BIOSIMILAR DEVELOPMENT: THE RACE TO MARKET CONTINUES…

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2012 will certainly be memorable as an Olympic year, but it has also seen a flurry of activity in the race to market biosimilar products. Keen to participate in this event, many countries throughout the world have established legal and regulatory pathways which allow manufacture of "copies" of a patent-expired biotherapeutic product. However, these are not simple generics as in the case of small molecules. The fundamental difference with these large, complex protein molecules is that they cannot be absolutely identical to the original. Instead companies developing these "copies" must demonstrate that they are similar by performing a side-by-side comparison with a reference sample of the originator molecule. Consequently, there are many challenges – legal, regulatory, non-clinical and clinical – which manufacturers must rise above to develop biosimilar products for global markets. This article introduces the concept of biosimilars, their importance in the global marketplace together with some historical background, and an update on the current regulatory situation. It will also address the issues involved in demonstrating physicochemical similarity of the biosimilar molecule to the originator – one of the first hurdles to be negotiated prior to biological and clinical testing.

INTRODUCTION

Since the first recombinant DNA-produced biologic, human insulin, was approved in 1982, the biotechnology industry has firmly established itself as a major source of new human therapeutic drugs. Now, many of these 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, preclinical and clinical data which show they are highly similar to the original drug. The potential market for these products is forecast to be substantial; IMS Health estimates that US$64 billion in global biologics sales will be off-patent by 2015. Hence, there are many factors encouraging this emerging pharma sector, but undoubtedly a key driving factor is the universal need for more affordable medicines in both developed and developing economies.

SMALL MOLECULE GENERICS VS LARGE MOLECULE BIOSIMILARS

By their nature, the structure of small synthetic molecule drugs and their impurities can be well defined chemically. This, together with rigorous testing by originators, enables generic manufacturers to avoid costly, full clinical evaluations as long as they are able to establish that their product is "bioequivalent" in pharmacokinetic studies to the brand/reference listed drug.

Unlike small molecule drugs, biologically-derived products are large, complex protein molecules, usually comprising of a mixture of closely related species-termed "microheterogeneities". When produced in mammalian expression systems, as many biotech drugs are, the protein can also be glycosylated- i.e. carbohydrate is attached to the protein backbone. This glycosylation pattern will depend on the cell type used and the physiological status of that cell and will increase the amount of heterogeneity (glycoforms). It is now widely agreed that glycosylation is extremely important for many reasons, including potential immunogenicity, and should be characterized thoroughly in addition to the protein moiety. Glycosylation is just one of many "post-translational modifications" which can occur which will alter the anticipated protein structure. Furthermore, the complexities of cellular expression and biomanufacturing make exact replication of the originator's molecule nearly impossible – the process will certainly be different. This is another reason why biosimilar proteins cannot be approved in the same way as simple generics.

REGULATORY ISSUES: EU LEADS THE FIELD…

The European Union (EU) established the first legal regulatory guidelines, which took effect in 2005, for "similar biological medicinal products" (i.e. biosimilars). The basis of these guidelines is that the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA, previously EMEA)

requires initial physical, chemical and biological characterization of the biosimilar in comparison to the originator reference product. If found to be "similar" during this extensive characterization, then subsequent non-clinical and clinical data are then required to demonstrate the same safety and efficacy profiles as the originator. However, the premise 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[1] was followed by guidelines on quality[2] and non-clinical /clinical issues[3]. Thereafter, specific product annexes, initially for somatropin (human growth hormone, rHGH), granulocyte colony stimulating factor (rG-CSF), epoetin (erythropoietin (rEPO) and insulin were published, followed more recently by interferon alpha (rINF alfa), low molecular weight heparins (LMWH) , monoclonal antibodies , follitropin-alpha (rFSH) and interferon-beta (rINF beta). Several of the original guidelines, both general and product specific, have been or are in the process of being revised in the light of nearly 8 years of initial experience. Currently, a draft update of the general Quality Guideline EMA/CHMP/BWP/247713/2012) has been released for public consultation, with a deadline for comments by 30 November 2012. All the above guidelines plus current revision concept papers and drafts are available on the EMA Multidisciplinary: Biosimilars website[4]

The first biosimilar molecule approved in Europe (April 2006) was Omnitrope, a version of somatropin. This was closely followed by another HGH, Valtropin. To date, the EU has approved 14 applications, all of which are versions of somatropin, epoetin or more recently, filgrastim (see Table 1). Some early applications, for example, for interferon alpha-2a, interferon beta-1a, insulin and more recently epoetin alfa were not successful, either rejected or withdrawn voluntarily, and commentary on this can be seen on the EMA web site.

USA CATCHING UP FAST…

It has now been over 2 years since President Obama passed the "Patient Protection and Affordable Healthcare Act" 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) provides a new abbreviated licensure pathway for biosimilars – the 351(k) route of the Public Health Service (PHS) Act. This pathway requires comparison of a biosimilar molecule to a single reference product which has been approved under the normal 351(a) route with reference to prior findings and existing scientific knowledge on safety, purity and potency of the originator. An aspect of the legislation which is unique to the US is the provision for two levels of product – Biosimilar and Interchangeable Biosimilar. However, the exact requirements for the latter option are still to be fully defined. It is described in the legislation as "can be expected to produce the same clinical result" in "any given patient" and if given more than once has no greater risk "in terms of safety or diminished efficacy" than the reference. Although a biosimilar may be accepted with less than the full complement of non-clinical and clinical data, the requirement of the 351(k) route calls for one or more clinical studies. In addition, there are very complex patent disclosure provisions with which manufacturers must contend.

Although not required by the statute, in February 2012, the U.S. Food and Drug Administration (FDA) published their long-awaited guidance to assist Biosimilar developers. The three draft documents contain FDA's current thinking on the key factors: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product[5], Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product[6] and Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009[7]. The consultation period for these guidelines has now ended and the industry

TABLE 1: APPROVED EU BIOSIMILAR APPLICATIONS

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

awaits the final versions.

OTHER RUNNERS...

In the meantime, many other countries including Brazil, Australia, Turkey, Taiwan, India, Malaysia, Argentina, Mexico, Japan, Canada and South Africa have established regulatory pathways and have licensed copies of biotech drugs. Some countries such as Australia, Japan, Canada and Malaysia have modelled their guidelines on those of the EMA, requiring a comparative approach. However, other less highly regulated countries have produced their own versions of guidelines, or use their standard authorization process. As this will not involve the scientific comparison against the original product, to differentiate these from true "Biosimilars" the term "non-comparable biologic" (NCB) is used. The World Health Organisation, WHO, although not a regulatory agency, adopted a "Guideline on Evaluation of Similar Biotherepeutic Products" in October 2009.

STRUCTURAL CHARACTERIZATION OF BIOSIMILAR PRODUCTS – THE BASIC GAME PLAN?

Any manufacturer seeking to develop and market a biosimilar product requires comprehensive physicochemical (glyco) protein structural characterization capabilities. This analytical task must be performed at distinct stages of development in a step-wise manner.

Initially, the aim is to determine the exact amino acid sequence of the target originator molecule. Considerable effort should be expended at this stage to ensure that the correct primary protein sequence is deduced. This is a critical step prior to cell lines and clones being selected and developed and the corresponding "targeted" protein produced. Without doubt, sensitive sequencing techniques, particularly de novo MS/MS sequencing will be required at this stage. In addition, post-translational modifications of the originator should be screened-for and assessment made, by study of various batches produced over time, of which may be potential "Critical Quality Attributes" or CQAs. Basically, it means establishing the goal-posts for the development of the biosimilar. The new EU Quality guidelines refer to this as determining the Quality Target Product Profile or QTPP.

The next stage, once the biosimilar product is produced, is to confirm its structure in the same way as one would for a new bioproduct to satisfy normal CMC requirements required by various regulatory agencies. In addition to this, studies should be conducted to provide comparative data for the biosimilar side-by-side with the originator molecule. Strategies at this stage must include assessment of primary and higher order structure and also batch-to-batch variation should be determined for both the biosimilar and the reference product. Finally, if structural biosimilarity is established using physicochemical methods, functional, safety and clinical studies can commence.

In practice, an analytical characterization strategy will follow the requirements of the ICH guideline Q6B[8], which are summarized below in the Annex from ICH Topic Q6B "Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products":

- Structural characterization 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
    7. Electrophoretic pattern
    1. Liquid chromatographic pattern
    2. Spectroscopic profiles

Over the last decade, and still today, the most important procedure for biomolecule structural characterization has been mass spectrometry (MS). As mass spectrometric techniques have advanced, the instrumentation has become more accessible. Usually several different types of instruments are used in the detailed study of a glycoprotein so that the overall structure can be elucidated. A variety of so-called "soft" ionisation techniques can be utilized, 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). Apart from the ability to study non-protein modifications such as sulfation 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 glycoforms.

Normally the first step in determining whether a biopharmaceutical product has the correct anticipated structure is a simple molecular weight measurement. Depending on the size of the molecule, this is usually 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 MS 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 anticipated peptides can be spotted and the corresponding peptides isolated and collected for further study if required. The variability of both the N- and C-terminal sequences can be analysed this way, and so called "ragged-ends", i.e. heterogeneity, assessed. An extension of the MS MAPPING strategy will also allow the assignment of disulfide bridges and/or free thiols. An additional benefit is that the MS technique relies on measuring mass changes, so that non-protein modifications such as sulfation, phosphorylation or addition of lipid or carbohydrate, can also be detected.

For biosimilars, the objective of the comparative study is to establish whether the biosimilar has the same primary protein sequence of amino acids as the

reference product. 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, characterization of the carbohydrate structure is essential too. 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 certainly depend upon the host cell type used, but it will also be a mixture of different "glycoforms", on the same polypeptide. Powerful MS-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 the types of oligosaccharide present. Chromatographic (anion exchange) methods can also be utilized for glycan profiling – the relative distribution of carbohydrate structures.

In addition to MS, a host of other analytical techniques should be used to compare the structure of both the biosimilar and originator at primary and higher order levels. Various chromatographic, spectroscopic, and electrophoretic methods can be used to interrogate and compare on the basis of size, charge and shape. Co- and post-translational modifications, fragmentation, aggregation, deamidation, oxidation, etc. should all be studied and compared. Furthermore, 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. Depending on the molecule, non-routine techniques such as protein NMR and X-ray crystallography may also be utilized. 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 new 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. So, 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. Likewise, the US FDA has adopted a similar approach, in that the analytical characterization should show that it is "highly similar to the reference product notwithstanding minor differences in clinically inactive components".

FUTURE DIRECTIONS?

In Europe, the biosimilar "revolution" marches on – many original published guidance documents have already been re-drafted, or are currently being revised, such as the three general guidelines and the Insulin and LMHW product annexes. More importantly, based on experience gained from the smaller protein molecules already assessed, a guideline on the "Development of Similar Biological Medicinal Products containing Monoclonal Antibodies"[10] has been published by EMA.

The concept of biosimilar monoclonal antibodies moves the challenge of establishing biosimilarity to another level. To date, the biosimilar molecules accepted under current guidelines have been small-medium sized proteins, albeit with some heavy glycosylation in the case of EPO. In contrast, monoclonal antibodies are considerably larger, at around 150,000 Daltons for an IgG. However, despite the vastly increased manufacturing costs and challenge, 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, there are over 30 novel therapeutic monoclonal antibodies which have been approved or reviewed in the EU and US, with many more currently undergoing the application process. The market for these products is forecast to reach nearly $58 billion by 2016 (9). 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.

In April this year, a South Korean company, Celltrion, filed the first application for a biosimilar mAb with the EMA. The product, CT-P13 is an infliximab and the patent for the original product, Remicade, expires in the EU in 2014. Celltrion had already filed in Korea and on July 23rd, the Korean FDA approved the product, "Remsima" for treatment of the following conditions: rheumatoid arthritis, ulcerative colitis, Crohn's disease, ankylosing spondylitis and psoriasis. It will be interesting to see what EMA does and whether this will then encourage a host of other Biosimilar mAb applications.

CONCLUSION

In essence, biosimilar drugs are fact - products are now available in many highly regulated markets and in some countries market share is already overtaking that of originator products. The legal and regulatory basis for authorization of Biosimilars is built on strong scientific and quality foundations coupled with appropriate clinical studies. It is debatable whether the approval process can be termed "abbreviated" given the extent of comparability required. Nevertheless, the requirement is for a stepwise head-to-head comparison against the reference product to establish "Biosimilarity" prior to (reduced?) pre-clinical and clinical studies.

Current analytical science can certainly rise to the challenge of Biosimilar comparability assessment. The structural analysis of highly complicated molecules such as glycoproteins requires a battery of analytical techniques, chemical and instrumental. The same techniques can

be utilized in the comparative approach for the assessment of biosimilar versus originator molecules. However, it is also important to understand the limitations of physicochemical characterisation techniques and that establishing "Biosimilarity" at the analytical level is just the first stage in the overall assessment.

So, 2012 has so far been a dynamic year for Biosimilars and as this article goes to print, other regulatory developments are imminent. Competition amongst biosimilar manufacturers is increasing with "originator" pharma companies entering the arena. How well each manufacturer is able to provide regulators with the necessary comparability data packages will determine how well they achieve the gold they seek.

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