EDQM & European Pharmacopoeia: State-of-the-art Science for Tomorrow’s Medicines

International Conference organised by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe
19-20 June 2019, Strasbourg, France

Workshop on Biotherapeutics

Moderator
Mr Peter MJM Jongen, Chair of the European Pharmacopoeia Group of Experts on P4 Biologicals
Biotherapeutic Products in the Ph. Eur.: Have all the challenges been tackled?

Dr Mihaela Buda
European Pharmacopoeia Department
EDQM, Council of Europe
Discussion focused on the role of public standards in the field of biosimilars.

- Opposition to individual monographs is often caused by an unfortunate misuse and misinterpretation of monographs (e.g., monographs being used to assess biosimilarity outside Europe) → enhance communication, promote trainings, scientific publications......

- Unanimous recognition of importance of establishment of continuity of biological activity → achieved by the choice of robust pharmacopoeial bioassays and appropriate reference standards.

- Flexibility built into individual monographs for biotherapeutic products allows for biosimilars not to be excluded from the market; however, too much flexibility can lead to meaningless standards.

- Further feedback on the current approach to address the elaboration of monographs for complex molecules is awaited following publication of Infliximab monograph.

Presentation Outline

- **Introduction**: some achievements since the Tallinn Conference
- **Ph. Eur. and flexibility**: the case of biotherapeutic product monographs
- **Monograph elaboration/revision process**:  
  - participation and role of stakeholders
- **Monograph implementation** – impact on already approved products:  
  - Infliximab case study
- **Summary and concluding remarks**
Since Tallinn Conference

New Section Dedicated to Biotherapeutics on EQDM Website
New Section Dedicated to Biotherapeutics on EQDM Website

Related news; Related articles; Related events (e.g. trainings, webinars); Additional information (e.g. Technical guide)...

Technical guide for the elaboration of monographs on recombinant DNA proteins and synthetic peptides (Edition June 2018):

- general update to take into account recent experiences on elaboration of monographs for complex proteins;
- new section ‘Flexibility’.

https://www.edqm.eu/en/biotherapeutics
Ph. Eur. Monographs for Biotherapeutics

- **Insulin glargine (2571)**
  - 175 aminoacids
  - 18799 Da

- **Filgrastim concentrated solution (2206)**
  - 20 aminoacids 150 KDa

- **Teriparatide (2829)**
  - 34 aminoacids 4118 Da
  - 53 aminoacids 6063 Da

- **Follitropin concentrated solution (2286)**
  - 175 aminoacids 18799 Da

- **Etanercept (2895)**
  - 934 aminoacids 150 KDa

- **Infliximab concentrated solution (2928)**
  - 1328 aminoacids 145 KDa (non-glycosylated)

- **Therapeutic proteins (hormons)**
  - Peptide
  - Glycoproteins

- **Fusion protein**
- **Monoclonal antibody**

Ph. Eur. Monographs for Complex Biotherapeutics

**Monograph specifications**

- **Flexibility** of expectations, so that they apply to a large variety of products:
  - Ph. Eur. General Notices (alternative methods; waiving of tests; enhanced approaches);
  - "Additional" flexibility.

- **Prescriptive** requirements so that the respective test procedures can be applied successfully in a control laboratory/allow independent testing:
  - method performance (system suitability) criteria; qualification of analytical methods using Ph. Eur. standards;
  - acceptance criteria; standardisation of potency/functionality.
Monograph for Biotherapeutics: Flexibility

**Production section**
(Ph. Eur. General Notices)
- Requirements related to process-dependent heterogeneity (e.g. glycan profile, charged variants)

**Test procedures**
- Generic methods of analysis (e.g. developed according to general chapters) – suitable methods
- Specific analytical procedures – example method

**Acceptance criteria for quality attributes**
- Numeric limits/ ranges (specific activity; primary structure; related proteins; HMW species)
- ‘As authorised by the competent authority’ (process-dependent quality attributes)

**Reference preparations**
- Ph. Eur. reference standards to evaluate method performance (system suitability)
- In-house reference preparation – for comparative purpose (e.g. matching LC profiles)

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### Monograph flexibility

Individual monographs can address complexity of biotherapeutics

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Monograph for Biotherapeutics: Additional Flexibility

**SUITABLE METHOD**

- **general indications** on the test procedure (main steps to be carried out, type of method, readout, cells, reagents...)
- the term "suitable" is a conventional term: ‘In certain monographs [...], the terms 'suitable' and 'appropriate' are used to describe a reagent, micro-organism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.’ (General Notices)

**EXAMPLE METHOD**

- **specific instructions**, quantities, concentrations, compositions of reagents/buffers, chromatographic conditions etc. together with **system suitability criteria**; method may be used as such but any other suitable validated procedure may be used without demonstrating its equivalence to the ‘example’ method (subject to approval by the competent authority);
- “The following procedure is given as an example.”
**Ph. Eur. Monograph Elaboration/Revision: the Process**

Monographs are based on quality described for registered products

- Call for interest

1. **Request for monograph elaboration/revision**
2. **Endorsement by the Ph. Eur. Commission**
3. **Assignment to a Group of Experts**
4. **Creation/revision of the text by the Group**
5. **Public enquiry in Pharmeuropa**
7. **Publication in the Ph. Eur.**

- **OMCLs, assessors, companies**

- **Participation of interested parties**

- **Responding to Pharmeuropa enquiry is a must**

- **Data package**
  - Current specifications, analytical procedures; validation data; batch and stability data
- **Material** for testing
- **Candidate material for RS establishment**

- **Review of data package**
- **Draft monograph development**
- **Laboratory study/collaborative testing** – all preparations (protocol preparation; method verification; data analysis)
- **Draft published for comments**
  - (testing of draft monograph) – 3 months commenting period
- **Evaluation of stakeholder feedback**
  - (technical comments, data)

Once a monograph is published and implemented, MAH’s of registered products have to assure their product meets the requirements of the monograph.

**Impact of Monographs on Already Approved Products**

If a monograph is revised/published, what is the **impact** on the already approved product(s)?

- Compliance with the Ph. Eur. monograph is mandatory, manufacturers have to meet the requirements of the (revised) pharmacopoeial text at the date of its implementation (6 months after publication of the new/revised text company).
  - Company evaluates and secures compliance with the monograph within 6 months.
- This is why it is important that key stakeholders get involved in the monograph elaboration (revision) process as early as possible.
- This is why monographs are published for consultation.
**Scenario 1: Charged Variants**

**Ph. Eur. Monograph**

**A. Isoelectric focusing** – gel electrophoresis (Ph. Eur. 2.2.54)
- **test procedure**: example method
  - system suitability: pI markers; infliximab CRS
- **acceptance criteria (isoforms)**:
  - comparison with in-house RS profile

Alternatively, use a suitable capillary isoelectric focusing method developed according to general chapter 2.2.47. Capillary electrophoresis.

**B. CEX-HPLC** (Ph. Eur. 2.2.29)
- **test procedure**: prescriptive requirements
  - system suitability: infliximab CRS
- **acceptance criteria (isoforms)**:
  - in-house reference preparation
  - limits: ‘as authorised by the competent authority’

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**Scenario 2: Potency/Specific Activity**

**Ph. Eur. Monograph**

**Potency – TNF-alpha neutralisation**

- suitable cell-based assay and a suitable readout for assessing the inhibitory effect of infliximab on the biological activity of TNF-alpha; infliximab BRP (assigned potency in IU)
- **example method**: WEHI-164 cytotoxicity assay; WST-8 colorimetric readout
  - **method performance/system suitability**: infliximab BRP
  - **estimated potency**: 80-120% relative to infliximab BRP (numeric range)

**Specific activity**
- $8 \times 10^3$ to $12 \times 10^3$ IU per milligram of protein

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**“Company A” (registered product)**

**“Company B” (registered product)**

- **U937 apoptosis assay**;
  - in-house RS
  - estimated potency: x-y% (relative to in-house RS)

- In-house RS (working standard) to be established by comparison with infliximab BRP to which it is traceable. (Ph. Eur. Reference standards (5.12))

**Protein content**

**Potency (IU)**

- Specific activity*: fulfills pharmacopeia requirements

* As indicated under section “Definition”
Scenario 3: Related Proteins by CE-SDS

**Ph. Eur. Monograph**

**Capillary electrophoresis** (2.2.47) under both reducing and non-reducing conditions

- **test procedure**: prescriptive requirements
  - system suitability: infliximab CRS
- **limits**:
  - reducing conditions: $\sum$ peaks other than HC and LC: $\leq 2\%$
  - non-reducing conditions: $\sum$ peaks other than IgG peak: $\leq 8\%$

**“Company C”** (registered product)

**Different CE-SDS method**

**Ph. Eur. General Notices**
- demonstration of compliance with the Ph. Eur.
- alternative methods (equivalence testing)

**Higher limits**

**Do not miss Pharmeuropa!**

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**Ph. Eur. Monographs for Biotherapeutics: Other Cases...**

**Physico-chemical characteristics**
- Specific activity
- % potency
- Relative to THRS (~ BRP)
- Relative to BDP
- QA3
- QA4
- QA2
- QA1
- Directive (EC) 2001/83, Annex 1

**Biological characteristics**
- Approved specifications
- Product A
- Product B
- Product C
- Product D

**Public standard**
- One part of a total control strategy designed to ensure product quality and consistency

**The Pharmacopoeia monograph ensures continuity of product quality**

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Concluding Remarks

- Individual monographs play a major role in ensuring a standardised level of quality for medicinal products, thus contributing to patient safety.
- Ph. Eur. has over 30 years experience in the elaboration of texts on biotherapeutic products.
- Elaboration of monographs for biotherapeutic products present a number of challenges due to their complexity (meaningful standard vs flexibility).
- The latter challenge has proven to be more difficult to overcome; still, with the recent adoption of Infliximab monograph, Ph. Eur. has paved the way for an important class of new generation biotherapeutics.

Discussion on how to continue develop these standards and ensure their effective implementation needs all stakeholders!

Towards Ph. Eur. 10th Edition: Major Milestones

- Finished Product Monographs
- Filgrastim injection (2848)
- P4Bio pilot phase
- Etanercept (2895)
- MAB pilot phase
- Infliximab (2928)
- UNDER ELABORATION
  - General methodologies ("horizontal standards")
  - Bioassay
  - CE-SDS
  - cIEF

- Golimumab concentrated solution (3103)
- Under Elaboration

- Towards Ph. Eur. 10th Edition: Major Milestones

- General methodologies ("horizontal standards")
- Bioassay
- CE-SDS
- cIEF

- Teriparatide injection (3130)
- Insuline glargine injection (3129)
- Human coagulation factor VIII (rDNA) powder for injection (3106)
- Human coagulation factor VIII (rDNA), B-domain-deleted, powder for injection (3108)
Acknowledgements

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Thank you for your attention

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Ph. Eur. Reference Standards for Biotherapeutic Products

Dr. Marie-Emmanuelle Behr-Gross
Scientific Officer
DBO Department

EDQM and European Pharmacopoeia:
State-of-the-art Science for Tomorrow’s Medicines
19-20 June 2019, Strasbourg, France
Biological Reference Preparations for Biotherapeutics

European Pharmacopoeia biological reference preparation (BRP). A substance or mixture of substances intended for use as stated in a monograph or general chapter of the European Pharmacopoeia. BRPs are either secondary standards calibrated in International Units or primary standards, which may be used to define a European Pharmacopoeia Unit (Ph. Eur. U.). Other assigned contents may also be used, for example, virus titre or number of bacteria.

63 BRPs are currently distributed by the EDQM see list

Example: Infliximab BRP batch 1
Infliximab Biological Reference Preparation (BRP) Batch 1

Use in Infliximab potency bioassay in monograph 2928

Principles

Suitable cell-based assay allowing to determine the inhibitory action of infliximab on the biological activity of TNF-α

Suitable read out for assessing this effect

Standard Infliximab BRP

Example procedure

Cytotoxicity assay, measuring the cytotoxic effect of TNF-α on WEHI-164 cells. Cell growth assessed through a colorimetric assay

Ability of Infliximab to block inhibition of proliferation of WEHI-164 assessed against BRP 1

Infliximab BRP batch 1 – Establishment Frame and Study

Frame: Biological Standardisation Programme (BSP) an applied research programme for the establishment of Biological Reference Preparations (BRP) carried out with funding by the European Union and by the Council of Europe

Establishement study for Infliximab BRP1: BSP146 performed jointly with 1st WHO International Standard (IS) establishment study through a collaborative study involving 26 laboratories (public and private sector) using different in vitro cell-based bioassays and binding assays

Infliximab BRP batch 1 – Establishment - Some Data

Materials tested

Candidate freeze dried reference preparation (16/170) prepared from a single batch of bulk recombinant “biosimilar” Infliximab samples A and C

Comparator freeze dried product (16/160) prepared from a commercial pharmaceutical preparation sample B

Where available in-house standards IH

Final outcome

Candidate freeze dried reference preparation 16/170 was adopted as the 1st WHO IS and as Ph. Eur. BRP1 with an assigned potency of 500 IU/ampoule

Infliximab BRP batch 1 – Establishment – Outcome example

Adapted from C. Metcalfe et al, The first World Health Organization International Standard for infliximab products: A step towards maintaining harmonized biological activity, MABS, 2018

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### Infliximab BRP batch 1 – Establishment - Some Data

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Potencies relative to Candidate A</th>
<th>Potencies relative to IH reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GM</td>
<td>LCL</td>
</tr>
<tr>
<td>WEHI-164 cytotoxicity assay</td>
<td>A</td>
<td>1.04</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.95</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Overall cell-based neutralisation assays*</td>
<td>A</td>
<td>1.04</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.95</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Cytotoxicity using L929, WEHI-13 cell-lines; U937 apoptosis assay and reporter gene assays used in the WHO study.

WEHI-164 cytotoxicity assay – individual laboratory results:
- potency estimates within labs are very similar for preparations relative to A with GCVs < 11.6%.

(EDQM article under preparation for publication in Pharmeuropa Bio & Scientific Notes)

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### Usefullness of BRPs for biotherapeutic testing

The Ph. Eur. reference **BRPs are qualified for the intended use(s)** described in Ph. Eur. monographs and the publication of the results of the collaborative studies run for their establishment allows to grant access to important information on

- **Methods**: critical reagents, SOPs, intra and inter-lab variations, stats...

- **References**: IH or commercial standard, BRP (candidates, former batch), IS

- **Products**: test panel (products of different origins; princeps & biosimilar(s),...)

Infliximab: [https://doi.org/10.1080/19420862.2018.1532766](https://doi.org/10.1080/19420862.2018.1532766)

Etanercept: [https://doi.org/10.1016/j.jim.2017.03.007](https://doi.org/10.1016/j.jim.2017.03.007)
Usefullness of BRPs for biotherapeutic testing

Their intended use is for the determination of **pharmaceutical quality** in line with compendial standards and to serve that purpose they are available for

- European Regulatory authorities e.g. EMA for Centrally Authorised Products (CAP) post marketing studies (feasibility study on the model of “generics studies” for Filgrastim completed, Etanercept planned; third to come)

- Official Medicines control laboratories (OMCLs) for batch release, national market surveillance studies, for internal and external QA purposes, ...

- Manufacturers for development and control, for batch release and stability studies...

As such they are **not to be used for other purposes and notably in vivo assays, clinical comparability studies nor for defining biosimilarity**, and BRPs should not be inferred as serving this purpose

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**Ph. Eur. Reference Standards for Recombinant Glycosylated Biotherapeutics**

Dr. Sylvie Jorajuria  
Head of the Biology Section  
Laboratory Department

**EDQM and European Pharmacopoeia:**  
State-of-the-art Science for Tomorrow’s Medicines  
19-20 June 2019, Strasbourg, France
Why is glycosylation important for biotherapeutics?

1) Glycosylation impacts properties of the protein:
   - Bioactivity
   - Pharmacokinetics
   - Stability
   - Immunogenicity

2) Glycosylation:
   - Non-template-driven enzymatic modification
     -> glycan heterogeneity

3) Heterogeneity impacts product quality:
   - Batch to batch variability
   - inconsistency of production
   - risk of out of specification

Glycosylation must be: qualitatively and quantitatively controlled at all stages of the lifecycle and therefore must be controlled by the monograph.

Heterogeneity of the protein glycosylation

1) Two major types of glycosylation: O- and N-linked

2) Microheterogeneity in N-linked glycans

3) Expression system for rDNA proteins
Glycan analysis procedures in the Ph. Eur.

**General chapter: 2.2.59. Glycan analysis of glycoproteins**

Heterogeneity in glycosylation is assessed by 4 distinct and complementary approaches:

<table>
<thead>
<tr>
<th>Analytical target</th>
<th>Structure</th>
<th>Resulting information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact glycoprotein</td>
<td></td>
<td>overall pattern of glycosylation of the glycoprotein, limited information when the molecule is large and contains multiple glycosylation sites</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td></td>
<td>site-specific glycosylation properties, degree of occupancy, oligosaccharide structures</td>
</tr>
<tr>
<td>Released glycans:</td>
<td></td>
<td>populations of glycans present on the protein (bi-, tri-, and tetra-antennary profile), degree of sialylation</td>
</tr>
<tr>
<td>labelled</td>
<td></td>
<td>monosaccharide composition of a glycoprotein</td>
</tr>
<tr>
<td>unlabelled</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Glycan analysis in Ph. Eur. individual monographs**

- **Complexity, including number of glycosylation sites**
  - Intact protein
  - Monosaccharide/sialic acid
  - Released glycans
  - Category of analytical target

- **Analytical targets**
  - Etanercept
  - Infliximab
  - rFIX
  - Follitropin
  - rFVIIa
  - IFN β-1a
  - EPO
Intended purpose of Ph. Eur. RS in glycan analysis

- Reference standards for glycan analysis may serve 2 functions:
  - to control the performance of the method, including glycan cleavage, recovery and analysis
    -> system suitability
  "The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system” Ph. Eur. 2.2.46.
  - to assess acceptance criteria (qualitative, quantitative)
    -> quality benchmark
Confirmation of identity of the analytical target

**Means:** chromatogram included in the monograph

**Etanercept monograph – Glycan mapping**

Identification of peaks: use the chromatogram shown in Figure 2895.-1

- > Documentary reference

**Etanercept CRS**

Confirmation of compliance with qualitative requirements

**Means:** CRS for system suitability and in-house reference preparation

**Infliximab monograph – Glycan mapping**

- System suitability:
  - the chromatogram obtained with infliximab CRS is qualitatively similar to the chromatogram supplied with infliximab CRS and peaks 1 to 7 are clearly visible

- Results:
  - the profile of the chromatogram and the retention times of the peaks obtained with the test solution corresponds to that obtained with the chromatogram obtained with a suitable infliximab in-house reference preparation

- > Consistency of production using a production process specific reference standard

**Infliximab CRS**
Confirmation of compliance with qualitative requirements

Monograph is applicable to Infliximab produced in mammalian cell expression system

**Sp2/0 cell line**
Infliximab, manufacturer 1

**CHO cell line**
Infliximab, manufacturer 3 (simulation, expected differences)

Infliximab, manufacturer 2

- Infliximab CRS is used for system suitability
- For consistency of production purpose, an in-house reference preparation is required

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Confirmation of compliance with quantitative requirements

- **Quantitative measurement** of analyte levels

**Means:** CRS for system suitability and quality benchmark

**Etanercept monograph – Test for Sialic acid**

- Reference standard for sialic acid (e.g. N-acetyleneuraminic acid, NANA) to prepare a calibration curve
- System suitability:
  - the peak due to sialic acid in the chromatogram **obtained** with etanercept CRS is visible and is similar to the corresponding peak in the chromatogram **supplied** with etanercept CRS
- Results:
  - the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with etanercept CRS

**Etanercept CRS**

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Confirmation of compliance with quantitative requirements

• Quantitative **expression** of separation profile

**Means:** no quantitative criterion of the CRS

<table>
<thead>
<tr>
<th>Inflimab monograph</th>
</tr>
</thead>
</table>

Calculate the percentage contents of fucosylated, afucosylated and sialylated glycans, by normalisation

-> only for test solution

-> Sustainable CRS strategy

Opportunities/lessons from the past

• Extend the CRS role to control the **performance** of the methods

• Acceptance criteria in the monograph must be **specific** (i.e. measurable attributes) and not embedded in a particular batch of candidate CRS

Otherwise when the Ph. Eur. RS is replaced, the new batch may not be able to satisfy the same need without impacting the acceptance criteria

• **Caution** when using Ph. Eur. RS as benchmarking tool because its quality attributes may be mistakenly considered as the monograph specification
Key messages

• Ph. Eur. RS is just "a" material:
  - not necessarily related to the reference product
  - not necessarily related to the monograph specifications
  - is fit for the intended purpose

• Flexibility is built in and monograph provides means for transferability of the analytical procedure and independent testing

Perspective: trends in glyco-engineering

Optimization and control of N-glycan profiles through:
• Cell culture engineering (media, parameters)
• Genetic modification of glycosylation mediators
  Ex: to enhance the desired glycoforms (afucosylated glycoforms: mAb ADCC)
• Mathematical models: to predict glycosylation

-> Recombinant glycosylated biotherapeutic monographs and corresponding CRS will have to keep pace with development
Thank you for your attention

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Complying with Public Standards for Complex Biological Molecules

What must a manufacturer do to ensure compliance with the PhEur?

- Adopt current PhEur. monograph
- Request for revision of PhEur. monograph
- Justification of equivalence and/or superiority of a testing in Divergence with PhEur. Monograph

What does this mean for a biopharmaceutical manufacturer?

- Substantial and challenging workload to adopt new methods and revise specifications or demonstrate equivalence of Alternative Methods combining with a risk of rejection
- Regulatory submissions to request approval from HAs for changes
**Typical Post CMC Changes having Impact on Quality Product Attributes / Characteristics**

- Process changes are inevitable during LCM looking for optimization due to market demand, patient-centric approach and competitive environment.

- **Cell Line**
  - MCB
  - WCB...

- **Cell culture Media**
  - Composition
  - High Cell density
  - Raw Materials suppliers...

- **Process**
  - Equipment (e.g. disposable)
  - Fed Batch to Perfusion
  - Scale Up...

- **Other(s)**
  - Formulation composition
  - Stability conditions
  - Manufacturing Sites

**Case Study: Introduction**

- Substantial disparities between product quality profiles/characteristics and/or analytical results doesn’t prevent demonstration of Comparability / Biosimilarity

- Different analytical strategy and methods are used between manufacturers

- The assessment of criticality depends on the demonstration of impact (or non-impact) on the biological activity and PK of the variant
Case Study: Comparability Results on Licensed Products

* Comparison of the different pre- and post-change batches of Rituxan


* Comparison of the different pre- and post-change batches of Enbrel

Heterogeneity due to glycosylation

Comparison of Galactosylation

Comparison of Afucosylated

Comparison of High Mannose
Case Study: Comparability Results on Licensed IgG1

* Heterogeneity due to glycosylation

Manufacturer 1

Comparison of Sialylation

Sialylation
Not specifically reported

Comparison of Glycation

Glycation
Not reported

Manufacturer 2

Comparison of Acidic Variants
CEX-HPLC

Comparison of Basic Variants
CEX-HPLC

* Product related Impurities_Charge Variants (covering Deamidation, C & N Terminal Variant, Oxidation)
Case Study: Comparability Results on Licensed IgG1

Comparison of Purity (aggregates and Product related impurities) Testing Strategy

<table>
<thead>
<tr>
<th>Quality Attribute</th>
<th>Test Method</th>
<th>Quality Attribute</th>
<th>Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer</td>
<td>SEC</td>
<td>Monomer</td>
<td>SEC &amp; AUC</td>
</tr>
<tr>
<td>HMW variants</td>
<td>SEC &amp; AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimers</td>
<td>AUC</td>
<td>Antibody fragments (HC,</td>
<td>SEC &amp; nrCE-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LW, MMW)</td>
<td>SDS</td>
</tr>
<tr>
<td>Intact IgG</td>
<td>SEC &amp; nrCE-SDS</td>
<td>Intact IgG + Pre peaks</td>
<td>nrCE-SDS</td>
</tr>
<tr>
<td>Acidic/Basic Variants</td>
<td>CEX-HPLC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Expectation of a Standard Monograph

- How do Standardization principles address the complexity of large biological proteins?
- Can a Biotech Monograph sufficiently describe ACCEPTABLE QUALITY for MARKET use?
- And be A Reliable PREDICTIVE MODEL of this ACCEPTABLE QUALITY?
Expectation of Standard Monograph_Benefice for the Patient?

Weight of interest of a Biotech. Monograph VS Frequence of Revision

- Prescription of Minimal Quality Standard
- Fast Pace Scientific / Technical Evolution
- Complex Molecule Intrinsic Nature of Heterogeneity
- Increased demand for Biosimilar Products

A proposed forward-looking Strategy to fulfill Ph.Eur. Core Mission

- Moving from “Product Specific” toward a “Modular Adaptative” approach involving Class & Performance based Monographs & General Texts

Expectation of a Standard Monograph

- A proposed forward-looking Strategy to fulfill Ph.Eur. Core Mission

(1) Similarity with Ph.Eur transversal Approach
WELCOME TO THE 10 Edition!
We encourage the European Pharmacopoeia to adapt the standardisation for complex biologicals in 21st Century
The Need for Monographs on Biotherapeutics: A Biosimilar Perspective

Dr. Emmanuel Rossy
Scientific Fellow, Analytical Characterization
EDQM and Ph.Eur.: State of the art Science for Tomorrow’s Medicines
19th June 2019, Strasbourg, France

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Agenda

1. Introduction

2. Infliximab case study
   - Complexity to define molecule specific attributes due to proteins natural heterogeneity
   - Need for flexible monographs
   - Need for a rapid monograph amendment process

3. Conclusion

Infliximab - a well characterized monoclonal anti-TNF antibody

- Infliximab is a recombinant chimeric monoclonal IgG1 kappa antibody composed of a human constant region sequence and murine anti human Tumor Necrosis Factor alpha (TNF-α) variable sequence
- First infliximab approved by EMA in 1999 (Remicade®, Janssen Biologics)
- Monograph for Infliximab concentrated solution (2928) of Ph. Eur. is the first monograph on a monoclonal antibody (effective Jan. 2019)
**Totality of the Data from comparative evaluation to the reference medicine**

- **Clinical Confirmation**
  - Confirmatory efficacy and safety study

- **Pharmacokinetics**
  - PK study in healthy volunteers; Serum concentrations of efficacy and safety study

- **Non-clinical**
  - Single-dose TK/tolerability study in rats

- **Comprehensive physico-chemical and biological characterization**
  - Structural and functional comparison using state-of-the-art technology

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**Natural Heterogeneity of Proteins is Complex to Define Adequately**

- Infliximab can be produced using a CHO cell line (e.g., Flixabi®), the reference medicine with a SP2/0 cell line
- Some differences are expected e.g.
  - SP2/0 cell lines produce glycans with the alpha-gal motif and N-glycolyneuraminic acid (NeuGc)
  - CHO cells produce typically low levels of glycans with alpha-gal motif or NeuGc (they produce N-Acetylneuraminic Acid - NeuAc)

Glycosylation profiling by 2-AB labeling and hydrophilic interaction liquid chromatography coupled with fluorescence detection

Changsoo Lee, et al. (2017), mAbs, 9:6, 968-977
Novartis GDD

**Totality of Data Demonstrated Absence of ‘Clinically Meaningful Differences’**

- A combination of in-depth *in vitro* biological characterization and comparative PK study showed that total of glycoforms act synergistically and confirmed absence of ‘Clinically Meaningful Differences’.
- Knowledge gained from structure-functions studies supports implementation of innovative approaches and latest technologies (e.g. expression system).

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**Monograph Flexibility for Class-, Molecule- and Product-Specific CQAs**

- Product Specific CQA understanding is gained through development and is reflected into e.g. specifications.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Limits sets from EP9.6</th>
<th>Potential Alternative Limits for a CHO product</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-linked Glycans</td>
<td>Percentage of fucosylated glycans</td>
<td>G0F (Major Species)</td>
</tr>
<tr>
<td></td>
<td>Percentage of afucosylated glycans</td>
<td>G0 (Surrogate for total afucosylation)</td>
</tr>
<tr>
<td></td>
<td>Percentage of sialylated glycans</td>
<td>G1F (Surrogate for terminal galactosylation)</td>
</tr>
<tr>
<td>Impurities by capillary</td>
<td>Sum of all peaks other than heavy chain and light chain: maximum 2 per cent</td>
<td>EMA approved limits broader</td>
</tr>
<tr>
<td>electrophoresis under</td>
<td></td>
<td></td>
</tr>
<tr>
<td>reducing condition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 relevant to ADCC
2 relevant to CDC

- Individual N-linked glycans and their levels are a product-specific CQA
- Need for monograph capable to cover both infliximab specifications
Rapid Monograph Amendment to Maintain Scientific and Regulatory Standards

• Technology, Scientific knowledge and Regulatory expectations are continuously evolving

Example glycan analysis method from monograph 2928 based on strongly basic anion-exchange chromatography coupled with pulse amperometry detection (example selected from monograph 2928)

Reference medicine analysis using hydrophilic interaction liquid chromatography coupled with fluorescence detection

• Example glycan analysis method from EP 9.6 is likely not capable to address current expectations in regards to critical glycan species

• Need for a rapid and simple amendment process to adapt to standards and include all infliximabs currently approved

Conclusion

• Current perspective on product specific monographs for biologics is aligned with IFPMA and Medicines for Europe

• Infliximab monograph provides flexibility but illustrates the shortfalls of molecule specific monographs, based on a small molecule format, for complex and large biologics

• Biologics have increased complexity compared to chemicals, and this is reflected in the regulatory pathways
  • Regulatory agencies determine approval of biosimilars as they review the totality of data (Process, Methods, Limits, Analytical, functional and clinical comparability to reference product)

• The single sponsor model has its limitation as limits and methods are based on the sponsor experience with its own process
  • Limits and methods are not necessarily aligned with other scientific and regulatory viable solutions, and limits not set based on clinical impact only

• Monographs should champion high quality products and methods but provide sufficient flexibility to facilitate innovation
  • Leveraging latest technologies and methods or enhancing manufacturability for processes contribute to develop high quality and cost effective biosimilars
Acknowledgements

– Martin Schiestl
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– Robert Ernst Meyer
– Martin Hartinger

Thank you
Any questions?
Pharmacopoeial monographs for biologicals – a regulatory view

Dr. R.M. van der Plas, Sr assessor MEB, disclaimers apply
19 June 2019

EDQM and EMA
A philosophical question: Half full or half empty?

- Interesting philosophical question, but limited practical meaning.
- ‘Can I drink the contents?’
- The same applies to monographs!
  - ‘Can I use the contents?’
• Definition from 2001/83
  – A biological [active] substance is a substance that is produced by or extracted from a biological source and that needs for its characterisation and the determination of its quality a combination of physico-chemical-biological testing, together with the production process and its control.

• A biological is complex
  – Complete characterisation difficult
  – Variable
    • Between batches and between manufacturers
  – Control through testing only, not feasible
  – Case by case approach

‘Minimum requirements’

• A ‘complete’ or ‘perfect’ monograph is an elusive goal

• Consistent EMA-BWP position that ‘compendial monographs are minimum requirements’.

• This actually should not hamper monograph development;
  – No need to aim for a ‘complete’ monograph covering all aspects
  – Instead, aim for a monograph, comprising a number of parameters which are both critical and amenable to standardisation
Biological monographs

- Monographs for biologicals (active substance and finished product) have been around for decades

- Examples
  - Most (common) childhood vaccines and influenza
  - Most (common) plasma derived products, and analogues
  - Several ‘classical’ biologicals
  - Several rDNA products

- In addition, many chapters on biological methods available
  - Biological tests 2.6
  - Biological; assays 2.7

Reminder: Meaningful standard vs flexibility

- Need for appropriate equilibrium
- Flexible > applicable to more products/situations
- Sufficiently detailed to be useful for laboratories
- A standard which is too flexible is meaningless
Meaningful standard - concerns

• Commonly voiced concerns are related to flexibility and maturity

• ‘Monographs hamper innovation’
• ‘Monographs hamper development of biosimilars’

Monographs do not hamper innovation

• This concern mainly relevant for very new (classes) of substances
  – CQAs not fully understood; or ranges insufficiently qualified
  – Methods not fully optimised
  – Standardisation needs a certain maturity

• Many biological monographs have been around for decades and have demonstrated their usefulness.
  – Maturity achieved
  – May rely on available flexibility (see e.g. General Notices) for implementation of innovative methods – CQAs are fixed/minimum requirement
Monographs do not hamper biosimilar development

- Concern valid but manageable
  - Need for rapid monograph amendments for new monographs
  - Will diminish with experience gained (better insights in CQAs, both general and product-specific).

- New monograph often based on data from one or few manufacturers
  - Are these data representative for all future manufacturers?
  - Is this a ‘true’ and reasonable quality profile?

- Example:
  - Reduced CE-SDS in Infliximab (currently 2% impurities allowed; seems unnecessarily tight)
  - Not all new manufacturers can comply

Successes

- EU regulators do apply the monographs!
- Some examples of meaningful standards:
  - Infliximab, potency (test and range)
  - Human Coagulation Factor VIII
    - Both plasma (<275>) and recombinant (<1643>) source
    - Associated method 2.7.4 ‘Assay of human coagulation factor VIII’: Standardisation of biological activity measurement (methods and standards = calibration)
    - Associated monographs, e.g. <853> ‘Human plasma for fractionation’.
  - Influenza
  - Somatropin, insulin
    - Fixed conversion IU/mg
- Method chapters utilised to full extent?
  - Host Cell Proteins (extremely difficult assay to validate/standardise, esp. Accuracy/Trueness)
Challenges

- **Maturity**
  - Is a substance/subject sufficiently well developed for standardisation?

- **Flexibility**
  - Example, glycans (link to potency?);
  - Example, Mab-particles

- **Identification of Critical Quality Attributes**
  - Example, sialic acids in Infliximab

- **Effort/resources**

- **Main users, different needs/requirements**
  - OMCLs, assessors, companies (cave: minimum requirements)

Challenges – finished product monographs

- **Finished products monographs for biologicals have been around for decades!**
  - See e.g. Influenza vaccine, whole virion, inactivated <159, >Human coagulation factor VIII <275>, Human normal IgG for IM use <338>
  - Monoclonal antibodies for human use <2031>

- **Long experience with such monographs >> elaboration not a fundamental challenge**
  - May be limited by practical considerations
  - May benefit from similar properties between DS and DP
Some points for future attention

- Assay standardisation/method chapters
  - ADCC and its (glycoform) determinants – mature?
  - Pro coagulant activity in human plasma immunoglobulins

- Protein concentration
  - Easy to determine in relative sense -compared to (paper) standard
  - Difficult to measure in absolute sense
  - Standardised measurement of paramount importance: labelling

Conclusion

- Monographs for biologicals have been around for decades
- Recently adopted monographs (etanercept, infliximab) demonstrate that new monographs can be successfully elaborated
- Balance between flexible and meaningful
  - “Case by case”
- Focus on what can be standardised
  - Mature aspects (methods, CQAs, etc) based on experience
  - Pragmatic approach
Half full or half empty? Celebrate!

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