Reference Standards to Support the Development of Biosimilars

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www.nibsc.org
What is a biosimilar?

EMA …… a biological medicine that is developed to be similar to an existing biological medicine (the ‘reference medicine’). ……… The active substance of a biosimilar and its reference medicine is essentially the same biological substance, though there may be minor differences due to their complex nature and production methods. EMA/837805/2011

FDA …. a biological product that is highly similar to an already approved biological product, notwithstanding minor differences in clinically inactive components, and for which there are no clinically meaningful differences between the biosimilar and the approved biological product in terms of the safety, purity, and potency. FDA NEWS RELEASE Feb. 9, 2012

How does the development of biosimilars impact the requirement for, and provision of, public reference standards?

• How have things been done previously?
• What factors are different for biosimilars?
WHO establishes International Biological Standards which define the unit of activity for a biological.


The National Institute for Biological Standards and Control is its principal collaborating laboratory in this activity. *(NIBSC is a centre of the Medicines and Healthcare Products Regulatory Agency, UK, since 01 April 2013)*

NIBSC produces >95% of the International Standards

>600 catalogue items

>125,000 ampoules/vials distributed per year to >60 countries

[www.nibsc.org/products.aspx](http://www.nibsc.org/products.aspx)
Examples of particular relevance to biosimilar development:

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>80/505</td>
<td>Growth Hormone, Human, Pituitary (1st WHO International Standard)</td>
</tr>
<tr>
<td>83/500</td>
<td>Insulin, Human (1st WHO International Standard)</td>
</tr>
<tr>
<td>95/566</td>
<td>Interferon alpha 2b rDNA (WHO 2nd International Standard)</td>
</tr>
<tr>
<td>95/566</td>
<td>Interferon beta rDNA (WHO 3rd International Standard)</td>
</tr>
<tr>
<td>00/572</td>
<td>Interferon beta Ser17 rDNA (NIBSC Reference Reagent)</td>
</tr>
<tr>
<td>86/504</td>
<td>Interleukin-2 Cell line derived (WHO 1st International Standard)</td>
</tr>
<tr>
<td>92/788</td>
<td>Interleukin-11 rDNA (WHO Reference Reagent)</td>
</tr>
<tr>
<td>96/602</td>
<td>Luteinizing Hormone, Human, recombinant (1st WHO International Standard)</td>
</tr>
<tr>
<td>95/646</td>
<td>Parathyroid Hormone, Human, recombinant (1st WHO International Standard)</td>
</tr>
<tr>
<td>98/580</td>
<td>Prolactin Human, recombinant, glycosylated (1st WHO Reference Reagent)</td>
</tr>
</tbody>
</table>

National and regional organizations such as the pharmacopoeias, for example United States Pharmacopeia and European Pharmacopoeia, establish reference standards for bio-pharmaceuticals


crs.edqm.eu/db/4DCGI/web_catalog_CRS

Comparison of WHO and pharmacopoeial biological standards: differences in form and scope
(some exceptions. eg somatropin, heparin mol wt calibrants)
Two approaches to standardizing the same bio-pharmaceutical

<table>
<thead>
<tr>
<th>THE SECOND INTERNATIONAL STANDARD FOR ERYTHROPOIETIN, RECOMBINANT</th>
<th>THE Ph. Eur. BRP for ERYTHROPOIETIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIBSC Code: 88/574</td>
<td>250μg EPO</td>
</tr>
<tr>
<td>1μg EPO</td>
<td>Protein-free formulation</td>
</tr>
<tr>
<td>Protein-containing formulation</td>
<td>Supports Bioassays and physico-chemical assays</td>
</tr>
<tr>
<td>Supports Bioassays</td>
<td>Secondary standard, with the unit traceable to the WHO IS</td>
</tr>
<tr>
<td>Primary standard, defining unit</td>
<td></td>
</tr>
</tbody>
</table>

*Only a few exceptions (e.g., somatropin, some vaccines), where content is defined in SI, and may be considered primary standard.*

*Where content in SI is assigned, are usually true primary standards value assigned using a reference method.*

*Where content in activity units is assigned, are usually secondary standards, defined in terms of IS using compendial methodology.*
1. International Standards (IS) and units of bioactivity were established for naturally occurring biological molecules

2. The first generation of recombinant bio-therapeutic products were recombinant DNA versions of naturally occurring molecules such as insulin, growth hormone, interferons, erythropoietin, etc.

3. Usually, separate International Standards for the recombinant DNA versions were established, with the unit in some way traced or related to the previous “natural” IS.

4. Recombinant therapeutic products were traced to the new recombinant IS’s.
Route of development of reference standards for erythropoietin

1\textsuperscript{st} IS for EPO (Human urinary extracted)

First recombinant products

First Pharmacopoeial standards

2\textsuperscript{nd}/3rd IS for EPO (recombinant)

Pharmacopoeial standards

Recombinant products

Early R & D Product development
What needs to be considered with biosimilars?

1) Comparison with the originator product.

Particularly for physicochemical analytical techniques, need to demonstrate capability of the system to detect differences.
For biological activity, need to compare originator and biosimilar to common reference standard “…. international or national standards and reference reagents should be used to determine the potency and to express results in IU or U…….” WHO Expert Committee on Biological Standardization, Geneva, 19 to 23 October 2009 Guidelines on Evaluation of Similar Biotherapeutic Products (SBPs)

2) Many will be “next generation”, modified molecules:

- Biosynthetic structural variants
- Chemically derivatized natural molecules (eg pegylated)
- “Natural” molecules with no natural equivalent (monoclonal antibodies)
- Artificial constructs which don’t exist in nature (receptor-Fc fusion proteins)

Unlike the first generation of biotherapeutics, these biological molecules do not exist in nature. There are no pre-existing standards: they cannot be standardized until after drug discovery. The products get to the market without the existence of WHO standards, or traceability to an international unit.
Biosimilars – importance of the issue

Growth predicted in

- Number of products
- Market volume and share
- Geographical distribution production and marketing

- Biosimilar monoclonal antibodies (mAbs) and insulins likely to see the fastest growth
- Emerging markets, especially China and India, currently account for majority of revenue
- Growth in developed markets expected with patent expiries on earlier biologics in next few years


WHO have recognised the need for global standardisation of biosimilar products (WHO technical report series, 56th report, 941: 12-13, 2007).
# Biosimilars in Europe

## Biosimilars approved by EMA

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Active Substance</th>
<th>Authorisation Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abseamed</td>
<td>epoetin alfa</td>
<td>28 Aug 2007</td>
</tr>
<tr>
<td>Binocrit</td>
<td>epoetin alfa</td>
<td>28 Aug 2007</td>
</tr>
<tr>
<td>Biograstim</td>
<td>filgrastim</td>
<td>15 Sep 2008</td>
</tr>
<tr>
<td>Epoetin alfa Hexal</td>
<td>epoetin alfa</td>
<td>28 Aug 2007</td>
</tr>
<tr>
<td>Filgrastim Hexal</td>
<td>filgrastim</td>
<td>6 Feb 2009</td>
</tr>
<tr>
<td>Filgrastim ratiopharm</td>
<td>filgrastim</td>
<td>15 Sep 2008</td>
</tr>
<tr>
<td>Nivestim</td>
<td>filgrastim</td>
<td>8 Jun 2010</td>
</tr>
<tr>
<td>Omnitrope</td>
<td>somatropin</td>
<td>12 Apr 2006</td>
</tr>
<tr>
<td>Ratiograstim</td>
<td>filgrastim</td>
<td>15 Sep 2008</td>
</tr>
<tr>
<td>Retacrit</td>
<td>epoetin zeta</td>
<td>18 Dec 2007</td>
</tr>
<tr>
<td>Silapo</td>
<td>epoetin zeta</td>
<td>18 Dec 2007</td>
</tr>
<tr>
<td>Tevagrastim</td>
<td>filgrastim</td>
<td>15 Sep 2008</td>
</tr>
<tr>
<td>Valtropin</td>
<td>somatropin</td>
<td>24 Apr 2006</td>
</tr>
<tr>
<td>Zarzio</td>
<td>filgrastim</td>
<td>6 Feb 2009</td>
</tr>
</tbody>
</table>

## Biosimilars under review by EMA

<table>
<thead>
<tr>
<th>Active Substance</th>
<th>Number of applications</th>
<th>Originator product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filgrastim</td>
<td>1</td>
<td>Neupogen</td>
</tr>
<tr>
<td>Follitropin alpha</td>
<td>2</td>
<td>Gonal-F</td>
</tr>
<tr>
<td>Infliximab</td>
<td>2</td>
<td>Remicade</td>
</tr>
</tbody>
</table>

*Data from GaBI Online, data collected 11 April 2013*
European Medicines Agency recommends approval of first two monoclonal antibody biosimilars
Recommendation marks extension of biosimilar concept to new product-class

Remsima and Inflectra both contain the same known active substance, infliximab. In the application dossiers, they have been shown to be similar to the biological medicine Remicade, a monoclonal antibody that has been authorised in the European Union since 1999.

Biosimilar monoclonal antibodies

Earliest mAbs are coming off patent

Biosimilar mAbs will have exact amino acid sequence but different manufacturing clone/process resulting in micro variations which might effect PK, PD and potency

*Biosimilar trastuzumab produced in tobacco plants by PlantForm, 2013*
*Biosimilar palivizumab (2012) & rituximab (2011) produced in non-transgenic green plants by iBio*

Complexity of the molecules means that it is not possible to produce exact copies of innovator mAbs. Concerns have been raised by WHO over their quality and safety.

In addition there are examples of falsified and counterfeit mAb products being marketed
Over 28 therapeutic mAbs approved for treatment of a wide range of diseases

Slide courtesy of Simon Hufton, NIBSC
Are potency standards needed……

…… when products are labelled and dosed by mass?

Yes: biological activity measured in a bioassay is included in the specification. Potency is measured against a reference standard.

“…. international or national standards and reference reagents should be used to determine the potency and to express results in IU or U…….” WHO Expert Committee on Biological Standardization, Geneva, 19 to 23 October 2009 Guidelines on Evaluation of Similar Biotherapeutic Products (SBPs)

Two products are shown to have similar biological activity by reference to a common standard. A comparator product is not a reference standard.
Are product-specific potency standards needed….

……. for next-generation biotherapeutics (whether biosimilar or originator) if an International Standard already exists for the “parent” molecule?
Activity of modified molecule may differ from parent molecule

Comparison of the IS for G-CSF (filgrastim) with G-CSF product & 2 pegylated G-CSF products, GNFS-60 bioassay

Figure courtesy of Chris Bird, NIBSC
Comparison of 95/650 IFN-α2a standard and a peg-IFN-α2a product B in antiviral- and reporter gene- assays:

In this case, defining the unit of the pegylated interferon product in term of the parent interferon molecule would give method-specific potency estimates.

Figure courtesy of Tony Meager, formerly NIBSC.
“Blood products” have a substantial history with these issues

Natural, recombinant & modified products ……example: Factor VIII

**products:**
- plasma derived (> 30 products)
- recombinant full-length (4 products)
- B-domain-deleted recombinant (reduce immunogenicity) (1 product)

**in development:**
- full length/B-domain-deleted pegylated/Fc-fusion (increase ½ life)

So far, have not used biosimilar route

*Data courtesy of Elaine Gray, NIBSC*
Are product-specific potency standards needed….

PRODUCT vs WHO IS

VALID
parallel / linear

PRODUCT vs WHO IS

INVALID
non-parallel / non-linear

BY 1 METHOD

BY ALL METHODS

ASSAYS POSSIBLE

ASSAYS IMPOSSIBLE

USE METHOD TO LABEL IU

NO DISCREPANCY

DISCREPANCY

LABEL BY EITHER METHOD IU

PRODUCT-SPECIFIC STANDARD product-specific units

NEW METHODS

LABEL BY MASS

PRODUCT-SPECIFIC STANDARD product-specific units

SELECT SINGLE METHOD IU

& / or

Are product-specific potency standards needed....

Note: If the relative potency determination requires specification of a particular method, the method may need to be very tightly defined and controlled.

For a biosimilar for which a potency standard of the same molecular form exists, the standard should be demonstrated to be suitable in the range of potency assays used.

For biosimilars which are modified molecules, it may be analytically possible to define the unit in terms of an existing standard of the parent product in some cases.

but

these molecules are designed to have different activities in man

use of the same units could be clinically misleading.
Guideline on the declaration of the quantitative composition / labelling of biological medicinal products that contain modified proteins as active substance

The strategy for declaring the quantitative composition that would be acceptable for a product containing a modified protein will likely have to be considered on a case-by-case basis. Therapeutic proteins constitute a large number of products applied in a wide range of therapeutic areas and as such it may be difficult to follow a “one fits all approach”. Three different situations are envisaged:

- **Product labelling in mass units**
- **Product labelling in “in-house units”, i.e. the unitage is product specific**
- **Product labelling in International Units**
Specifications for biological therapeutics include a range of attributes requiring physico-chemical analyses. These, typically, include:

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>SDS-PAGE, Size –exclusion HPLC, Mass Spectroscopy</td>
</tr>
<tr>
<td>Charge</td>
<td>Ion exchange HPLC, Capillary electrophoresis, non-denaturing PAGE</td>
</tr>
<tr>
<td>Amino-acid composition</td>
<td>Reverse Phase HPLC</td>
</tr>
<tr>
<td>Sequence</td>
<td>Peptide mapping</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Glycan analysis, Capillary electrophoresis</td>
</tr>
</tbody>
</table>
Reference standards to support method validation and system suitability criteria

For biosimilars, need to meet specifications and demonstrate similarity to originator product.

Consider a size exclusion test for dimers and aggregates.

If the reference material and test samples look like this:

- Are all samples entirely monomers?
- Is the system incapable of resolving monomers, dimers and aggregates?
- Are the dimers and aggregates below the limit of detection of the system?

➢ Need to test system with impurities under investigation
System validation reagents are often provided by treating the CRS using defined procedures

-Oxidised products
  Hydrogen peroxide or chloramine-T treatment

-Aggregates
  Agitation or heat treatment

-Deamidation
  High pH treatment

Such procedures:

- are non-defined and irreproducible (“vortex for about 30s)

- are one of the most frequent sources of “it doesn’t work” user-feedback

- Cannot support defined limits of detection
The need to support validation of the method performance and system suitability criteria

Returning to the size exclusion test for dimers and aggregates:

If the prescribed system suitability test (70degC, 1h) produces this, it isn’t much use
Reference standards to support method validation and system suitability criteria

This preparation was prepared by controlled chemical cross-linking, thermal aggregation, and reformulation.

It is freeze-dried, apparently stable, and could have assigned identities and quantities.
Reference standards for physicochemical analytical methods

- A reference material which is similar to drug substance can serve to identify and quantify the drug substance in the sample.

- Additional molecular entities are required to identify and quantify impurities and demonstrate system suitability.

- Drug substance spiked with characterized impurities can serve multiple functions.
<table>
<thead>
<tr>
<th>Test</th>
<th>Standard</th>
<th>Function</th>
<th>Replaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size – exclusion HPLC</td>
<td>Stable dimerized preparation</td>
<td>Demonstrate column performance (separation and/or Limit of detection)</td>
<td>Defined method: Agitation/heat treatment methods</td>
</tr>
<tr>
<td>Ion-exchange HPLC</td>
<td>Deamidated preparation</td>
<td>Demonstrate column performance (separation and/or Limit of detection)</td>
<td>Defined method: High pH treatment</td>
</tr>
<tr>
<td>Reverse Phase HPLC</td>
<td>Oxidised preparation</td>
<td>Demonstrate column performance (separation and/or Limit of detection)</td>
<td>Defined method: Hydrogen peroxide treatment</td>
</tr>
<tr>
<td>Peptide mapping</td>
<td>Single amino-acid mutants</td>
<td>Demonstrate system resolution</td>
<td>Nothing</td>
</tr>
<tr>
<td></td>
<td>Target peptides</td>
<td>Support quantitative applications</td>
<td>Nothing</td>
</tr>
<tr>
<td>Glycan analysis</td>
<td>Glycan preparations</td>
<td>Demonstrate system resolution</td>
<td>Test substance</td>
</tr>
<tr>
<td></td>
<td>High/low pl preparations</td>
<td>Support quantitative applications of Z number</td>
<td>Nothing</td>
</tr>
</tbody>
</table>
Glycoprotein glycan reference standards

Glycosylation patterns - determined by the producing cell line, growth and purification conditions, rather than the introduced gene.

Analysis - usually by cleavage of the glycan chains from the protein backbone

- Analysis is independent of protein backbone
- There is a heterogeneous mixture of glycans
- The glycan mix can be profiled by chromatographic, electrophoretic or MS analyses

Proposed USP glycan standards – developed in collaboration with NIBSC

- Four mixtures of different types of glycan, designed to cover different groups of recombinant proteins
- The analytical method should be able to resolve this mixture into (at least) the specified number of components with appropriate relative amounts.
- Suitable methods are given in (draft) USP chapter <212>, but…….
- Usage of the standard should be independent of the analytical method

CMC Strategy Forum July 2013
HPLC separation of fluorophore-labelled glycan standards of proposed USP standards relevant to mAbs

Figure courtesy of Chris Jones, NIBSC
Three dimensional structural similarity?

Folding and three dimensional structural integrity:

- Often analysed by circular dichroism or Fourier transform infra-red
- Often not clear whether minor differences are significant
- Replace subjective visual comparison with statistical approach
- Use a reference standard (e.g. one protein spiked with varying amounts of another) to understand when spectra are significantly different.
## Logistical issues in development of public reference standards for biosimilars

- **Identification of requirement, selection of types of material(s)**  
  - *data contribution & collation*

- **Decision to proceed**  
  - *cooperation, coordination, harmonization, MoUs*

- **Procurement of materials**  
  - *drug material: drug manufacturers, originator/biosimilar impurities, fragments, other moieties – manufacturers/other laboratories*  
  
  (potency 1ug drug per vial? Physiochemical 10-50 ug ?)

- **Processing development**  
  - *utilizing (usually extensive) existing data*

- **Processing, testing**

- **Collaborative study**  
  - *time and effort should not be underestimated*

- **Agreement on outcome**

- **Storage, distribution**  
  - *cost and infrastructure*
Reference standards for biosimilars

In conclusion

- The need for reference standards to support development of biosimilars has already arrived and is expected to increase rapidly
- This requires additional and different types of reference materials from those required previously
- A cooperative, structured approach and harmonization will benefit all
- WHO and pharmacopeias will need to modify and adapt their current approaches to meet the changing requirements
- Guidance and feedback from regulators will help focus efforts appropriately
- Progress will depend on input from manufacturers and CROs: information on anticipated requirements, materials, data on materials, contribution to testing candidate standards