Austrian Agency for Health and Food Safety

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Identification of an appropriate similarity condition

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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 assessment 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

Schuster et al., 1998
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
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 studies
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 controlled 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 analytical and process variability
• Robust towards outliers
• Focussed on mean

90% c.i. of means & w/i 1.5 \( \sigma \) 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 – moieties 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 $7.2 \times 10^{16}$ possibilities

- Physicochemical characteristics
  - 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

Unravelling Glycobiology by NMR Spectroscopy, Pomin 2011
Clinical relevance of 158 F/V polymorphism

Dinutuximab

- 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

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

Assessing Glycosimilarity of Biotherapeutics, A. Guttman et al.
rhFVIIa – 406 AA and $4.5 \times 10^{15}$ possibilities

Which QA is uncritical?

- Multiple interaction partners
- Post translational modifications
  - 2 N- and 2 O-glycosylation sites
  - 1 Phosphorylation site
  - 1 β-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
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

Comparable?

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 variability
  - 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

Continuously Gaussian distributed random variables

Increased variance

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

Shifted mean

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

Random distribution by analytical variability?

- Same mean, reduced variance
  - Improved analytical precision
  - Reduced process variability
  - 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?

![Graph showing the impact of changes on safety and efficacy](image)
What is behind the peaks?

Random distribution by analytical variability?

- 2 species, similar amounts
  - Broad gaussian distributed peak
  - Improved analytical resolution
  - Orthogonal methods
  - 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
  - Identified 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.