BIOSIMILAR DEVELOPMENT: FROM SCIENCE TO MARKET

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The biotechnology industry has established itself as a major source of new human therapeutic protein drugs which can be described as complex biological entities. The majority of these in production, such as erythropoeitin (EPO), interleukin-2 (IL-2), interferon (IFN), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF) and tissue plasminogen activator (TPA), occur naturally in the form of glycoproteins.

The first approved recombinant DNA-produced biologic drug was human insulin, in 1982. Since then, the global biopharmaceutical market has grown considerably and was valued at an estimated $125 billion in 2010[1]. Many of the first generation products have reached, or are about to reach, patent expiry and this has led to the advent of "Biosimilars" - legally approved versions of an existing branded biologic which are granted marketing approval on the basis of analytical, pre-clinical and clinical data which show they are highly similar to the reference product.

SMALL MOLECULE GENERICS VS LARGE MOLECULE BIOSIMILARS

By their nature, small molecule drugs are able to be well defined chemically. This, together with rigorous testing by originators, enables generic manufacturers to avoid full, costly, clinical evaluations by establishing that their product is "bioequivalent" to the originator.

Unlike small molecule drugs, biologically derived products are large, complex molecules, usually comprising of a mixture of closely related species. When expressed in recombinant form in mammalian systems, the protein backbone of a biological may be identical to the native, however, the glycosylation pattern will depend on the cell type used and the physiological status of that cell. Although there are examples where glycosylation of the recombinant molecule does not appear to have an in-vivo influence (eg G-CSF), in others, most notably TPA and EPO, glycosylation is certainly required for biological activity. It is now widely agreed that protein glycosylation patterns and their degree of microheterogeneity are extremely important for many reasons, including potential immunogenicity and should be characterised thoroughly in addition to the protein moiety.

The complexities of biomanufacturing make exact replication of the originator's molecule nearly impossible. Indeed, for the originator, despite years of experience with the patented process, the manufacture of consistent batch-to-batch material may still pose a challenge. As it is not considered possible to manufacture "copy" versions of bioproducts using a process which will certainly be different to the originator, biosimilar proteins cannot be approved as simple generics.

REGULATORY ISSUES

EU blazes the trail. . .

In 2003, the EU first held discussions around the concept of follow-on versions of recombinant proteins and subsequently established the first guidelines, which took effect in 2005, for "similar biological medicinal products"-i.e. biosimilars. The basis of the guidelines is that the Committee for Medicinal Products for Human Use (CHMP) requires physical, chemical and biological characterisation of the biosimilar in comparison to the reference product. In addition to this extensive characterisation, non-clinical and clinical data are required to demonstrate the same safety and efficacy profiles as the originator. However, the premise of the published guidelines is that the amount of non-clinical and clinical data required will be much less than for a novel stand-alone application.

The initial "overarching" guideline, CHMP/437/04[2] was followed by guidelines on quality[3] and non-clinical /clinical issues[4], with additional product specific annexes, initially for somatropin (human growth hormone, HGH)[5], G-CSF[6], EPO[7] and insulin[8]. These were then followed more recently by additional guidelines regarding interferon alpha[9], low molecular weight heparins[10] and monoclonal antibodies[11].

The first biosimilar molecule approved in April 2006 in Europe by the European Medicines Agency (then EMEA, now EMA) was Omnitrope, a version of somatropin. This was closely followed by another HGH, Valtropin two weeks later. To date, the EU has approved 14 biosimilars, all of which are versions of somatropin, epoetin (EPO) or more recently, filgrastim

(see Table 1). Some early applications, (e.g. interferon alpha-2a and insulin) were not successful; either rejected or withdrawn voluntarily. Commentary on these applications can be seen on the EMA web site (see Table 2).

US PERSPECTIVE

It has been just over one year (23rd March 2010) since President Obama passed the "Patient Protection and Affordable Healthcare Act". This was the foundation of regulatory legislation designed to pave the way to cut spiralling healthcare costs by creating a potentially less costly route for approval of certain biotherapeutics. The Biologics Price Competition and Innovation Act (BPCIA), part of the Affordable Care Act provides a new pathway for biosimilars- the 351(k) route. It requires comparison to a single reference product which has been approved under the normal 351(a) route with reference to prior findings on safety, purity and potency. The legal pathway provides for two levels of products-Biosimilar and Interchangeable Biosimilar. The exact requirements for the latter option are still to be fully defined. In addition there are very complex patent disclosure provisions with which manufacturers must contend. The Act, however, passes responsibility for defining the guidelines for regulation to the US Food and Drug Administration (FDA) and so far there has been silence on their part as to what data will be required. The FDA Commissioner, Margaret Hamburg, has been quoted as saying these rules will be communicated "very soon".

In the meantime, many other countries including Brazil, Australia, Turkey, Taiwan, India, Malaysia, Argentina, Mexico, Japan, Canada and South Africa have established regulatory pathways. In Japan, the term "Subsequent Entry Protein Products (SEPP)" is used. Some countries such as Australia and Malaysia have modelled their guidelines on those of the EMA. The WHO adopted a "Guideline on Evaluation of Similar Biotherepeutic Products" in October 2009.

STRUCTURAL CHARACTERISATION OF BIOSIMILAR PRODUCTS - WHAT TO DO?

Any manufacturer seeking to develop and market a biosimilar product must perform comprehensive physicochemical structural characterisation of the (glyco)protein. This task will have to be performed at three distinct stages of development. Initially, the exact sequence of the target originator molecule needs to be determined. Then, once the biosimilar product is produced, confirmation of its structure must be performed. Finally side-by-side comparative data for the biosimilar and the originator molecule must be obtained as required by various regulatory guidelines. Strategies should include assessment of primary and higher order structure and batch-to-batch variation should be determined for both the biosimilar and the reference product. In practice this requires the same types of analytical techniques which have been used for characterising the molecule, but now they are conducted side-by-side

TABLE 1: BIOSIMILARS APPROVED BY THE EU

TRADE NAME	ACTIVE SUBSTANCE	REFERENCE PRODUCT	DECISION	OWNER OF TRADE NAME
Omnitrope	somatropin	Genotropin	12/04/2006	Sandoz
Valtropin	somatropin	Humatrope	24/04/2006	BioPartners GmbH
Epoetin alfa Hexal	epoetin alfa	Eprex	28/08/2007	Hexal
Binocrit	epoetin alfa	Eprex	28/08/2007	Sandoz
Abseamed	epoetin alfa	Eprex	28/08/2007	Pütter Medice Arzneimittel GmbH & Co
Silapro	epoetin zeta	Eprex	18/12/2007	Stada Arzneimittel
Retacrit	epoetin zeta	Eprex	18/12/2007	Hospira
Tevagrastim	filgrastim	Neupogen	15/09/2008	Teva Generics GmbH
Ratiograstim	filgrastim	Neupogen	15/09/2008	Ratiopharm
Filgrastim Ratiopharm	filgrastim	Neupogen	15/09/2008	Ratiopharm
Biograstim	filgrastim	Neupogen	15/09/2008	CT Arzneimittel GmbH
Zarzio	filgrastim	Neupogen	06/02/2009	Sandoz
Filgrastim Hexal	filgrastim	Neupogen	06/02/2009	Hexal
Nivestim	filgrastim	Neupogen	08/06/2010	Hospira

TABLE 2: : UNSUCCESSFUL BIOSIMILAR APPLICATIONS IN THE EU

TRADE NAME	ACTIVE SUBSTANCE	REFERENCE PRODUCT	STATUS	DECISION	APPLICANT
Alpheon	Interferon alfa-2a	Referon-A	Negative Opinion	28/06/2006	BioPartners GmbH
Insulin Human Rapid Marvel	human insulin (soluble insulin)	Humulin Insulin S	Withdrawn	20/12/2007	Marvel LifeSciences Ltd
Insulin Human Long Marvel	human insulin (isophane insulin)	Humulin Insulin I	Withdrawn	20/12/2007	Marvel LifeSciences Ltd
Insulin Human 30/70 Mix Marvel	human insulin (30% soluble insulin and 70% isophane insulin)	Humulin Insulins M3	Withdrawn	20/12/2007	Marvel LifeSciences Ltd
Biferonex	Interferon beta-1a		Withdrawn after negative opinion	28/05/2009	BioPartners GmbH
Ratioepo	epoetin theta		Withdrawn after positive opinion due to administrative reasons	25/02/2010	Ratiopharm GmbH
Epostim	epoetin		Withdrawn	April 2011	Reliance Genemedix

with a reference product. In essence, an analytical strategy will follow the requirements of the ICH guideline Q6B[12], which are summarized below.

Annex from ICH Topic Q6B "Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products"

Structural characterisation and confirmation

1. Amino acid sequence
2. Amino acid composition
3. Terminal amino acid sequence
4. Peptide map
5. Sulfhydryl group(s) and disulfide bridges
6. Carbohydrate structure

Physicochemical properties

1. Molecular weight or size
2. Isoform pattern
3. Extinction coefficient
4. Electrophoretic pattern
5. Liquid Chromatographic pattern
6. Spectroscopic profiles

Over the last decade, and still today, the most important procedure for bioproduct structural characterisation has been mass spectrometry. An overview of the development of mass spectrometric techniques for proteins and glycoproteins has been presented in a previous article by Professor H.R.Morris[13]. As mass spectrometric techniques have advanced, the instrumentation has become more accessible. Various "soft" ionisation MS techniques have been utilised for glycoprotein analysis, including Electrospray Mass Spectrometry (ES-MS), on-line ES-MS (where the MS is coupled to an HPLC), Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) and for derivatised carbohydrates, Gas Chromatography Mass Spectrometry (GC-MS). Usually different types of instruments are used in the detailed study of both the protein and the carbohydrate moieties of the molecule, so that the overall structure of glycoproteins can be elucidated. Apart from the ability to study non-protein modifications such as sulphation and phosphorylation, the other major strength of an MS approach is in the analysis of mixtures. This has obvious applications in the analysis of heterogeneous glycosylation states-which give rise to different glycoforms.

The first step in determining whether a biopharmaceutical product has the correct anticipated structure - in other words, confirming that the gene sequence has been correctly translated and that no errors, insertions, deletions and mutations have occurred – is usually a simple molecular weight measurement. Depending on the size of the molecule, this is often performed by MALDI-TOF or ES-MS. This measurement would "flag" any discrepancy between the theoretical mass and the actual mass, and, depending on the mass range and resolution of the technique, may provide a clue to the type of modification(s).

However, in order to take a closer look at any potential modifications, MS-MAPPING procedures must be carried out. Analogous to LC peptide mapping, the molecule is initially digested into smaller parts using enzymic or chemical means and then the mixture of peptides produced is analysed using ES or MALDI MS. If the mixture is too complex, then it can be analysed using on-line LC-MS, bringing the additional dimension of molecular weight to the peptides separated in the UV profile. In this experiment, differences between the measured masses and the theoretical masses of the antici-

pated peptides can be quickly observed and even the corresponding peptides isolated and collected for further study. An additional benefit to an MS approach is that the technique relies on measuring mass changes, so that non-protein modifications such as sulphation, phosphorylation or addition of lipid or carbohydrate, can also be detected.

The objective of the comparative study is to establish whether the biosimilar has the same primary protein sequence of amino acids as the reference. This can be done by using classical protein sequencing (automated Edman degradation), peptide MS-Mapping, MS/MS sequencing and amino-acid analysis. For products which are glycosylated, characterisation of the carbohydrate structure is essential. The ICH guideline Q6B[1] states,

"For glycoproteins, the carbohydrate content (neutral sugars, amino sugars and sialic acids) is determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile) and the glycosylation site(s) of the polypeptide chain is analysed, to the extent possible."

Glycosylation is arguably the most important of the numerous post-translational modifications, but what is undeniable is that it presents a unique challenge for analytical methods. The population of sugar units attached to individual glycosylation sites on any protein will depend on the host cell type used, but it will be a mixture of different "glycoforms", on the same polypeptide. Powerful mass spectrometric based strategies can be used to analyse both free (un-derivatised) and derivatised samples to determine sites of glycosylation of both N- and O-linked structures, the identity of terminal non-reducing ends (potentially the most antigenic structures) and to identify the type of oligossaccharide present[14]. Chromatographic (anion exchange) methods can also be utilized for glycan profiling. In addition to mass spectrometry, a host of other analytical techniques can be used to compare primary and higher order structure. Various methods can be used to interrogate and compare on the basis of size, charge and shape. Techniques such as near and far UV Circular Dichroism provide information on the folding and secondary and tertiary structure of the protein and can be used in a comparative sense. In fact, a whole panel of methods should be employed, including orthogonal techniques to analyse particular quality attributes.

HOW SIMILAR IS SIMILAR?

A question which is often asked is: "how similar to the originator molecule must the biosimilar be"? It is clear from the EU guidelines that the primary protein structure, the amino-acid sequence, must be the same, otherwise it will not be considered as a "Biosimilar". The guidelines anticipate that minor differences in post-translational forms or product-related impurities may exist and that these should be investigated with regard to their potential impact on safety and efficacy. Products (epoetins) have already been assessed which have different glycosylation patterns to the originator, but it is the total package of data which will be taken into account on a case-by-case basis. The impurity profile is not expected to be the same, due to the differences in the manufacturing process.

FUTURE DIRECTIONS?

In Europe, the biosimilar "revolution" marches on – some previously published guidance documents have already been re-drafted, for example on EPO. And, more importantly, based on experience gained from the smaller protein molecules already assessed, a concept paper on the "Development of Similar Biological Medicinal Products containing Monoclonal Antibodies"[11] has been published. This draft guideline followed a consultation meeting held with industry and other stakeholders by the EMA in London, on the 2nd July 2009. The consultation period for the guideline ended on May 31st 2011 so we await the publication of the final paper.

The concept of biosimilar monoclonal antibodies moves the challenge of establishing biosimilarity to another level. To date, the molecules accepted under current guidelines around the world have been small-medium sized proteins, albeit with some heavy glycosylation in the case of EPO. Monoclonal antibodies are considerably larger, at around 150,000 Daltons for an IgG. However, there are schools of thought that contend that these molecules will also be 'copied'. One of the main driving reasons for this is that this class of drug is extremely successful. At the moment, in the EU and US there are over 30 novel therapeutic monoclonal antibodies which have been approved or reviewed, with many more currently going through the application process. The market for these products is forecasted to reach nearly $50 billion by 2013. The "best sellers" such as Avastin, Herceptin, Humira, Remicade and Rituxin, which account for over half of all global revenues, are about to fall over the "patent cliff" and are attractive targets for biosimilars.

CONCLUSION

In essence, the structural analysis of highly complicated molecules such as glycoproteins requires a battery of analytical techniques, chemical and instrumental. The same techniques can be utilised in the comparative approach for the assessment of biosimilar versus originator molecules.

ABOUT SGS LIFE SCIENCE SERVICES

As the leading pioneer in Biosimilar comparability testing, SGS M-Scan has broad expertise in bio/pharmaceutical characterization using high-end mass spectrometry and ancillary techniques to analyze the primary and higher-order structure of (glyco)proteins. At the end of 2010, M-Scan joined SGS Life Science Services, a leading contract service organization providing clinical research services, analytical development, biologics analysis, and quality control testing. With 17 laboratories across Europe, North America and Asia, SGS represents the broadest network of contract analytical and bioanalytical laboratories. In addition to testing services for the bio/pharmaceutical market, SGS also provides clinical trial management (Phase I to IV) and services encompassing clinical pharmacology studies, data management, pharmacovigilance and regulatory consultancy.

We have seen that characterizing the molecule is key but it is not sufficient to market a "biosmilar". Performing the complete range of assays and clinical trials to demonstrate biosimilarity of the compound is also challenging. It includes the following measurements and validations:

- Comparative PK/PD studies in healthy and target population: dose-response trials, distribution, density, avidity and other characteristics of the indication receptors
- Clinical efficacy in randomized parallel group clinical trials in sensitive populations
- Safety data and risk management program
- Potency assay(s) and in-vitro assays covering the functionality of the molecule
- Bioassays: Antibody-dependent cytotoxicity (ADCC)
- Immunogenicity and biomarkers

Helping clients to optimize drug development timeliness and decision making processes is our promise. Delivering results is our commitment.

Team up with SGS for your Biosimilar drug development to streamline Analytical, Clinical and Regulatory intelligence synergies.

REFERENCES

1. Frost and Sullivan Report, May 2011

2. CHMP/437/04 Guideline on Similar Biological Medicinal Products. September 2005. http://www.emea.eu.int/pdfs/human/biosimilar/043704en.pdf

3. CHMP/49348/05 Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Quality Issues. February 2006 http://www.emea.eu.int/pdfs/human/biosimilar/4934805en.pdf

4. CHMP/42832/05 Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Nonclinical and Clinical Issues. February 2006 http://www.emea.eu.int/pdfs/human/biosimilar/4283205en.pdf

5. CHMP/94528/05 Annex to Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Drug Substance- Nonclinical and Clinical Issues containing Recombinant Human Growth Hormone. February 2006 http://www.emea.eu.int/pdfs/human/biosimilar/9452805en.pdf

6. CHMP/31329/05 Annex to Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Drug Substance- Nonclinical and Clinical Issues containing Recombinant Granulocyte-Colony Stimulation Factor. February 2006 http://www.emea.eu.int/pdfs/human/biosimilar/3132905en.pdf

7. CHMP/32775/05 Annex to Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Drug Substance- Nonclinical and Clinical Issues containing Recombinant Human Insulin. February 2006 http://www.emea.eu.int/pdfs/human/biosimilar/3277505en.pdf

8. CHMP/94526/05 Annex to Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Drug Substance- Nonclinical and Clinical Issues containing Recombinant Human Erythropoietin. March 2006 http://www.emea.eu.int/pdfs/human/biosimilar/9452605en.pdf

9. CHMP/102046/06 Guideline on Similar Biological Medicinal Products Containing Recombinant Interferon Alpha. October 2007 http://www.emea.eu.int/pdfs/human/biosimilar/10204606en.pdf

10. CHMP/118264/07 Guideline on Nonclinical and Clinical Development of Similar Biological Medicinal Products Containing Low Molecular Weight Heparins. March 2009 http://www.emea.eu.int/pdfs/human/biosimilar/11826407en.pdf

11. EMEA/CHMP/BMWP/632613/2009 CHMP Concept Paper on Development of a Guideline on Similar Biological Medicinal Products Containing Monoclonal Antibodies. October 2009 http://www.emea.eu.int/pdfs/human/biosimilar/en.pdf

12. ICH Harmonised Tripartite Guideline, Topic Q6B. Specifications: Test Procedures and Acceptance Criteria for Biotechnological/ Biological products. Step 4, Consensus Guideline, March 1999 (CPMP/ICH/365/96).

13. Protein and Glycoprotein Characterisation by Mass Spectrometry. Morris, H.R. (2011) SGS Newsletter, March 2011.

14. Glycoprotein Structure Determination by Mass Spectrometry. Dell, A. and Morris, H.R. (2001) Science, 291, 2351-2356.

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