International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 11 No. 3 Jun. 2015, pp. 728-733

http://www.ijias.issr-journals.org/

Technical hindrances in establishing biosimilarity - the final lap in the race

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ABSTRACT: Therapeutics by means of complex biological molecules is currently dominating the total drug space. Several such complex biologics nearing patent expiry, various attempts to launch their generic equivalents is on-going. With stringent regulatory guidelines in place, there is a necessity to understand the basic challenges and differences in the area of establishing comparability. A perspective on existing methods is provided which highlights key gaps in the existing technologies. The coming years would see more developments in the tools and platforms that enable comparability studies and statistical methods that can help extrapolate a quantitative measure for biosimilarity.

KEYWORDS: Biosimilars, Micro-heterogeneity, Post-translational modifications, interchangeability

1 INTRODUCTION

The pharmaceutical industry has undergone significant transformation with the introduction of biotechnological drugs such as hormones, growth factors, interferons and more specifically mAbs. Most of the existing biologics have been in the market for about 20-30 years dominating their presence particularly in immunology and oncology therapeutic areas [1]. With their patents about to expire, their generic versions are expected to enter the market. With escalating health care expenditures, the generic versions of biologics are likely to bring down costs. In a study in 2009, it was estimated that in US, 75% of drugs in small molecule category were generics which brought down the costs by nearly 77% [2].They are referred to by different terminologies in different countries such as biosimilars in Europe, follow on biologics in US and subsequent entry or “me too” products in Canada. However, there are differences in the generic versions of biologics which cannot be treated in the same way as generics for small molecules. This has led to a major challenge in establishing a molecule to be a generic equivalent of a biologic. In additional to the technological challenges, there exist many unresolved matters in the regulatory aspects concerning the government bodies and the major players in this space. The core reasons that contribute to current scenario are discussed in the subsequent sections.

2 CHALLENGES IN ESTABLISHING BIOSIMILARITY

2.1 BASIC CONCEPTS IN UNDERSTANDING THE DIFFERENCES BETWEEN TWO DRUG VERSIONS

Understanding the differences in definitions of basic terminologies is a major challenge. Various regulations and guidelines have led to differences in terminologies, sometimes even counterintuitive. This is a key factor which influences the objective of comparability between the drugs such as:

Comparable: An internal comparison which applies to changes within the innovator product due to changes in manufacturing processes.

Similar: This is an external comparison between an innovator and biosimilar indicating their similarity.

Highly similar: The definition is same as the above however indicates more similarity in a crude quantification.

Interchangeable: Change from one medicine to another decided by the prescriber

Substitution: Change the prescribed biologic to an equivalent one at the level of the pharmacy without the involvement of the prescriber.

Switching: The decision to change one medicine to another lies with the treating physician.

2.2 ACHILLES HEEL: UNDERSTANDING THE INNOVATOR'S PRODUCT

A big vulnerable spot for biosimilar manufacturers is to understand the innovator product. This can be mainly due to technological knowledge on the behavioral properties of the drug and secondly the availability of the innovator drug for comparability studies. The source of the innovator biologic is usually from the open market. Most biologics have specific formulations with a combination of inert and active compounds. Therefore, use of improper extraction methods, or not placing the biosimilar drug in a similar formulation, would adversely affect the validity of the conclusions drawn from comparability studies [3-6]. In addition, certain countries may have jurisdiction regulations for obtaining the innovator drug and conducting studies on them [7].

Once there is a basic understanding of the innovator product, various analytical tools need to be employed to establish similarity between the two drugs. This is highly important and non-trivial in case of biologics because of the sheer size of the biologic in comparison to small molecule drugs. Biologics, especially protein molecules, undergo post translational modifications which are crucial in differentiating between two molecules. The exact link between the modification that leads to a specific clinical outcome is yet to be deduced but the below section quantifies the magnitude of the problem that needs to be dealt with.

Considering a mAb, which belongs to the immunoglobulin IgG class, there are totally six different known post translational modifications that occur at multiple sites in the protein [8] (Figure 1 shows the different post translational modifications that are known in IgG). In a very conservative estimate, the number of forms that can exist is two for an indication at one site (example: truncated or not). Therefore, assuming each of these indications occur at only one site, the total number of variable forms is 2^6 which is 64. However, in most cases the number of sites and the number of indications is far more and hence the possible number of variable forms is humungous (x^n, where x is the number of forms per site and n is the number of sites undergoing some form of post translational modification), making it nearly impossible to achieve the same form of the antibody by random sampling (with a probability of occurrence as $1/x^n$). So achieving an identical molecule every time is nearly impossible.

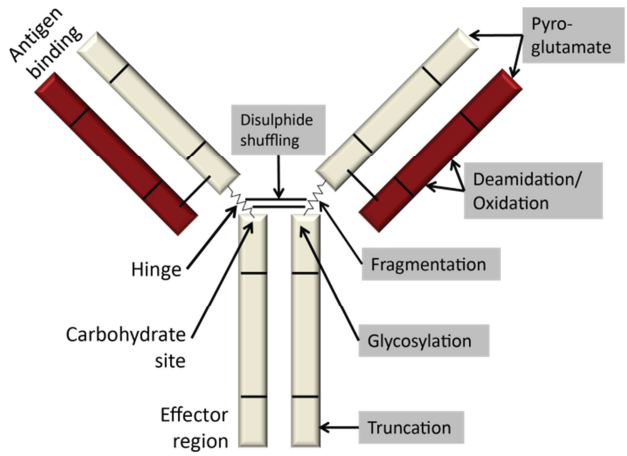

Figure 1. mAbs are potentially affected by one or more forms of heterogeneity which is dependent mainly on the nature and extent of post translational modifications. The effect of such heterogeneity could lead to major clinical ramifications. Depicted above is a cartoon representation of an IgG, with the heavy chain in tan, the light chain in red and disulfide bridges in black. Antibody function that can be potentially affected by heterogeneity, and structural features involved in giving rise to heterogeneity are listed on the left. The different forms of heterogeneity are denoted in grey boxes. Although deamidation/oxidation is depicted exclusively in the Fab region, there are examples of other susceptible residues dispersed throughout the molecule. Glycosylation heterogeneity can have multiple carbohydrate species (e.g., G0, G1, G2). Fragmentation aggravated by proteolytic susceptibility of the hinge region. These forms of heterogeneity can in principle affect additional classes of protein products as well.

2.3 OVERVIEW OF AVAILABLE ANALYTICAL TOOLS

There are several types of PTMs that occur in proteins and the most commonly occurring types have been discussed in the earlier section. PTMs, in most cases occur through chemical changes *in vivo* but some chemical changes can also occur *in vitro*, during various stages of manufacture such as purification and storage. PTMs lead to change in protein function and therefore the differences in PTMs need to be studied. The differences in PTMs need to be analyzed both internally (batch variations of the same product) and externally (between the innovator product and the biosimilar). Various types of analytical tools need to be employed which give information on different parameters. Sometimes, a combination of tools needs to be selected for measuring a single parameter due to the inherent short-comings of some of the techniques.

The most basic requirement in the analysis of PTMs is estimation of composition. Glycosylations are very common and usually the differences occur in the nature, number and linkages of sugar moieties. Therefore a technique like MS would be very useful in determining variability across batches. However, the link between glycosylation composition and clinical ramification is not directly established except for few cases such as addition of sialic acid which is known to cause immunogenicity [9,10]. Another serious concern that leads to immunogenicity is amino acid isomerization which can be estimated by latest versions of MS such as electron transfer dissociation (ETD) [11] and electron capture dissociation (ECD) [12]. O-Glycosylations are usually very difficult to determine and MS based tools are not suitable. While NMR is very useful in such cases, it cannot be used for big proteins and also requires large concentrations for analysis. More sophisticated versions such as flow and microcoil NMR address the concentration issues and are extremely sensitive even in picomolar range.

The proteins usually assemble into specific 3D folds and also form higher order structures. Several techniques are useful in determining higher order structures which enlisted in the table. However, this step is usually complicated. Accurate and highly sensitive techniques are extremely time-consuming and require highly skilled analysts to decipher the information accurately. Techniques such as crystallography provide accurate 3D structures and sometimes higher order information as well. A major limitation of this method is that the protein needs to be crystallized. Many times to obtain crystals, only parts of the protein would be used, or the PTMs would be removed therefore not painting a complete picture. High accuracy X-ray data can be measured only in synchrotrons which are not accessible to emerging countries and is a very expensive tool. Other techniques such as AUC, CD, DSC, etc. do give information on higher order structure. The signals obtained in these instruments are usually a summation of signals from individual parts of the protein and therefore gives only an overall picture on the folded state or oligomerization of the protein. Another issue with these techniques is that there is a lot of noise that needs to be filtered to obtain meaningful signals and the data obtained is usually only a subset of the total ensemble of the various proteins forms that exist in solution. Techniques such as HDX-MS monitor real time dynamics on the basis of deuterium exchange and are highly sensitive in capturing subtle protein dynamics [13]. The existing situation is that no single technique provides complete picture on the protein as summarized in Figure 2. Therefore, to attribute high confidence, multiple tests would be necessary for a given parameter as mentioned in table 1. Therefore, when probing is deeper to obtain highly accurate information, the larger picture is never really captured. The finer details of the gross picture thus captured are usually missed. The situation warrants development of more sophisticated techniques.

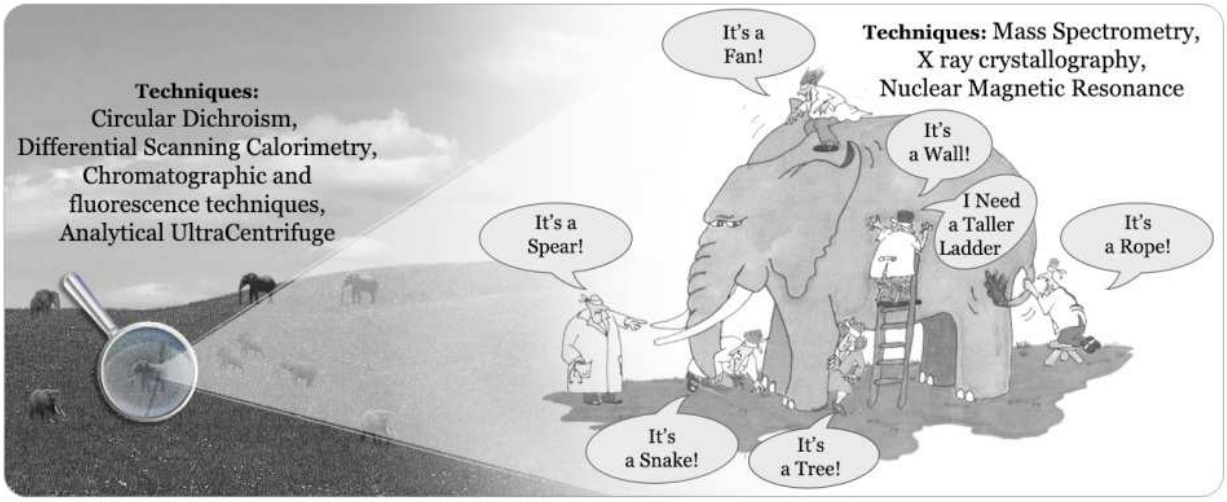

Figure 2. A cartoon representation of existing analytical techniques. The left section depicts and eagle's eye view of elephants which represents the techniques that give gross level picture. The right section is the famous blind man and elephant experiment. Each description is individually accurate but far from reality. In some cases the technique falls short in capturing the complete fine level information as well. So what are needed is taller ladders or techniques that exhaustively capture information at fine level and magnifying lenses or techniques that can appropriately help fitting of gross and fine level data such that the gap is bridged.

Table 1: Overview of various analytical tools available that can be used for examination of various parameters during comparability studies.

Parameter	Analytical tests
Primary structure Amino acid composition analysis,	MS, N/C-terminal sequencing, TPM and sequencing/MS
Higher order structure	CD, NMR, X –ray crystallography, immunoreactivity with conformational-dependent antibodies, TPM/MS, DSC, fluorescence, dye binding assays, Cryo-EM
Size	AUC, FFF, MALDI-TOF MS, LC-ESI, SDS-PAGE, SEC-HPLC
Charge	CE, IEC, IEF
Hydrophobicity	HIC-HPLC, RP-HPLC
Immunoreactivity	Immunoprecipitation, western blot analysis
For glycosylated products	Glycosylation pattern/sequence CE, HPAEC-PAD, LC-ESI, MALDI-TOF MS, RP-HPLC
Identification of glycosylation sites	TPM/MS
Aggregates	SEC, FFF, AFFFF

Legend: *MS, Mass Spectrometry; TPM, Trypsin Peptide Mapping; CD, Circular Dichroism; NMR, Nuclear Magnetic Resonance; AUC, Analytical Ultra Centrifuge; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; LC-ESI, liquid chromatography-electrospray electrospray; SEC, size exclusion chromatography; HPLC, high performance liquid chromatography; HIC, hydrophobic interaction column; RP, Reverse phase; HPAEC-PAD, high pH anion exchange chromatography with pulsed amperometric detection, DSC, Differential scanning calorimetry, EM, Electron microscopy; FFF, field flow fractionation; AFFFF, Asymmetric flow field flow fractionation.*

2.4 REGULATORY HURDELS

The US government authorized a regulatory pathway for the approval of biosimilars under the BPCI Act. However, in comparability studies, the first step is to establish biosimilarity which is difficult given the current technological shortcomings faced by most companies. The further step which requires far stringent review is to establish interchangeability that allows alternating between the innovator and biosimilar. On the other hand the companies that are into manufacture of biologics (either innovators or biosimilars) also have a crucial role to play depending on their opinions and validation of their opinions. It is interesting to note that few companies which are only into manufacturing innovators are strongly opposing the idea of biosimilars due to safety concerns. The large corporations that are into biosimilars are in strongly in favor of interchangeability (opinions are indicated in Table 2). The interesting aspect is the stance of companies such as Amgen which

are players on both sides exhibiting contrasting opinions both in favor/against use of biosimilars interchangeably. Amgen is in the process to launch 6 major biosimilars by 2020. Having the expertise in technological capabilities acquired through development of innovator biologics or acquiring companies with relevant technologies, companies like Amgen are likely to have maximum chance in succeeding in this space.

Table 2: Opposing opinions on advocating interchangeability of biosimilars

Advocating interchangeability **Amgen and Sandoz**	**Opposing interchangeability** **Amgen, Janssen, Genentech and Abbott**
Names sharing a common root but having a unique suffix and/or prefix to denote biosimilarity and interchangeability	Physician involvement in interchangeability is mandatory
Companies submitting guideline proposals to demonstrate interchangeability	Non-jurisdiction approved product cannot be used to demonstrate interchangeability
	Stringent regulations to exhibit interchangebility on any given patient

3 CONCLUSION

The understanding of living systems and their complex mechanisms is still nascent. Therefore the gaps that would arise during drug development involving living systems have to be acknowledged. Many such issues were raised during the introduction of small molecule generics while only some are relevant to date. A more sensible path would be to work in a concerted manner with the known and unknowns to develop better strategies to handle clinical trials/pharmacovigilance. The general accepted norm in case of small molecule generics is to measure the mean and variances of various parameters between the reference and test samples; usually a sample size of 18-24 is accepted if the parameters do not show significant variance. Considering biomolecules are far more complex and pose more serious reactions, a revision in this number, with acceptable compromise from the conventional clinical trials would provide a better risk management plan which is the main concern in the introduction of biosimilars.

To bridge the existing gaps in comparability studies, more sophisticated analytical tools need to be developed which are capable of precise measurements with high confidence. However, a critical deterrent for companies is the high cost, in many cases, low profit margins. The industry is shifting more towards biologics and its generic versions, therefore, the requirement of analytical instruments would increase. However, the existing methods for comparability studies between innovator biologics and biosimilars are not very sensitive and need more improvement to achieve the desired level of sensitivity and accuracy. An example of the lack of progress in this field is of Beckman coulter, which manufactures analytical ultracentrifuges under monopoly. Although there have been advancements in electronics, detectors, computer hardware and software as well as in the detection of product quality issues, there hasn’t been a significant improvement to the instrument. The Open AUC project [14] is an initiative in this direction to consolidate findings across various research groups to improve the existing instrumentation. At the level of regulators, the development of more such platforms are to be incentivized by promoting such projects and their use be made mandatory by biosimilar manufacturers to claim the similarity associated with their biosimilars.

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