THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)
Development of New Ph. Eur. Bioassay "Horizontal" Standards: An Insight into the Anti-TNF-alpha Product Class Collaborative Study

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EDQM, Council of Europe
Presentation Outline

- Ph. Eur. and bioassays approaches
- MAB pilot phase:
  - ‘Bottom-up’ approach – horizontal standards development
  - TNF-alpha product class case study: bioassay collaborative study
  - Elaboration of a general chapter on Cell-based assays for potency determination of TNF-alpha antagonists: points to consider
- Concluding remarks and future perspectives
### Bioassay Approaches in the Ph. Eur.

<table>
<thead>
<tr>
<th>General monographs</th>
<th>Individual monographs</th>
<th>General chapters</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ classes of substances or products (defined by production method, intended use);</td>
<td>▪ based on approved specification(s) backed up by batch data;</td>
<td>▪ general recommendations for analytical procedures;</td>
</tr>
<tr>
<td>▪ mandatory requirements for all the products within the scope of definition section.</td>
<td>▪ validated analytical procedures*; acceptance criteria (*unless otherwise stated).</td>
<td>▪ guidance for design of analytical methods and for analysis of their results;</td>
</tr>
</tbody>
</table>

#### Chapter 5.12

**Reference Standards**

Biological Reference Preparations (BRPs)

Established specifically and exclusively for use in monographs, as prescribed in the procedures given.
### Bioassay Approaches in the Ph. Eur. – Therapeutic MAbs

#### Products of recombinant DNA technology (0784)

- **PRODUCTION – Characterisation:**
  - **Structure**: biological assays based on functional activity may serve as additional confirmation of the higher-order structure.
  - **Biological activity**: assessed by biological, biochemical assays,... as appropriate.
  - **Mechanism of action**: investigated and preferably reflected in the potency assay.

- **ASSAY – Potency**:
  - potency assay established using a suitable reference standard and carried out against this reference standard;
  - general chapter 5.3. may be used to design the assay and calculate the results.

#### Monoclonal antibodies for human use (2031)

- **PRODUCTION**:
  - **Product characterisation**
  - **Biological assay** chosen in terms of its correlation with the intended mode of action of the monoclonal antibody.

- **IDENTIFICATION**: the assay also contributes to identification.

- **ASSAY**:
  - carry out a suitable biological assay compared to the reference preparation;
  - design of the assay and calculation of the results: usual principles (for example, 5.3).
MAB Pilot Phase: A ‘Bottom-up’ Approach

Explore flexible concepts of standardisation:
- focus on key quality attributes and associated testing strategies;
- establish suitable common expectations and general methodologies with broad applicability.

- General monograph on *Monoclonal antibodies for human use* (2031)
- Product classes/sub-classes QAs
- HORIZONTAL STANDARDS: general methods to be used for purity testing, potency determination
- Product-specific QAs: flexibility; method performance criteria; examples of suitable procedures; establishment of Ph. Eur. reference preparations

* Published in Ph. Eur. Supplement 9.6 (July 2018)
** Under elaboration
Bioassay Standards: the Case of Anti-TNF-alpha Products

**Anti-TNF Biologic Drugs**

- prevent TNF-alpha receptor activation by binding to TNF-alpha, thereby neutralising the biological activity of TNF-alpha;
- biological activity evaluated in **cell-based potency assays** using different approaches for TNF-alpha neutralisation.

Adapted from Robert S. Woodrick & Eric M. Ruderman

**Ph. Eur. monograph for Etanercept (2895)**

- suitable cell-based assay based on the inhibitory action on the biological activity of TNF-alpha and a suitable readout for assessing this inhibitory effect.

- **Ph. Eur. Etanercept BRP**
- Example procedure: **U937 cell apoptosis assay**

**Ph. Eur. monograph for Infliximab concentrated solution (2928)**

- **Ph. Eur. Infliximab BRP**
- Example procedure: **WEHI-164 cytotoxicity assay**
Development of Bioassay "Horizontal" Standards

Anti-TNF-alpha Bioassay Collaborative Study

Assay Methodology

WEHI-164 cytotoxicity assay
HEK-Blue CD40L reporter gene assay
U937 apoptosis assay

Collaborative study: experimental verification of selected bioassay models

⇒ AIM: to evaluate suitability of candidate assays to be applied as universal methods for potency determination of TNF-α antagonists.
Anti-TNF-alpha Bioassay Collaborative Study

Study design

- **Study participants**: 9 labs, 8 countries (Official Medicines Control Laboratories, National Control Laboratories and EDQM Lab)
- **Sample panel**:
  - Etanercept: samples A, B
  - Infliximab: samples C, D
  - Adalimumab: samples E, F
  - Certolizumab pegol: sample G
- **Reference standards**:
  - Ph. Eur. Etanercept BRP (10000 IU/amp)
  - Ph. Eur. Infliximab BRP (500 IU/amp)
  - Adalimumab in-house RS (I)
  - Certolizumab in-house RS (H)
- **Bioassays** (based on common study protocol):
  - WEHI-164 cytotoxicity assay
  - HEK-Blue CD40L reporter gene assay
  - U-937 apoptosis assay
- **EDQM statistical analysis** (dose-response: 4-parameter logistic model; ratio-of-slopes approach for parallelism)
Bioassays Performed in the Collaborative Study

<table>
<thead>
<tr>
<th>Cell types</th>
<th>rhTNF-alpha</th>
<th>Assay</th>
<th>Readout</th>
<th>No. labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEHI-164</td>
<td>60 IU/mL</td>
<td>Cytotoxicity</td>
<td>Absorbance: WST-8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>15 IU/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-40 IU/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U937</td>
<td>40 IU/mL</td>
<td>Apoptosis</td>
<td>Luminescence: Caspase/Glo 3/7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>37.5 IU/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEKBlue CD40L</td>
<td>40 IU/mL</td>
<td>Reporter gene</td>
<td>Absorbance: QUANTI-Blue</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0.4 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WHO International Standard 3rd International Standard for Tumour Necrosis Factor-alpha (Human, rDNA derived)

WEHI-164 cytotoxicity assay

U937 apoptosis assay

HEK-Blue CD40L reporter gene assay
Study Design

- Preliminary assay:
  - TNF-alpha control curve
  - Reference standards:
    - Infliximab BRP (WEHI-164 cytotoxicity assay)
    - Etanercept BRP (U937 apoptosis assay)
    - Certolizumab pegol IHRS (HEKBlue rep. gene assay)
  - Controls:
    - specificity control (non-TNF-alpha antibody)
    - ‘cells only’; ‘cells + TNF-alpha’

- Final assays:
  - three assays (each assay spread over 2 days)
  - four plates per day (total of 24 plates/assay type/ lab)

- Reporting and evaluation of:
  - specificity
  - precision (plate, assay, lab)
  - recovery (mean vs. target)
  - cells only / cells + TNF-alpha
  - coefficient of correlation
## Bioassay Verification Study: Results Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WEHI-164 cytotoxicity assay</th>
<th>U937 apoptosis assay</th>
<th>HEK-Blue CD40L reporter gene assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
<td>no detectable activity of non-TNF-alpha antibody</td>
<td>no detectable activity of non-TNF-alpha antibody</td>
<td>no detectable activity of non-TNF-alpha antibody</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>cells only/cells +TNF-alpha &gt; 3 ( (n = 96) )</td>
<td>cells +TNF-alpha/cells only &gt; 2.5 ( (n = 96) )</td>
<td>cells +TNF-alpha/cells only &gt; 3 ( (n = 96) )</td>
</tr>
<tr>
<td><strong>Correlation</strong></td>
<td>( r \geq 98.5% ) in 90% of plates</td>
<td>( r \geq 97.5% ) in 90% of plates</td>
<td>( r \geq 99.5% ) in 90% of plates</td>
</tr>
<tr>
<td><strong>Mean bias(^1)</strong></td>
<td>( \leq 2.5% )</td>
<td>( \leq 2.5% )</td>
<td>( \leq 2.5% )</td>
</tr>
<tr>
<td><strong>Repeatability(^2)</strong></td>
<td>( \leq 10% )</td>
<td>( \leq 10% )</td>
<td>( \leq 5% )</td>
</tr>
<tr>
<td><strong>Intermediate precision(^3)</strong></td>
<td>( \leq 15% )</td>
<td>( \leq 10% )</td>
<td>( \leq 10% )</td>
</tr>
<tr>
<td><strong>Reproducibility(^4)</strong></td>
<td>( \leq 20% )</td>
<td>( \leq 10% )</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

\(^1\) Reference standard.
\(^2\) between plates within an assay; \(^3\) between different plates and assays within a lab; \(^4\) between different plates, assays and laboratories. GCV% are averaged over the results of all labs, assays & plates.
Product Cluster/Bioassay Format: Overall Results (1/2)
Product Cluster/Bioassay Format: Overall Results (2/2)

**ADALIMUMAB**

<table>
<thead>
<tr>
<th>Sample E</th>
<th>Sample F</th>
<th>Sample I (Ref.)</th>
</tr>
</thead>
</table>

**CERTOLIZUMAB PEGOL**

<table>
<thead>
<tr>
<th>Sample G</th>
<th>Sample H (Ref.)</th>
</tr>
</thead>
</table>

[Graphs showing relative potency for different samples and labs for ADALIMUMAB and CERTOLIZUMAB PEGOL.]
Anti-TNF-alpha Bioassay Collaborative Study: Conclusions

- **Proof of concept** demonstrated.

- **Each assay procedure works equally well for all anti-TNF-alpha products tested:**
  - **suitability** in terms of specificity and precision has been demonstrated for all other substances outside the scope covered by the initial validation;
  - **concentration range** may need to be modified for different products;
  - **curve fitting** for all curves very good;
  - assay procedure variability considered acceptable.

- Experimental data generated in the collaborative study **set the basis for defining:**
  - system suitability parameters and criteria to be included in the general chapter;
  - specific procedures to be described in the general chapter, including sufficiently descriptive conditions to facilitate successful independent analysis;
  - a common set of analytical expectations and approaches.

- **Critical parameters** and possible sources of variation identified:
  - level of details/prescriptive conditions to be suitably reflected in the draft chapter.
INTRODUCTION AND SCOPE

PRINCIPLE [different assay models]

GENERAL RECOMMENDATIONS

- assay qualification/controls

PROCEDURES

- preparation test/reference solution; TNF-alpha working solutions;
- cells preparation; plate preparation;
- addition of staining reagent

ADJUSTEMENT OF ASSAY CONDITIONS

SYSTEM SUITABILITY

- test and standard dose-response relationships similar (see general chapter 5.3 for methodologies on assessing similarity/parallelism of four-parameter logistic curves);
- standard curve: sigmoid curve; lower and upper plateaus; number of dilution points
- standard curve: coefficient of determination calculated for the standard curve ($r^2$);
- ratio ‘cell+ TNF-alpha control’ to ‘cells only’.

Scope of validation/verification

<table>
<thead>
<tr>
<th>Anti-TNF-alpha antagonist</th>
<th>U937 apoptosis assay</th>
<th>WEHI-164 cytotoxicity assay</th>
<th>HEK-Blue CD40L rep. gene assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etanercept</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Infliximab</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Certolizumab pegol</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

● signifies originally validated procedure
● signifies that suitability has been demonstrated during verification experiments
Future Perspectives

Ph. Eur. Bioassay Approaches – Horizontal standards:

- Draft chapter *Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)*: to be published for comments (Pharmeuropa) soon.

- **New frontiers** (different mAbs/shared functionality; types of bioassays; Fc-effector functions....)?

Discussion on how to develop these standards needs all stakeholders!
Acknowledgements

❖ Experts of the Ph. Eur. MAB Working Party

❖ Special thanks to the participants in the anti-TNF-alpha bioassay collaborative study

❖ EDQM Colleagues

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Emmanuelle Charton
Thank you for your attention

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