Strategies to Improve Comparability Assessments Across Bioanalytical Data for Biosimilar Studies

Gwen Eak

Associate Director – Bioanalytical Lab, PPD Clinical Research Business, Thermo Fisher Scientific

Kelli Phillips

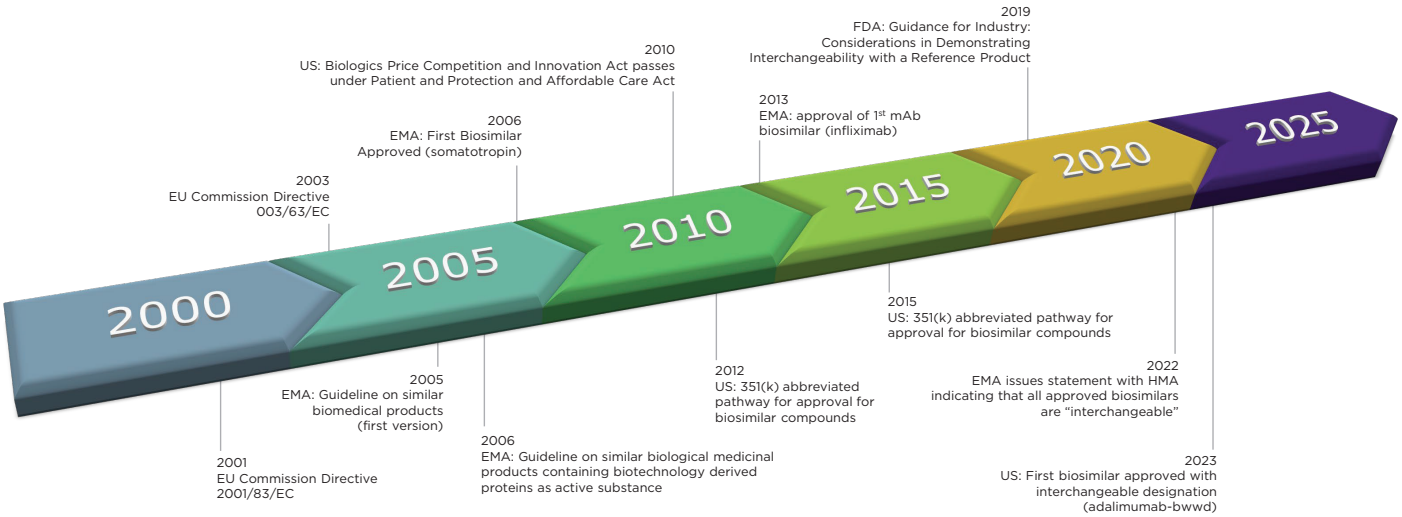
Director – Bioanalytical Lab, PPD Clinical Research Business, Thermo Fisher Scientific

It has been over two decades since the European Union (EU) issued the first Commission Directive (2001/83/EC) regarding biosimilar drugs (referred to as similar biological medicinal products). Five years later, the small biologic Somatotropin was approved (2006).[1, 2] It was still two years until the United States (U.S.) entered the biosimilar market, triggered by (U.S.) Congress approval of the Biosimilar Price Competition and Innovation Act (BCPIA). BCPIA was enacted as a provision of the Affordable Care Act the following year (2010) creating a regulatory approval pathway for biosimilars for Americans. The U.S. Food and Drug Administration (FDA) issued an abbreviated licensure pathway with submission under 351(k) (2012) for approval of biosimilar drugs. This licensure pathway permits a biosimilar biological product to be licensed under the Public Health Service Act (PHS Act) based on less than a full complement of product-specific preclinical and clinical data. The first biosimilar (Zarxio, Filgrastim-sndz) was approved by the FDA three years later.[3, 4] As in the EU, the first approved biosimilar in the U.S. was a small biologic (18.8 kDa). Incrementally, the biologics approved as biosimilars increased in complexity/size. The first monoclonal biosimilar drug product approved was for reference product Remicade (infliximab) and was approved by the EU in 2013 and the U.S. in 2016.

In the U.S., guidance for establishing interchangeability was finalized in 2019. Per this guidance, approved biosimilars are not automatically considered Interchangeable. Instead, the interchangeable designation is applied after biosimilarity is confirmed and following the review of additional data needed to support a demonstration of interchangeability. Products that will be administered multiple times are recommended to be assessed using switching studies that cycle patients between the reference product and the biosimilar.[5, 6] In late 2023, adalimumab-adbm was the first biosimilar approved with the Interchangeable designation in the U.S., which allows for pharmacy-level substitutions between the reference product and an approved biosimilar.[7]

Prior to 2022, the European Medical Agency (EMA) did not provide biosimilars with an interchangeable designation, allowing each country to establish individual recommendations. However, to harmonize recommendations within the EU, the EMA and the Heads of Medicines Agencies (HMA) issued a joint statement on July 22, 2022 that confirmed that all biosimilar drugs were interchangeable. The level of approval required for substitution is still decided by each country.[8]

Overall, the EU is the clear leader in biosimilar adoption with 86 approvals, compared to 46 in the U.S. (as of March 2024).



i. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official Journal L 311, 28/11/2001 P. 0067 – 0128.
ii. Public Law 111-148, Patient Protection and Affordable Care Act, 2010
iii. Public Law 78-410, Public Health Service Act, 1944
iv. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER). Considerations in Demonstrating Interchangeability with a Reference Product. May 2019.
v. European Medical Agency and Health Medical Authority. Statement on the scientific rationale supporting interchangeability of biosimilar medicines in the EU. July 2022

Method Development and Validation to Support Biosimilar Programs Pharmacokinetic Assays

Bioanalytical assays that measure the concentration of reference and biosimilar drug products in patient samples ensure that the pharmacokinetic (PK) characteristics of both drug products are comparable by evaluating the pharmacokinetics/potency of targeted epitopes. Differences in the drug products at this level are not observed in chemistry and manufacturing controls (CMC) comparability testing but may be observed during PK method development. PK assays also allow for comparisons of drug clearance and biodistribution of the reference product versus the biosimilar.

Marini et al. discussed the one-assay-approach in 2014.[9] The goal of method development and validation is to establish bioanalytical comparability using a single assay designed to detect each drug product equally. This approach eliminates the biases that may result from methodology differences and allows the bioanalytical scientist and clinical pharmacologist to remain blinded to dosing. Concentration-response curve comparison was also recommended by Marini et al.[9] To support the one-assay approach, this assessment must occur very early in development. Current recommendations now suggest that curve overlay experiments must also be conducted during validation.[10]

Immunogenicity Assays

Anti-drug-antibodies (ADAs) may change the rate of drug clearance or potentially cause severe reactions or adverse events including anaphylaxis and cytokine release syndrome. As a result, similar immunogenicity profiles for the reference standard and biosimilar are critical when establishing biosimilarity. Two drug products may have differential immunoreactivity in patients due to glycosylation or post translational modifications. Evaluating immunogenicity ensures that the magnitude (incidence and severity) of immune response is the same in patients dosed with the reference product versus biosimilar. Immunogenicity is also key when establishing interchangeability in the U.S. Switching studies are recommended to evaluate the immunogenicity impact as patients switch between a reference product and biosimilar over an extended treatment time.

Regulatory agencies only require that the biosimilar product is no more immunogenic than the reference product when evaluated head-to-head. Due to the evolution of assay methodologies, ADA incidence in patients assigned to the reference product may be higher than reported during historical trials. This is expected, as advances in methodologies over the last decade have improved assay sensitivity. Consequently, comparative immunogenicity assessments during the biosimilar clinical trial provides the most meaningful data. In circumstances when ADA incidence is lower than previously reported for the reference product, it may suggest that the bioanalytical method used for biosimilar assessment is not fit-for-purpose.

The bioanalytical industry extensively debated using one versus two assays for biosimilar immunogenicity assessments.[11] In 2019, the one-assay approach was determined to be most appropriate.[12] In addition to providing rationale for using a single assay for comparative assessments, evaluations such as drug tolerance and confirmatory evaluations were also recommended to provide evidence for antigenic equivalence. As a final step, during validation, confirmatory cut points were established with both drugs, and similarity is assessed using a statistical approach; ANOVA and LEVENES test were suggested.

Method Development and Validation to Support a Biosimilar Program at a CRO

When contracting bioanalytical method development with a contract research organization (CRO), it is important that a sponsor has identified the target markets for biosimilar approval. As discussed above, the U.S. and EU have different requirements, as do many of the other regulatory agencies world-wide. Early market identification will enable the CRO to better scope and frame expectations. Each market has specific regulations that must be followed, and additional drug products will also extend development time and complexity of the validation. It is also important to know that even if a CRO has an in-house assay for reference product analysis, this should be considered a starting place and pre-validation runs will be necessary to confirm comparability of the biosimilar and the reference product. And full validation will be necessary for the required head-to-head comparisons.

In addition, final certificate of analysis (CoA) for the biosimilar is essential; changes in concentration will trigger full scale recalculations during the validation phase. Finally, as with all bioanalytical assays, good capture and detection reagents are essential to a quality method. As noted in Thway et al., reagents for reference products off-patent are often commercially available and are used in 80% of cases that were evaluated.[10] However, caution should be used; these reagents may not detect the reference product and biosimilar in an identical manner, as commercially available reagents are not specific to the biosimilar structure and may result in under recovery due to post translational modifications present on the biosimilar.

Sample Analysis to Support Biosimilar Programs

Bioanalysis for a biosimilar program's patient samples can be daunting; however, if planned correctly keeping regulatory requirements, shipment logistics and analysis strategies top of mind, these large quickly–enrolling studies can go very smoothly. Published reference product results permit method development to be tailored appropriately, ensuring the assay range, sensitivity, drug tolerance and throughput of each assay are optimal for method performance and clean, high quality data submissions.

Strategic planning for each study ensures that requirements are met for each regulatory authority that will review the data and can reduce variability between the reference product and reference product dose groups. The assay acceptance controls, drug products and statistical evaluations are just some of the aspects affecting comparability assessments that are required to ensure submissions schedules are met in the highly competitive biosimilar segment.

Controls

Per EMA guidance all controls included on sample analysis assays must have criteria applied to pass or fail a run.

The sponsor will have access to characterization data for the proposed biosimilar drug product and has the ability to extend product stability over the life of the program. Therefore, when using a single assay approach, after comparability of the two drug products is proven in method development and validation, all controls used to monitor PK assay performance should be prepared with the biosimilar. For immunogenicity assays, the biosimilar should be used in the inhibition solution for all samples, regardless of dosing product.

In addition, setting upper bounds for the assays negative control (NC) and lower bounds on the low positive control (LPC) for immunogenicity methods is important to ensure the LPC/NC signal to noise ratio remains within the bounds established during validation.

Qualifying a new lot or preparation of critical reagents is important to ensure the bioanalytical methods consistent performance. A target interference control (TIC) can be used to monitor the performance of new lots of reagents used to reduce

target interference. A TIC can be used to qualify new lots of the reagent which is used to limit the formation of drug-target complexes which may lead to false positives for immunoreactivity. New lots of reagents should be bridged into the method prior to use to ensure the TIC meets acceptance criteria and the TIC can be included in each sample analysis assay (but is not required).

PK Considerations

Reference products are packaged with a nominal concentration for dosing; however, the actual concentration of each lot is within an allowable range. Whereas the biosimilar is dosed at a known concentration for the individual lots. This may contribute to a perceived comparability issue in the PK data.

This actual versus the package concentration difference for reference product drugs is also a reason to use the biosimilar to prepare calibrators, quality controls and inhibition solution once comparability is proven in method development and validation.

Immunogenicity Considerations

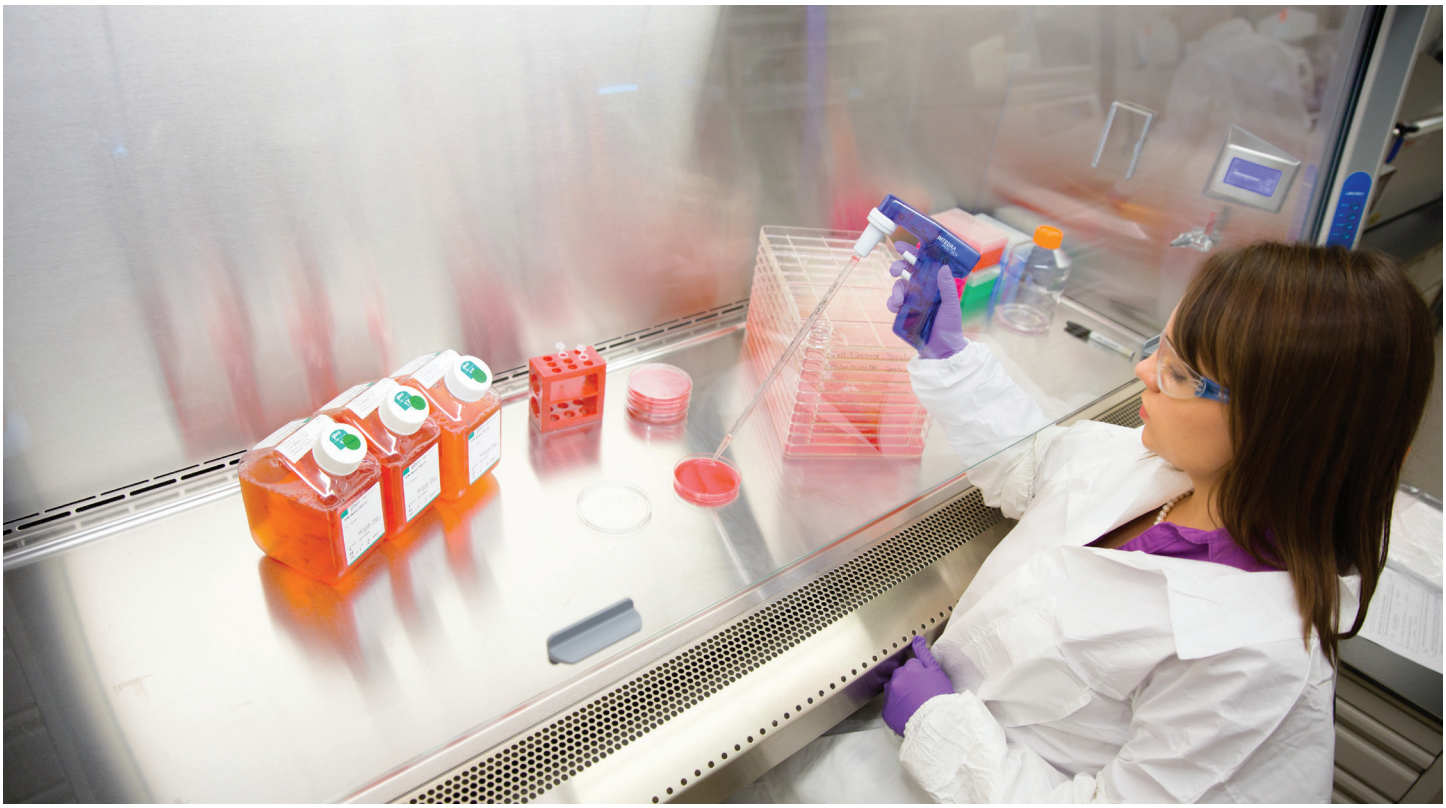
Immunogenicity data is generated to determine if the immune response is the same in patients dosed with the reference product versus the biosimilar. The data is considered qualitative so ensuring the assay performance is consistent with validation data and that assay variability is low is important for accurate and high-quality analysis.

The patient population should be evaluated to determine if it is consistent with the commercially purchased individuals used to generate the cutpoints during validation. The two populations are considered equivalent if the study predose sample screening and confirmatory results generates a false-positive rate of 2%-11%.

An in-study cutpoint may be required if the patient population differs from commercially available individuals used to determine the cutpoints during validation. If an in-study cutpoint is needed, samples collected during patient screening may be used instead of the day 0 predose samples if available. This reduces the handling and volume stress of analyzing predose samples in multiple panels for cutpoint statistical evaluations.

It is also important to ensure the ADA drug tolerance is sufficient to cover sample Cmax to minimize risk of false-negative immunogenicity results, potentially impacting comparability between dose groups.

Analysis Strategies to Limit Artificial Variance Between Biosimilar and Reference Product Patient Results.



"The observed bioanalytical bias differences exceeded 10% in nine out of 18 comparisons between biosimilar product and US-reference product and in seven out of 15 comparisons between biosimilar product and non-US comparator product. These data demonstrate that understanding the potential bias difference that may exist between products may be important and suggest that minimizing the absolute bioanalytical bias difference among products to less than 10% could be beneficial."[10]

One strategy to reduce bioanalytical bias is to batch samples by patient into a single assay from dosing to washout timepoints to reduce assay-to-assay variability.

This allows for the most accurate area under the curve calculations for PK assays. This reduction in variability also allows for a meaningful comparison of ADA results, particularly titer results, across timepoints for a patient. This does increase the amount of long-term stability needed to cover PK samples and the complexity of selecting samples for analysis by subject ID as full profiles become available at the BioA lab for testing.

Employing automation, especially for the PK analysis, is another way to decrease assay-to-assay variability it results in an electronic audit trail of sample volumes used in sample dilutions and plating which can be reviewed to find assignable cause for any results questioned by the PK scientists.

Neutralizing antibody (NAb) analysis may be optional depending on how many patients generate an ADA response. Very low immunogenicity rates may not be balanced between dosing groups and the data may not be meaningful for comparability between drugs. The NAb method should be validated so the data can be obtained quickly if needed.

Reanalyzing samples after generating an initial reportable result is highly discouraged by guidance. If reanalysis is required, the assignable cause must be well documented and very transparent in the final bioanalytical report. The lab should also ensure samples and controls are within long-term, freeze thaw and thawed matrix stability parameters established during validation. Method performance trending can also be very important for ADA and NAb analysis to ensure critical reagents are maintaining validation performance and any deteriorating critical reagents are replaced promptly.

Looking to The Future

Over the next several years many of the biggest drugs that will come off market are biologics and therefore candidates for biosimilar products. Biologics coming off patent leading up to 2030 include but are not limited to Keytruda®, Revlimid®, Eliquis®, Eylea®, Stelara®, Opdivo®, Trulicity®, Prolia®, Cosentyx® and Entyvio®. More than 15 large pharma companies are major players in the Biosimilar business and will be targeting development and testing of biosimilar products to align with patent expirations.

Market research estimates growth from $35.47 billion to $82.27 billion over the 5-year period from 2024 to 2029. Asia Pacific is expected to be the fastest growing biosimilar market with North America being the largest market. , As biosimilar companies compete to grab a share of the market the cooperation between pharma and CROs to create and execute analytical methods with highest quality comparability assessments will be key to gaining approval and licensure for new biosimilars products.

References

1. Romer, T.; Peter, F.; Saenger, P.; Starzyk, J.; Koehler, B.; Korman, E.; Walczak, M.; Wasik, R.; Ginalska-Malinowska, M.; Solyom, E. Efficacy and safety of a new ready-to-use recombinant human growth hormone solution. *Journal of Endocrinological Investigation* **2007**, 30, 578-589.
2. Saenger, P. Current status of biosimilar growth hormone. *International Journal of Pediatric Endocrinology* **2009**, 2009, 1-6.
3. Awad, M.; Singh, P.; Hilas, O. Zarxio (Filgrastim-sndz): the first biosimilar approved by the FDA. *Pharmacy and Therapeutics* **2017**, 42 (1), 19.
4. Raedler, L. A. Zarxio (filgrastim-sndz): first biosimilar approved in the United States. *American health & drug benefits* **2016**, 9 (Spec Feature), 150.
5. Biosimilars and the era of interchangeability. *The Lancet Rheumatology* **2023**, 5 (9), E495.
6. Barbier, L.; Vulto, A. G. Interchangeability of biosimilars: overcoming the final hurdles. *Drugs* **2021**, 81, 1897-1903.
7. Bhat, S.; Patel, M.; Duly, K.; Choi, D. Adalimumab-adbm: the first interchangeable biosimilar for the treatment of inflammatory diseases. *Annals of Pharmacotherapy* **2022**, 56 (12), 1356-1364.
8. Gherghescu, I.; Delgado-Charro, M. B. The biosimilar landscape: an overview of regulatory approvals by the EMA and FDA. *Pharmaceutics* **2020**, 13 (1), 48.
9. Marini, J. C.; Anderson, M.; Cai, X.-Y.; Chappell, J.; Coffey, T.; Gouty, D.; Kasinath, A.; Koppenburg, V.; Oldfield, P.; Rebarchak, S. Systematic verification of bioanalytical similarity between a biosimilar and a reference biotherapeutic: committee recommendations for the development and validation of a single ligand-binding assay to support pharmacokinetic assessments. *The AAPS journal* **2014**, 16, 1149-1158.
10. Thway, T.; Wang, Y.; Booth, B.; Maxfield, K.; Huang, S.; Zineh, I. Current perspectives on ligand-binding assay practices in the quantification of circulating therapeutic proteins for biosimilar biological product development. *The AAPS Journal* **2020**, 22, 1-6.
11. Ryding, J.; Stahl, M.; Ullmann, M. Demonstrating biosimilar and originator antidrug antibody binding comparability in antidrug antibody assays: a practical approach. *Bioanalysis* **2017**, 9 (18), 1395-1406.
12. Civoli, F.; Kasinath, A.; Cai, X.-Y.; Wadhwa, M.; Exley, A.; Oldfield, P.; Alvandkouhi, S.; Schaffar, G.; Chappell, J.; Bowsher, R. Recommendations for the development and validation of immunogenicity assays in support of biosimilar programs. *The AAPS Journal* **2020**, 22, 1-9.

vi. https://www.mordorintelligence.com/industry-reports/global-biosimilars-market-industry
vii. https://www.mordorintelligence.com/industry-reports/global-biosimilars-market-industry/companies

For more information visit our website at **ppd.com**

twitter.com/PPDCRO

facebook.com/PPDCRO

youtube.com/PPDCRO

linkedin.com/company/ppd

PPD®
Part of Thermo Fisher Scientific