW aggregates & dimer induced by tungsten

Tungsten-induced denaturation/aggregation of rhEPO during primary packaging

Temperature- & concentration-dependent influence of tungsten on aggregate formation

Seidl A *et al*; Pharm Res 2011

Table V Aggregate Content of Epoetin Alfa Samples Spiked with Tungsten Pin Extract (0, 2 or 20 ppm) and Stored for 6 Months at 5°C or 25°C (Determination by HP-SEC)

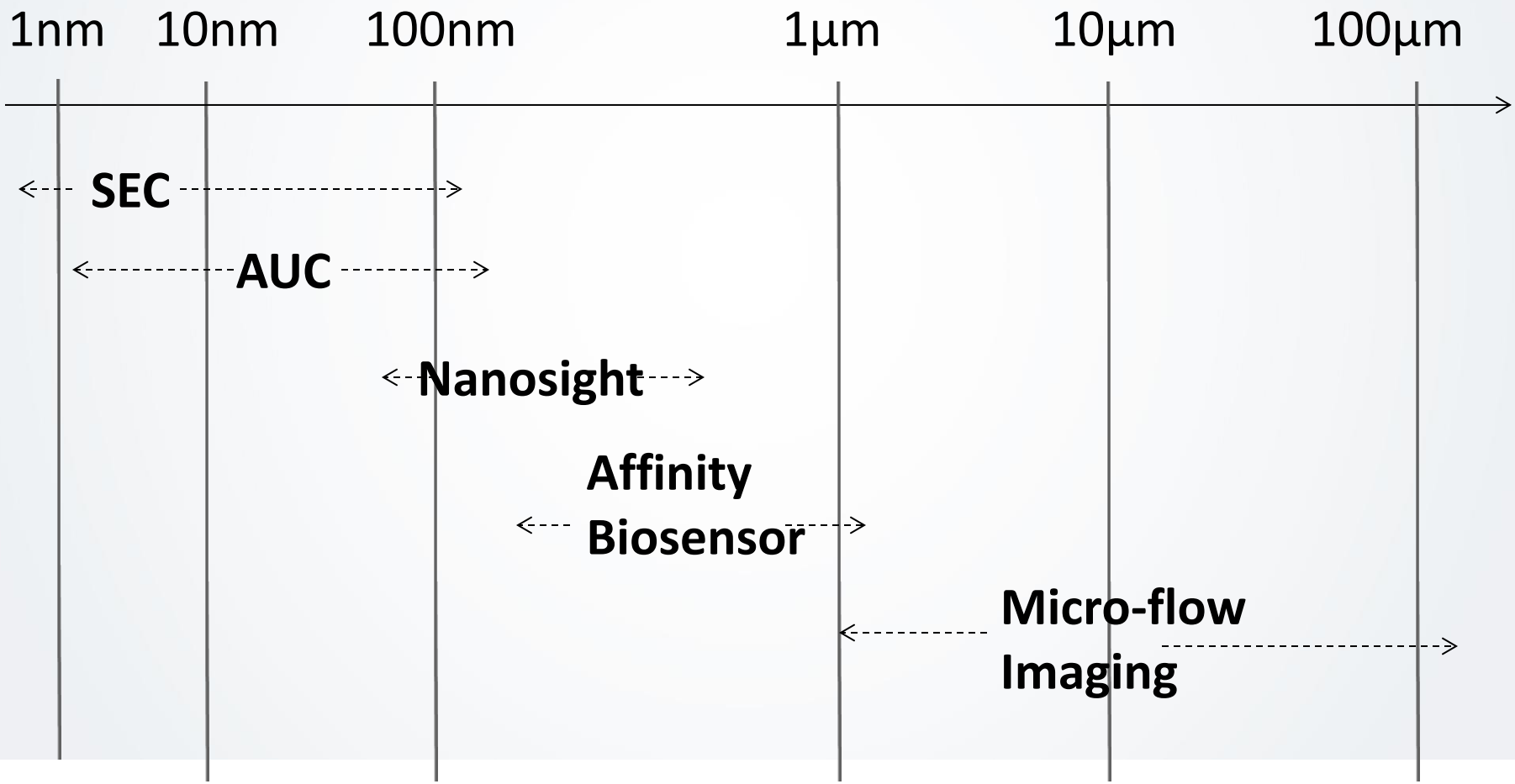
Tungsten concentration (ppm)	Storage temperature (°C)	Epoetin alfa monomer (relative area, %)	Epoetin alfa dimer (relative area, %)	Epoetin alfa higher aggregates (relative area, %)
0	5	100.00	ND	ND
2	5	99.95	0.05	ND
20	5	99.33	0.42	0.25
0	25	99.48	0.52	ND
2	25	99.41	0.59	ND
20	25	97.67	1.72	0.61

Tungsten-induced protein aggregation:

Bee JS, Nelson SA, Freund E, Carpenter JF, Randolph TW:. J Pharm Sci. 2009; 98:3290–301
Jiang Y, Nashed-Samuel Y, Li C, Liu W, Pollastrini J, Mallard D, *et al*:. J Pharm Sci. 2009; 98:4695–710
Liu W, Swift R, Torraca G, Nashed-Samuel Y, Wen ZQ, Jiang Y, *et al*:. PDA J Pharm Sci Tech. 2010; 64:11–9

Analytical Methods for Characterizing & Quantifying Aggregates and Particles

Courtesy of John Carpenter, Univ Colorado



Global approach: Choice of RMP

EU + USA + Japan

Biosimilar *vs*. RMP marketed in different regions:

➔ Directly comparative analytical data
➔ Bridging clinical data, e.g. comparative PK

Do ***not*** need to repeat:
- Non-clinical *in vivo* studies
- Therapeutic equivalence

Canada & Australia

Biosimilar *vs*. ICH RMP:

➔ Demonstrate linkage of regional product to RMP marketed in ICH regions

Conclusion

The biosimilar is as highly similar to the innovator product as the innovator is to itself over time

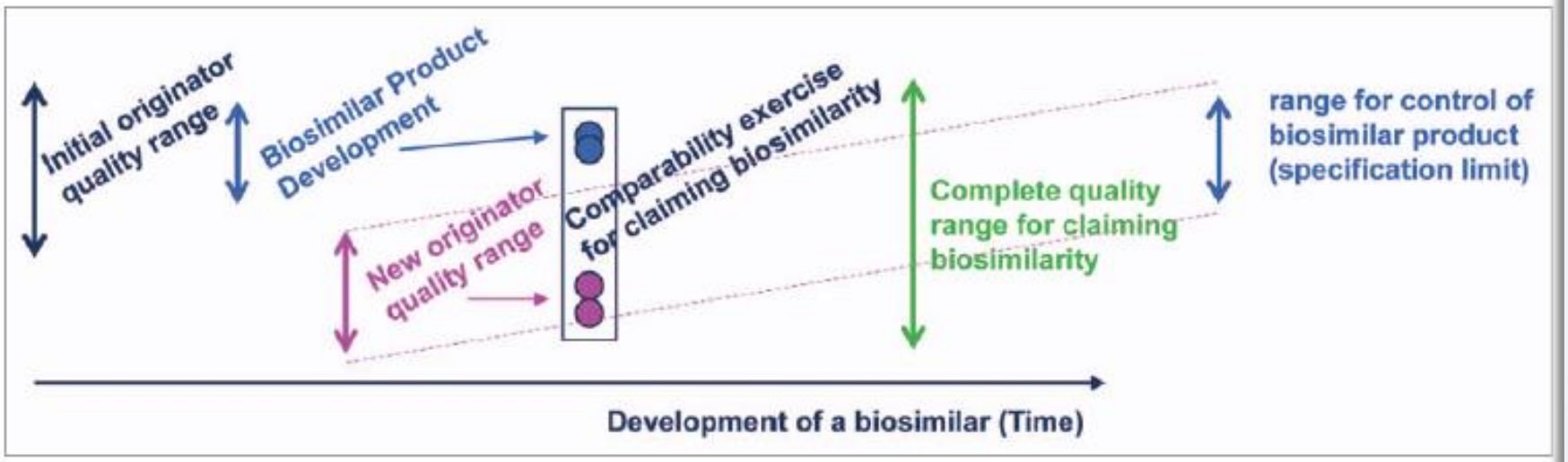


Figure 4. Biosimilarity goal posts. The "goal posts" of biosimilarity are established by the biosimilar sponsor by their analysis of the distribution of product attributes present in the reference product pre- and post-manufacturing change. They then use these to select the design space for their biosimilar candidate. While the complete quality range may be quite broad for the life time of the reference product, the biosimilar sponsor will select a tighter range of control for their biosimilar product.

McCamish & Woollett; mAbs 2011, 3:2, 209-217