Austrian Agency for Health and Food Safety

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Identification of an appropriate similarity condition

Disclosure and Disclaimer: I attend this meeting/conference to represent the AGES. The views expressed here in no way shall be binding for the AGES. My remarks do not necessarily reflect the official view of AGES, BASG, EMA or EC.

Manfred Schuster

Assessor for Biologics

Overview of this talk

Setting a similarity condition

- Understanding the Active Pharmaceutical Ingredient
  - Analytical characterisation using state of the art orthogonal methods
  - Isolation and in-depth assesment of variants
- Identification of Critical Quality Attributes
- Establishment of a dedicated control strategy
  - Setting of IPC's and Specifications
  - Analytical validation
  - Process Performance Qualification runs
- Comparability protocol
  - Setting similarity conditions
- Comparability study

When do we assess comparability?

- Changes to (adaptations of) established manufacturing processes
  - Process transfer
  - Scale up / down
  - Adaptations – optimisations
    - Optimisation of Amino acid sequence
    - Switch to different expression host
    - Changes w/i USP – media / process / scale
    - Changes w/i DSP – media / process / cycling
    - Changes to formulation – Excipients / process
- Development of a Biosimilar

What means comparable?

- No change in CQA's
  - Initially only focused on changes of manufacturing processes
  - Extended to confirmation of biosimilarity
- Comparability according to ICH Q5E
  - Science driven approach
  - Flexible – case by case
  - Pre- and post-change product highly similar but not necessarily identical
  - Statistical flexibility
    - Comparability ranges
    - Historically justified quality range
  - Differences have no adverse impact on clinical safety (including immunogenicity) and efficacy (including pharmacology)

What is the meaning of biosimilarity?

No change of CQA's

- Highly similar quality profile, notwithstanding minor differences in clinically inactive components
- No clinically meaningful differences between the biologic product and the reference product in terms of safety, purity, and potency
- Demonstrated by extensive comparability exercise using orthogonal methods – not restricted to release and stability specification
- Analysis of degradation pathways
- Method status: Qualified for the intended purpose!
- Any differences
  - Will have to be appropriately justified with regard to their potential impact on safety and efficacy
  - Might trigger further non-clinical assessment

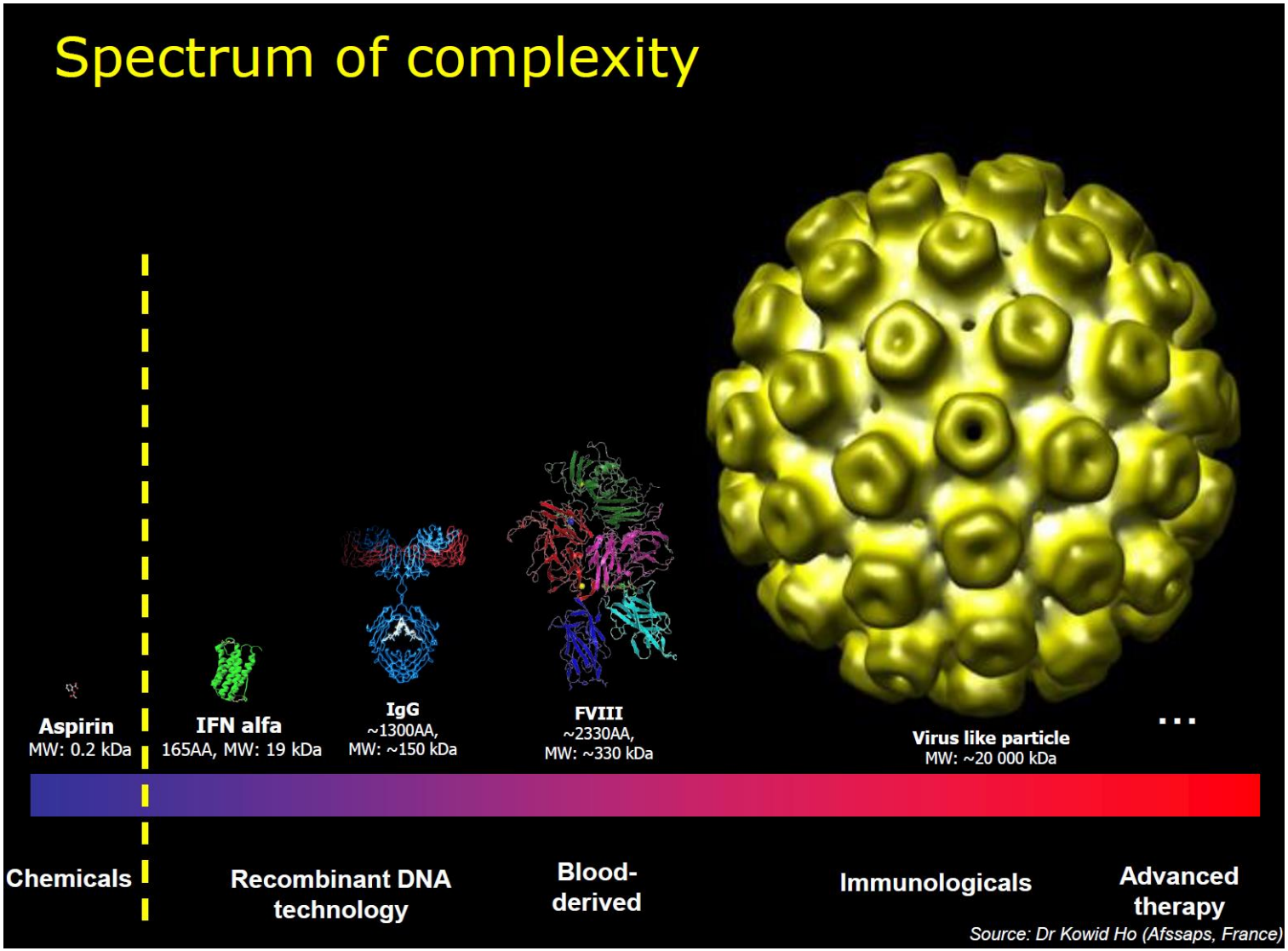
Understanding of the API

Identification of CQA's

- Efficacy related properties
  - Biological functions
    - Do we understand the MOA?
    - Do we know all the interaction partners?
  - Understanding of the physiological milieu
  - Effects of post-translational modifications
  - Micro-heterogeneity triggered alteration of biological functions
  - Stability profile – stressed degradation studies
- Safety related properties
  - Impurity profile
  - Immunogenicity (product related and process related)

Spectrum of Complexity

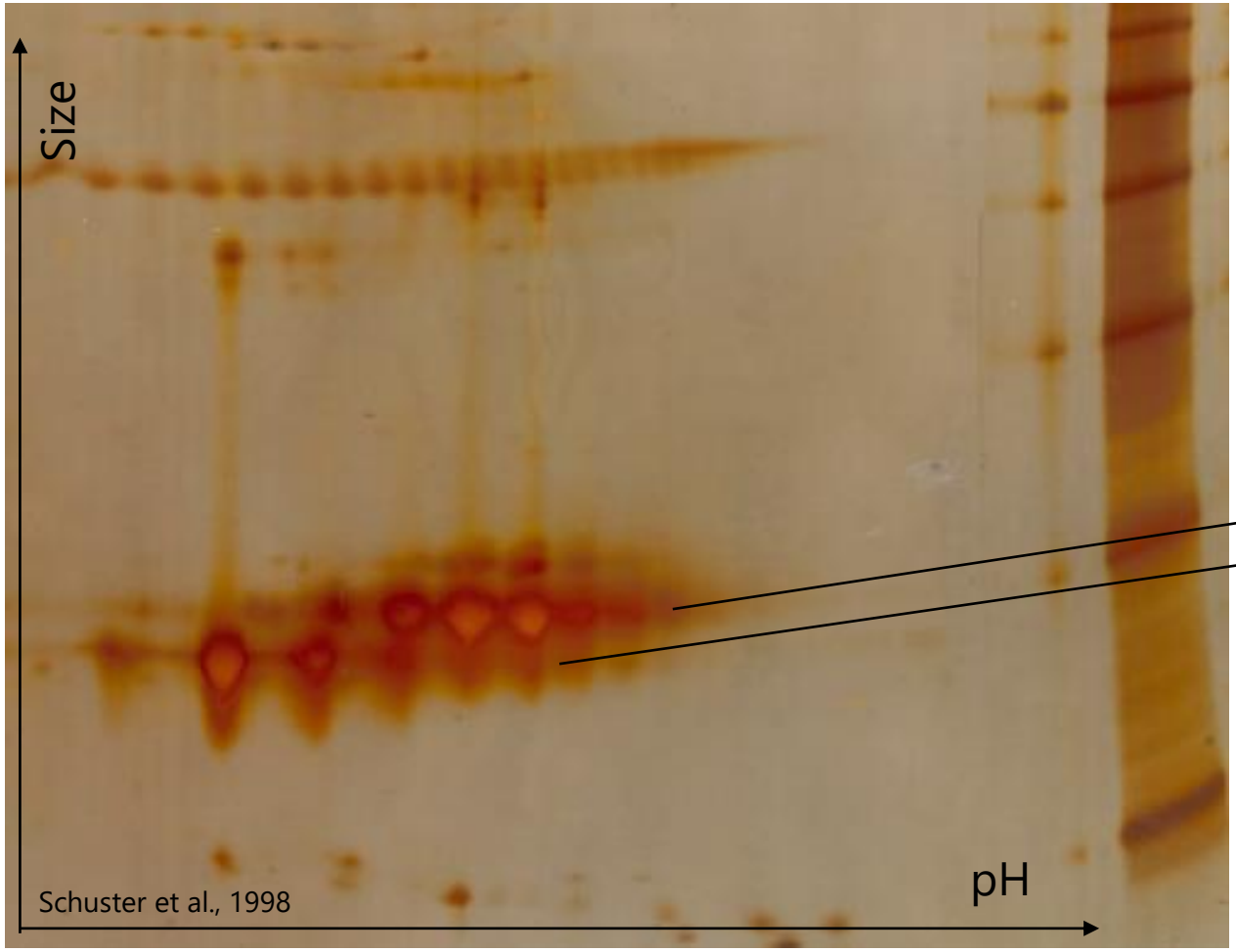
Large molecules

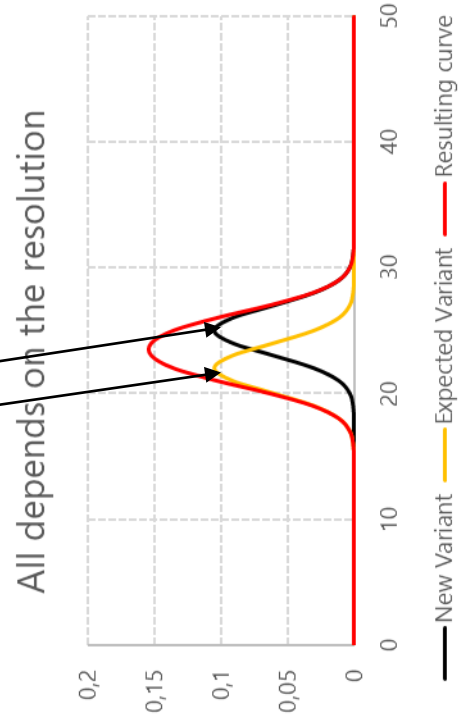


Spectrum of Complexity

Complex molecules

rec. human Eukaryotic Elongation Initiation Factor 5A



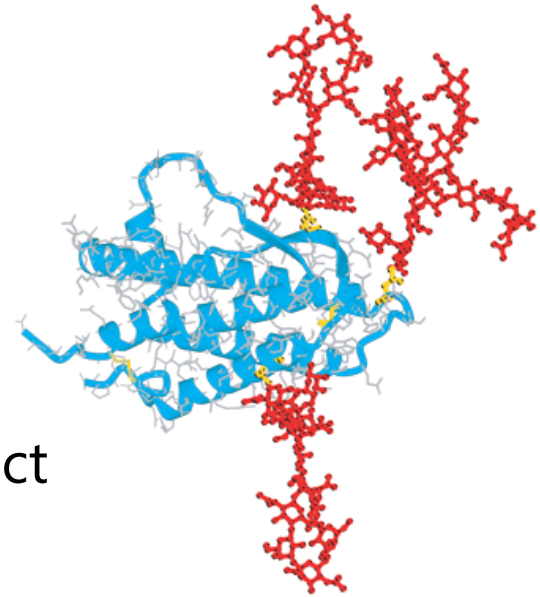


Spectrum of Complexity

Understanding the API

- Complex manufacturing process
  - Multiple steps
  - Black box of the expression host
- Large size and complex molecules
- Small process changes may have a high impact
- Variability
  - At process level – batch to batch variability
  - Analytical variability – assay precision
  - Moving target: time dependent variability

  Instability of the API (pH, temperature, oxidation, mechanical stress,…) –
- No characterization at molecular level
  - micro-heterogeneity in structure



Courtesy of M.R. Wormald and R.A. Dwek, Oxford Glycobiology Institute, and P.M. Rudd, NIBRT

rec. human EPO

Analytical toolbox

Understanding the API

- Primary, secondary, tertiary and quaternary structure

  Peptide map, disulfide bridges, CD, FT-IR, crystallisation,…
- Purity: size, hydrophobicity, charge distribution

  CE, HPLC, PAGE, AUC, DLS,…
- Post-translational modifications

  N-and O-linked glycosylation, phosphorylation, proteolysis, ubiquitynation, oxidation, deamidation,…
- Content and identity
- Impurities and adventitious agents

  CE, HPLC, sterility, qPCR, endotoxins, ELISA,…
- Degradation pathways – stress strudies

Biological activity

Understanding the API

- Set of binding and cell-based assays for characterisation and comparison of biological activity
- Assessment of all known biological functions necessary
- Ideally inclusion of all interaction partners in the physiological milieu
- Assay format
  - Bioassays, SPR, Enzymatic assays, ECL based displacement assays
- Side by side analysis required
- For mAb's
  - Fab-associated functions (e.g. neutralisation of a soluble ligand, receptor activation or blockade)
  - Fc-associated functions (e.g. ADCC, CDC, complement activation)

Setting of similarity conditions

Managing process changes

- Setting ranges
  - Quantitative ranges where possible
  - Not be wider than variability of representative RMP batches
  - A descriptive statistical approach to establish ranges for quality attributes could be used, if appropriately justified
- Statistical approaches
  - No regulatory requirement to use any specific statistical method
  - Proposals for statistical evaluation need to be justified
  - Raw data should always be provided to enable assessment of comparability independently from applied statistics
  - Statistical package assessed on a case by case basis

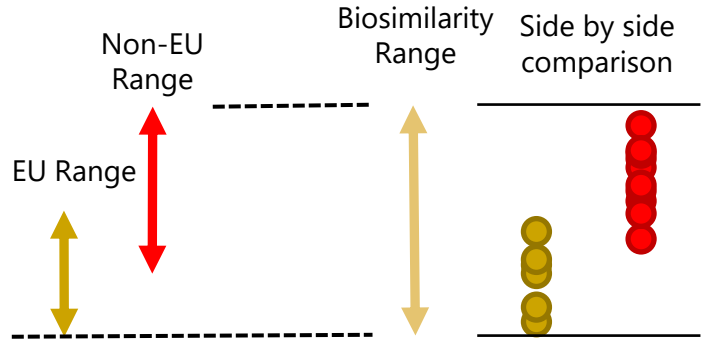
Justification of equivalence ranges

- Understanding of variability
  - Micro-heterogeneity of biologics
  - Analytical variability – precision and accuracy
  - Batch to batch variability
  - Stability – aging of biologics
- CQA's are controled by limits
  - IPC's
  - Specification
- Acceptable variability
  - Clinically justified – safety & efficacy
  - Experienced with pre-change material or RMP
  - Limitations by analytical performance

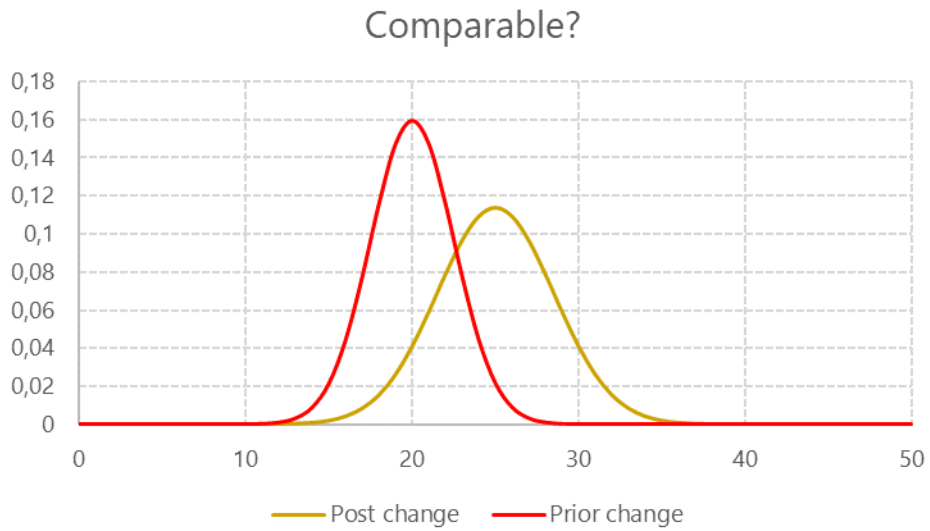
How do we compare

Establishment of acceptance criteria

- Treatment of patient occurs via an individual batch (≠mean)
- Pre-defined comparability range
  - Min-max approach
  - Mean +/- k x SD
- Equivalence testing
  - Δ of means
  - Δ of variance



Cesar dog food: Perhaps we search out a companion that reminds us of ourselves



Equivalence testing - comparability range

Pros and cons

- **Equivalence testing** – pure statistical evaluation
  - Complex!
  - Increases objectivity
  - Assumes normal distribution of analtical and process variability
  - Robust towards outliers
  - Focussed on mean

  90% c.i. of means & w/i 1.5 σ of the reference product
- **Comparability range** – scientifically justified
  - Reflects control strategy (upper and lower acceptance limits)
  - Clinically justified, thus scientific rationale
  - In line with EU biosimilarity guidance - should not be wider than the range of variability of RMP unless otherwise justified
- 3 tiered approach
  - Specific tier based criterion: equivalence, ranges, descriptive

Biosimilarity

Selected concerns raised during the review process of biosimilar applications and in scientific advice procedures

- Setting of biosimilarity ranges – statistical approaches

  Tolerance interval based on analysis reduced number of RMP batches resulted in too wide ranges for biosimilarity assessment

- Use of non - EEA authorised RMP requires full quality comparison of non - EEA RMP with EEA RMP

- Differences in defined quality attributes needs to be justified and might require isolation and characterisation of the isolated variants

Limitations

- CQA's
  - Number of CQA's
  - Understanding of CQA's
  - Inter-relations of CQA's – moeities within the API
  - Clinical relevance – dose relationship mostly not established
  - CQA's controlled by process & IPC's
- Sample numbers – impossible to sample all RMP lots
- Sampling might be biased
  - Age of batches – shelf lifes – stability indicating QA's – comparison @ EOS, normalisation?
  - DP's from identical DS lots
  - RMP process changes
  - Batches from other markets – supportive only

Limitations

- Statistical limitations
  - Data BQL or „no new peaks“ above detection limit
  - „Presence of major peaks only“
  - Comparison of fingerprints
- Process-related impurities are process specific
- Statistical tools
  - Data size and distribution driven
  - CQA driven

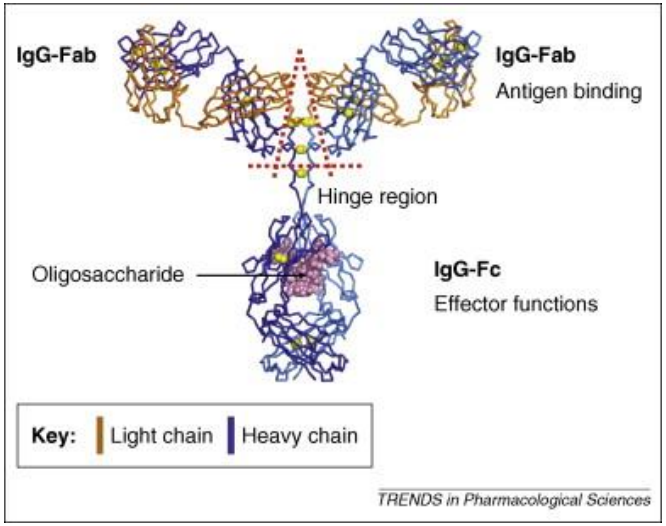
Examples

IgG – 150 kDa and 7.2×10^{16} possibilities

- Physicochemical characterisitcs
  - N-glycosylation sites (2)
  - Disulphide bridges (16) – shuffling/cleavage
  - Deamidation, acetylation, glycation
  - Methionine oxidation sites
  - Pyro-Glutamic acid
  - C-term lysine
  - Fragmentation, aggregation
- Interaction with target antigen

Affinity, avidity, crossreactivity

- Fc related interaction
  - Effector functions
  - Pharmacokinetics



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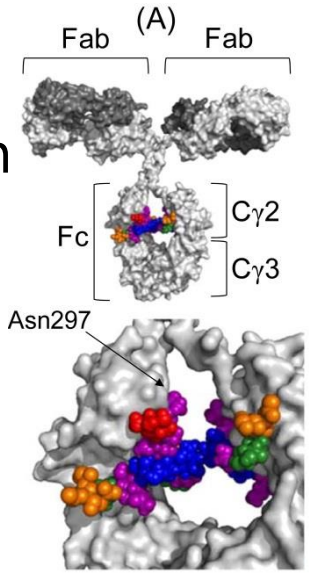
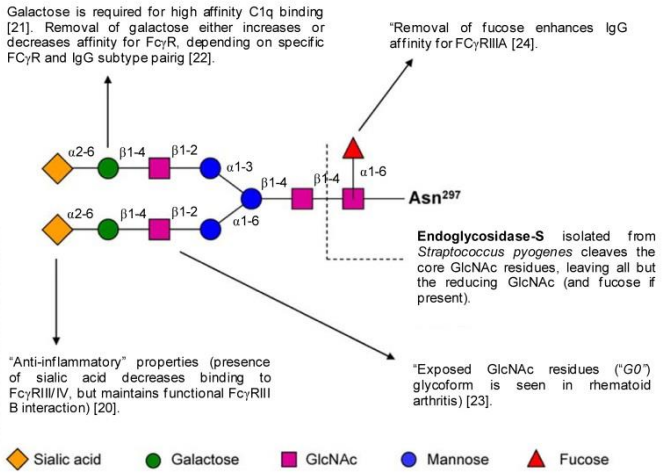
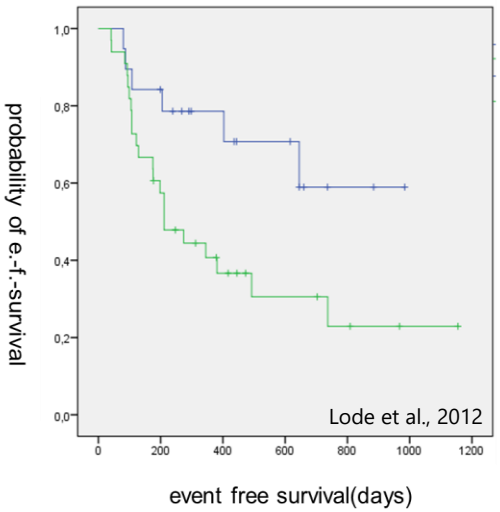




Unravelling Glycobiology by NMR Spectroscopy, Pomin 2011

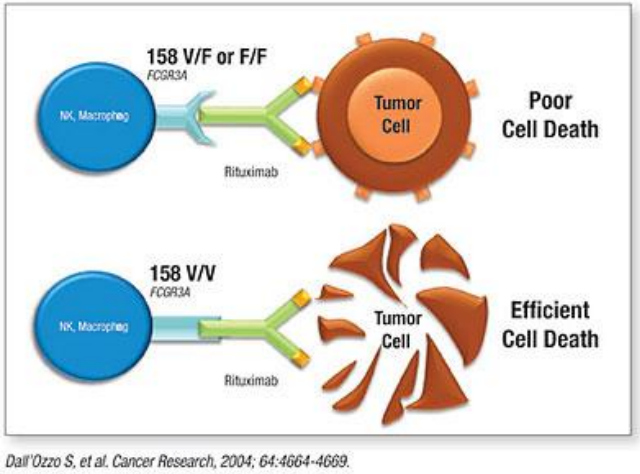
Clinical relevance of 158 F/V polymorphism

Dinutuximab



3A \ 2A	H/H	H/R	R/R
V/V			
V/F			
F/F			

Log-rank test: p=0.025



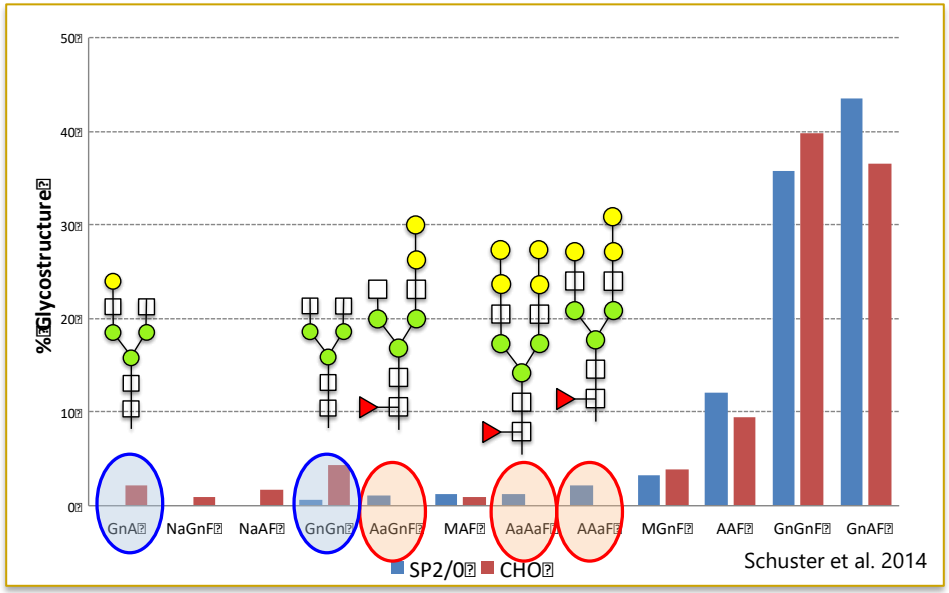
Dall'Ozzo S, et al. Cancer Research, 2004; 64:4664-4669.

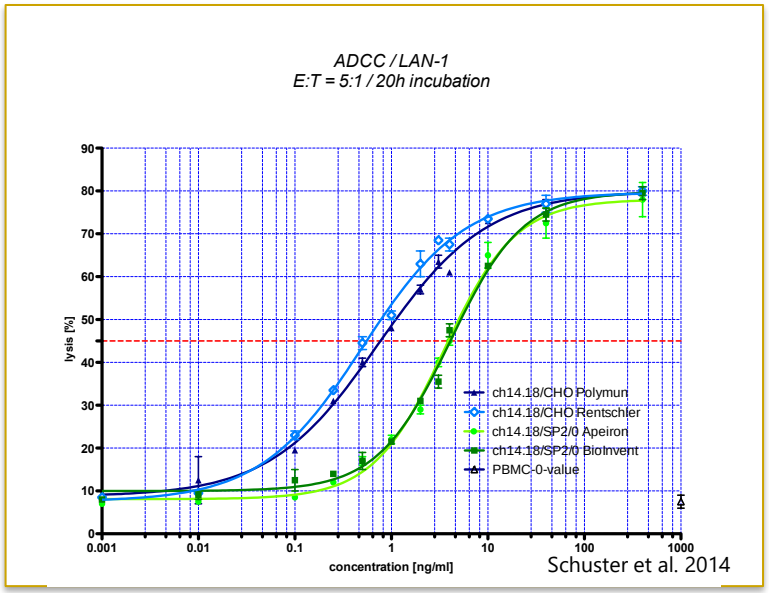
- Increased affinity of mAb binding in 158 V/V genotype
- Increased ADCC for 158 V/V genotype
- Reduced overall survival for F/F genotype

Importance of CD16 binding affinity

Small differences – considerable effects

N-linked oligo saccharides - Dinutuximab





- Presence of Galili epitopes on SP2/0 material
- Presence of afucosylated expression products on CHO material
- Clinical confirmation not feasible
  - No head to head comparison
  - Patient numbers - Orphan indication

Monoclonals are complex molecules

But well studied product-class

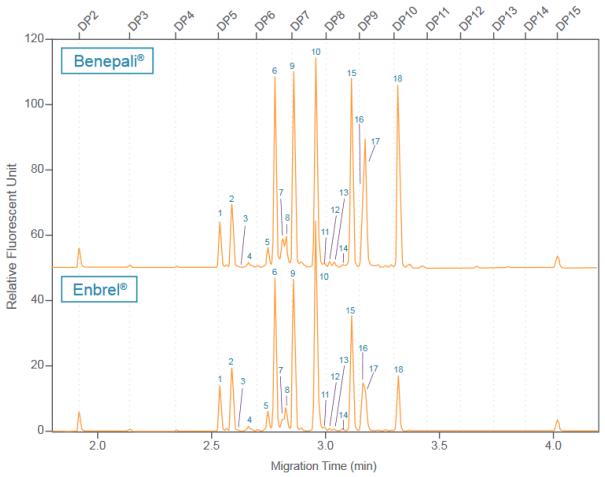
- The mode of action is complex and may involve contributions from multiple mechanisms
- High level of microheterogeneity
  - There will always be differences
  - Even small differences may have significant effects
  - Need to combine physicochemical results with functional assays (e.g. antigen-antibody binding assays and cell-based assays)
  - Qualification in preclinical and clinical studies
- Demonstration that differences do not impact on clinical efficacy and/or safety challenging
- But: We meanwhile know what to look at

Biosimilar to Etanercept

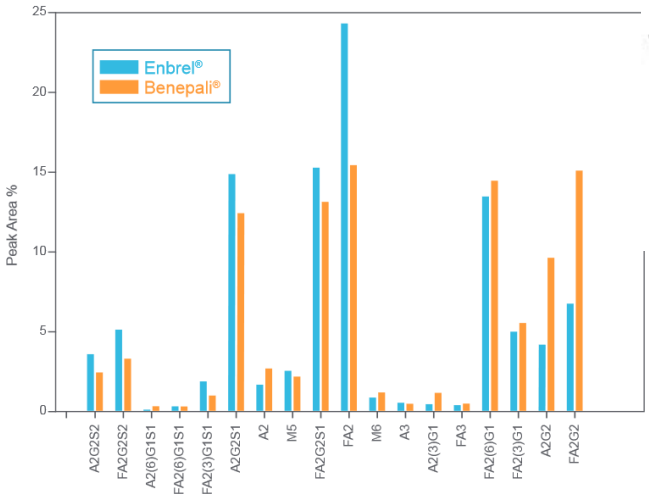
AGES

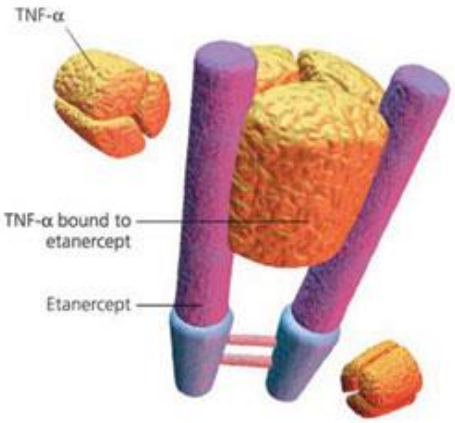
- Different N-glycosylation profile of BS
  - A-fucosylated glycan content in BS higher
  - CD16 binding and ADCC affected – Critical?
- TNF-alpha trap: ADCC not relevant MOA
  - Conclusion: Fucose content in this case not a CQA
  - Differences not clinically meaningful
  - No impact on the safety/efficacy

Cesar dog food: Perhaps we search out a companion that reminds us of ourselves



Assessing Glycosimilarity of Biotherapeutics, A. Guttman et al.



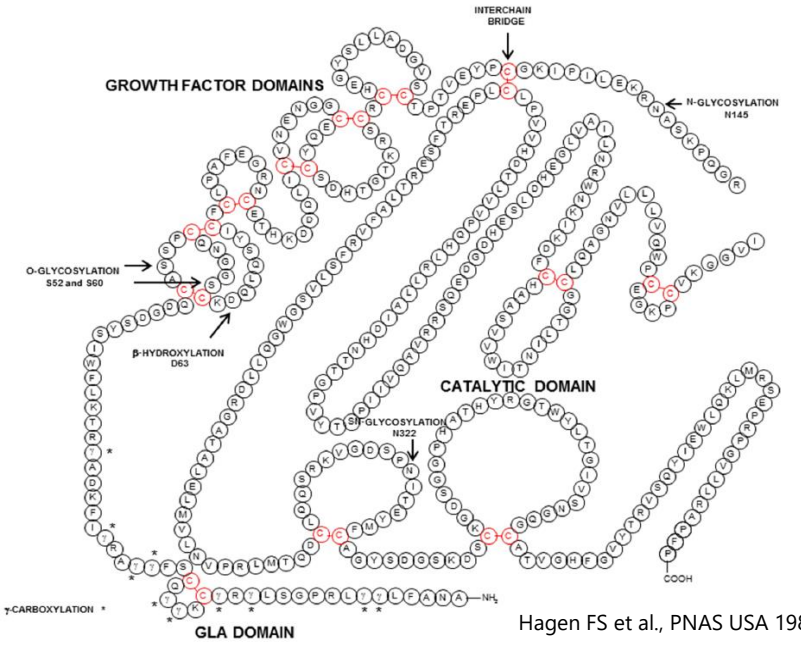


Biosimilars/News, 03.04.2020

rhFVIIa – 406 AA and 4.5×10^{15} possibilities

Which QA is uncritical?

- Multiple interaction partners
- Post translational modifications
  - 2 N- and 2 O-glycosylation sites
  - 1 Phosphorylation site
  - 1 ß-hydroxylation site
  - 12 disulphide bridges
  - Activation by proteolytic cleavage
  - Light chain – 152 AA, 20 kDa
    - N-terminal gamma-glutamic acid-rich domain – 9 γ-carboxylation sites with multiple calcium-binding sites and
    - 2 epidermal growth factor (EGF)-like domains (kringle domains)
- Heavy chain – 254 AA, 30 kDa
  - Catalytic domain and a single calcium-binding site



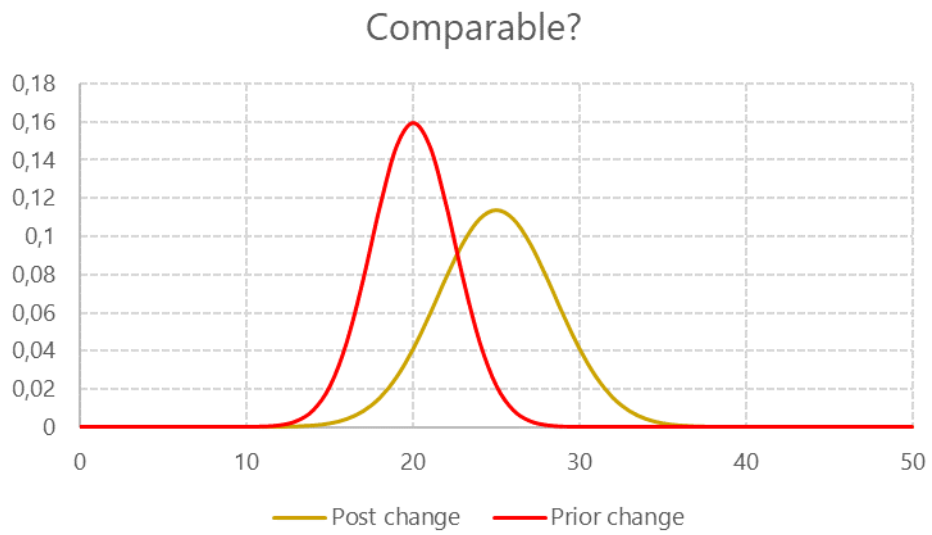
Hagen FS et al., PNAS USA 1986

rhFVII a - Complex molecule

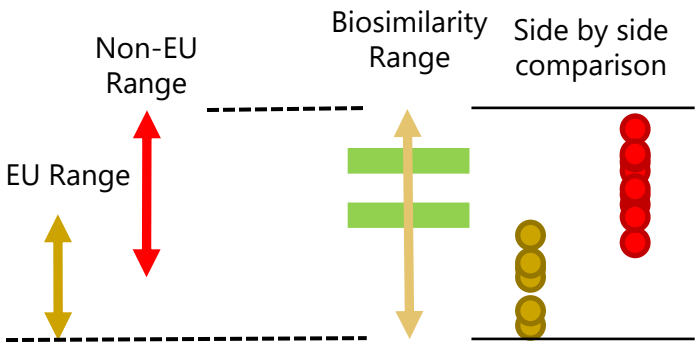
- Despite long lasting experience – less expertise
- Relevance of some post-translational modification unclear
  - Singular modification - interplay at molecular level
- So far no biosimilar approved
- What do we look at
  - In-depth characterization of the API and identification of CQA's
  - Understanding of process variability
  - Quantitative assessment of every CQA
  - Extensive assessment by functional assays
  - Detailed in-depth characterisation of isolated variants and structure-activity relationship studies
  - Clinical validation

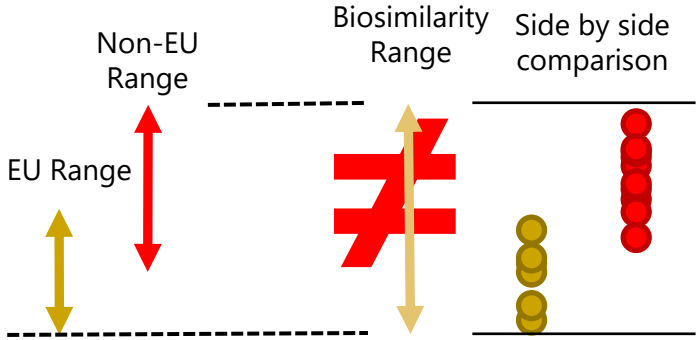
What do we compare

Establishment of acceptance criteria



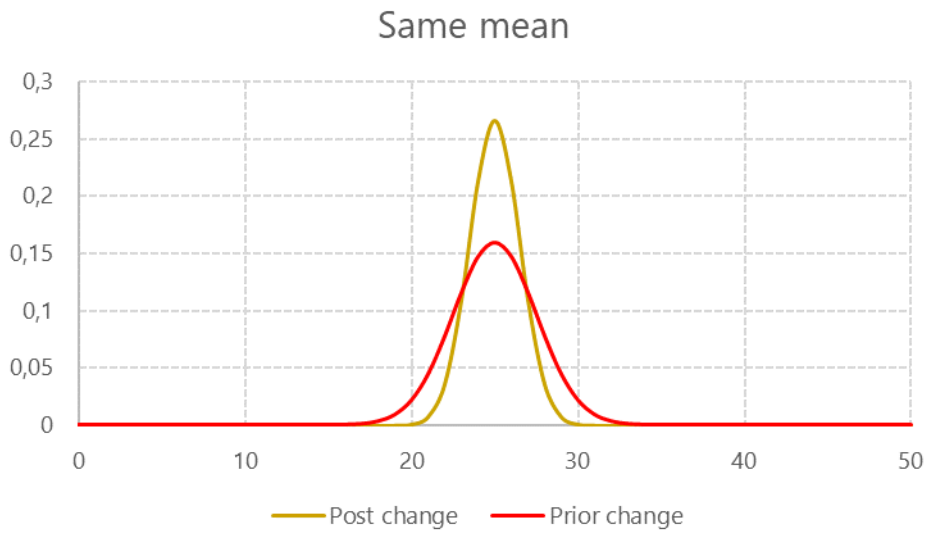
Cesar dog food: Perhaps we search out a companion that reminds us of ourselves



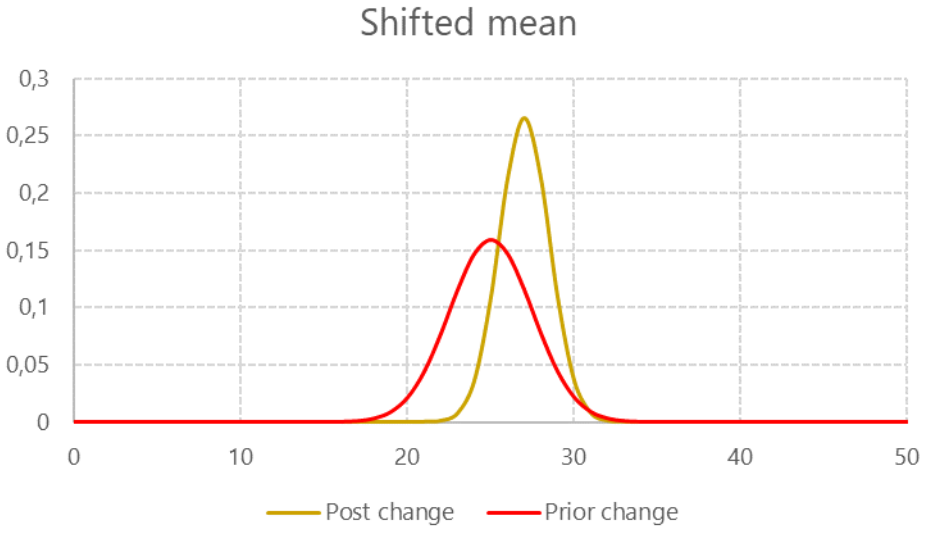


Macroscopic point of view

Continously Gaussian distributed random variables



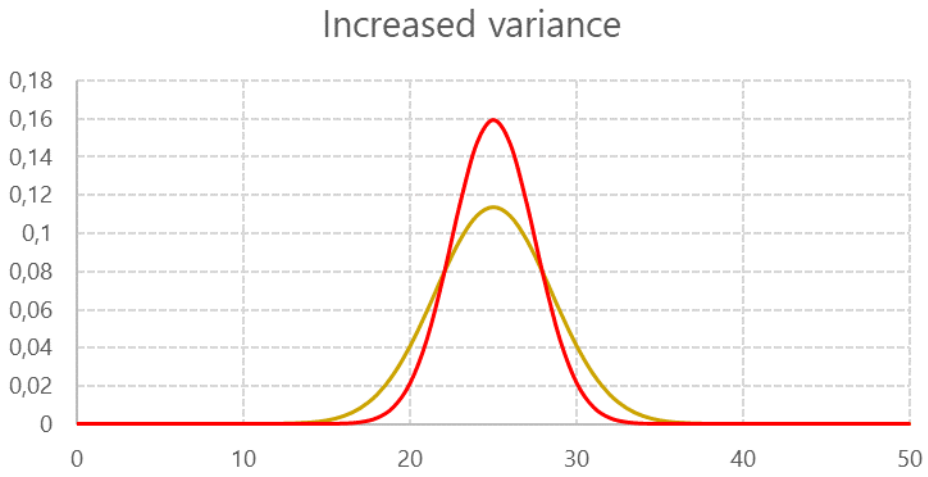
- Same mean, reduced variance
  - Improved analytical precision
  - Improved process variabiltiy
  - Clinically justified
  - Loss of variants unlikely



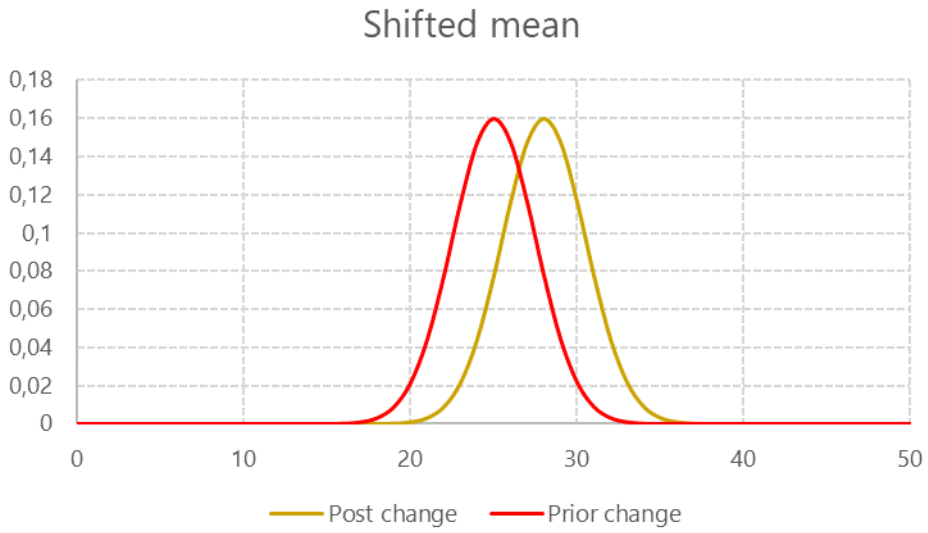
- Shifted mean, reduced variance
  - Improved analytical precision
  - Other Reference Std?
  - Reduced process variability
  - Loss of variants?
  - Increase of other variants?
  - Clinically justified?

Macroscopic point of view

Continously Gaussian distributed random variables



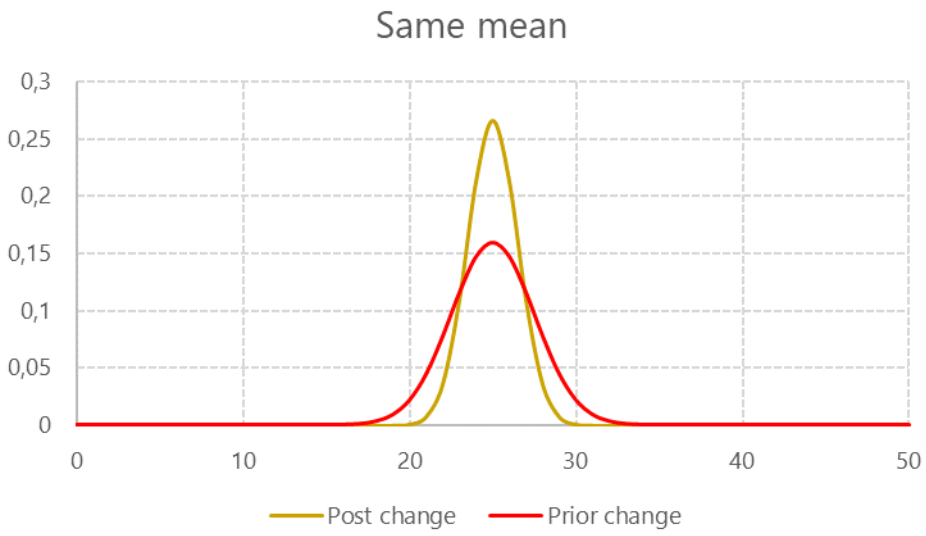
- Same mean, increased variance
  - Reduced analytical precision
  - Increased process variabiltiy
  - Clinically not justified



- Shifted mean, identical variance
  - Change in process
  - Clinically not justified

In-depth analysis

Random distribution by analytical variabilty?



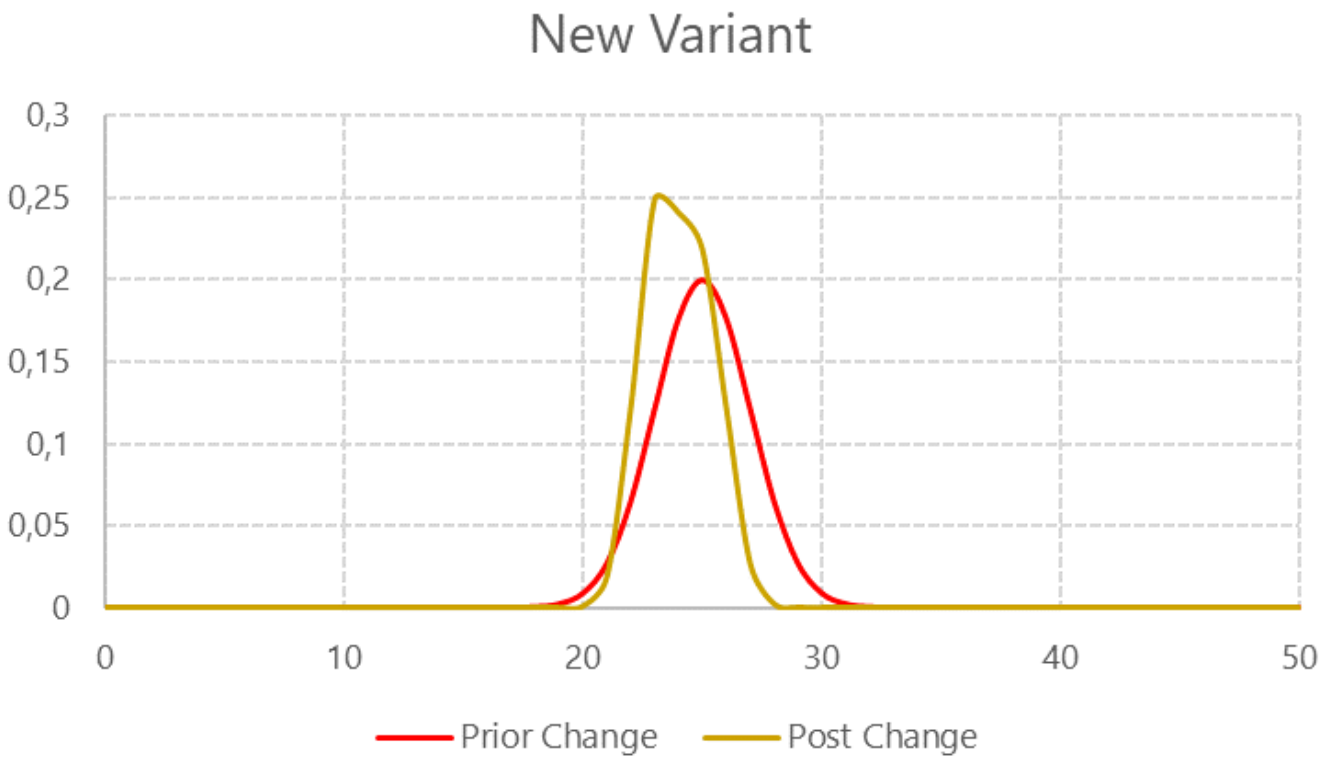
- Same mean, reduced variance
  - Improved analytical precision
  - Reduced process variabiltiy
  - Clinically justified

- Shifted mean, reduced variance
  - Improved analytical precision
  - Reduced process variability
  - Shifted mean contained within the original data range
  - Is it really clinically justified?

In-depth analysis

Different peak shape

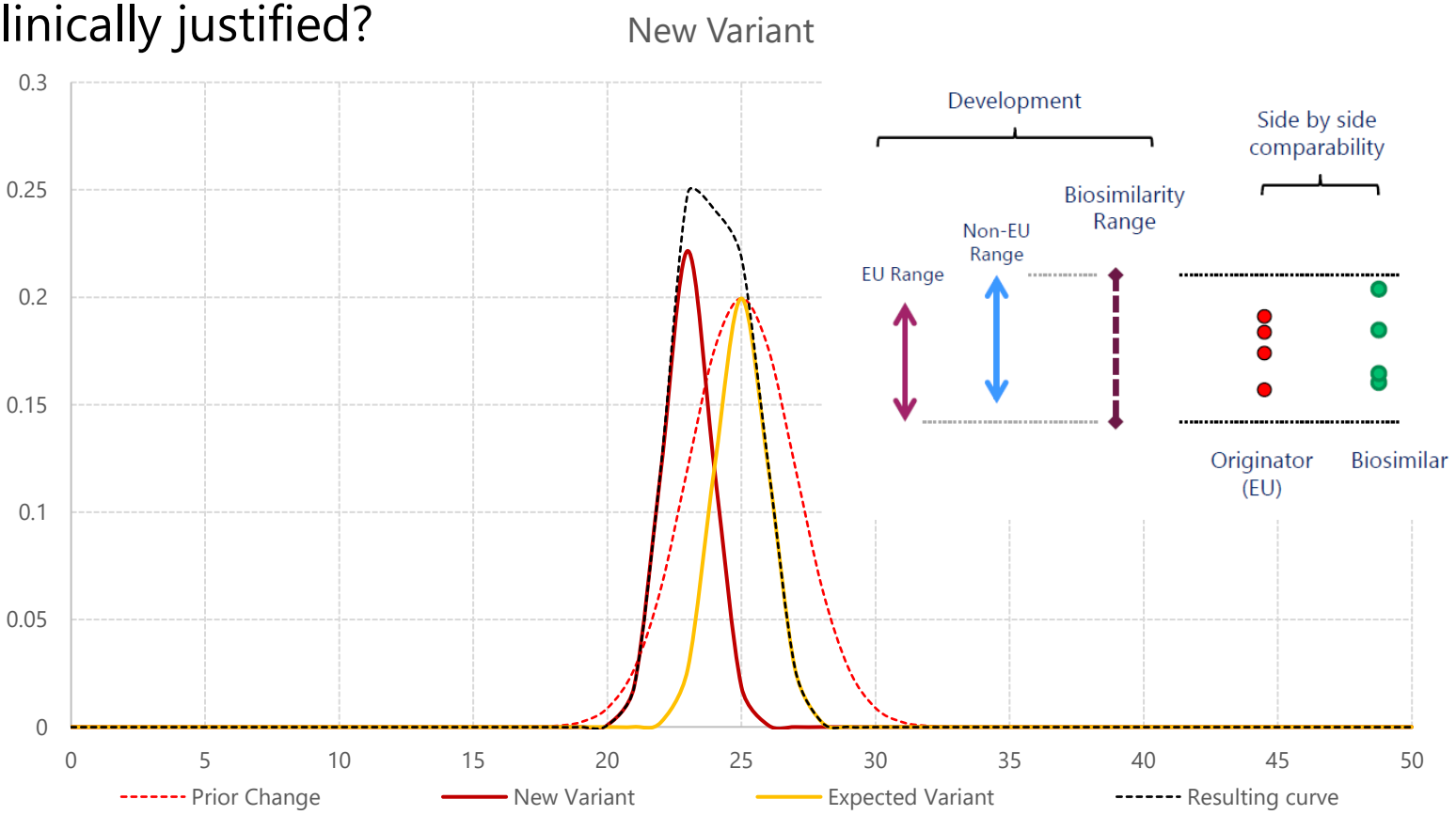
- Shifted mean, same variance?
  - Peak shape slightly different
  - Clinically justified?



In-depth analysis

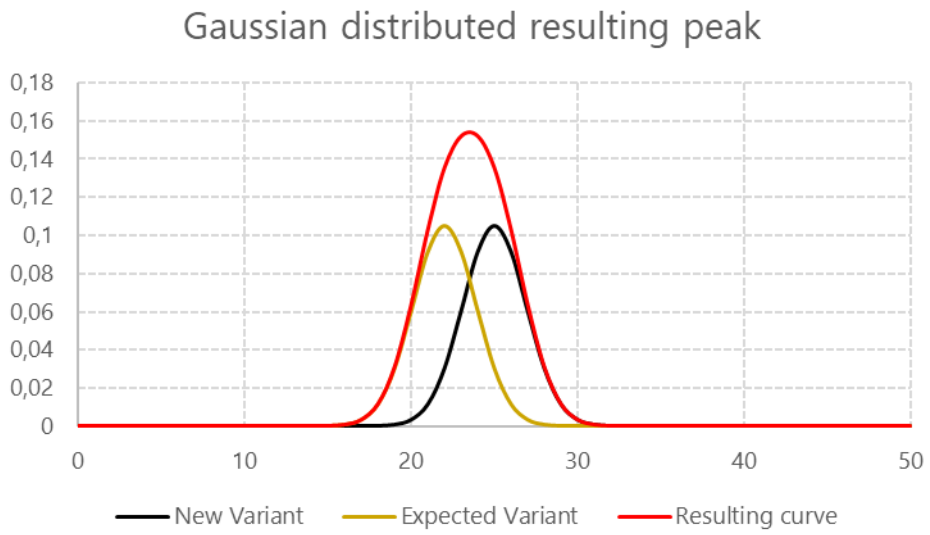
Discrete molecular variants!

- Change in manufacturing process leads to new variant
- Impact on safety and efficacy?
- Clinically justified?

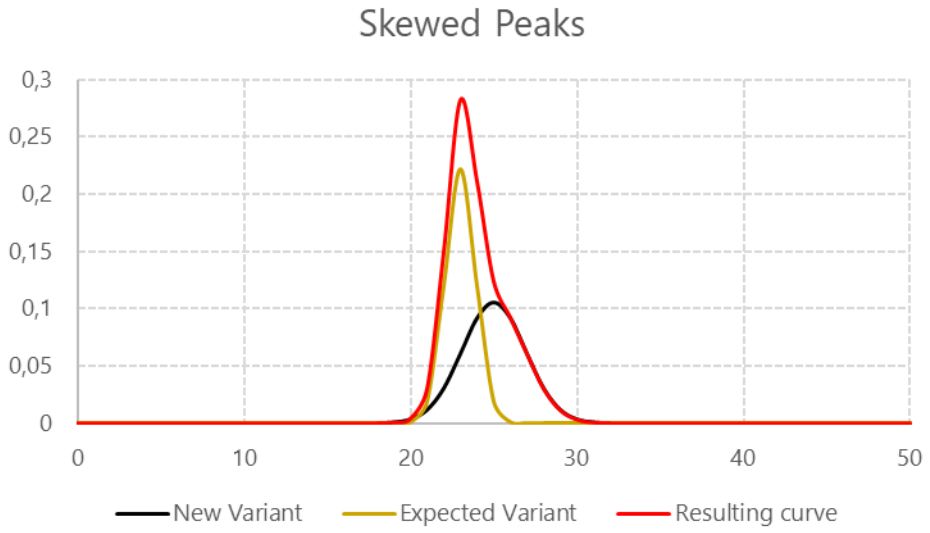


What is behind the peaks?

Random distribution by analytical variabilty?



- 2 species, similar amounts
  - Broad gaussian distributed peak
  - Improved analytical resolution
  - Orthogonal mehtods
  - Clinically justified?



- 2 species, different amounts
  - Further investigations required
  - Criticality?
  - Improved analytical resolution
  - Reduced process variability
  - Clinically justified?

It depends!

Defining a similarity condition

- Efficacy - what is the MOA
  - Antigen traps / neutralisation of ligand-receptor interactions
  - Cellular effector functions / activation of complement cascade
  - Impact of pharmacology – recycling via FcRn
  - Biological / cellular assays
- Safety - specific process and product related impurity profile
  - Immunogenicity – aggregates, oligosaccharides
  - Side effects triggered by complement activation
  - Charge variants, de-amided, oxidized, C-terminal Lysine variants
- mAbs with Fc-triggered effector functions
  - Binding to antigen and to Fcγ, FcRn and to C1q
  - N-linked oligo-saccharides

Establishment of a similarity condition

Summary

- Selection of multiple API based comparability criteria
  - Scientifically justified for substance class by literature
  - Indentified through in-depth product characterisation, isolation and analysis of defined variants and criticality assessment

  Examples: content of variant A, amount of defined oligo-sacch. structure,…
- Analytical performance
  - Identification and establishment of the analytical portfolio
  - Assessment of analytical suitability and variability
- Process performance
  - Assessment of process variability
- Definition of acceptance criteria for head to head assessment
  - Justification by RMP / (pre-)clinical studies with representative batches

Cesar dog food: Perhaps we search out a companion that reminds us of ourselves

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