Article

Early phase biologics characterization; a key factor for risks mitigation in biologics product developments

Rafael Sande MSc., Head Operations Characterization Large Molecule / ATMP Services, Solvias AG
Contact: Rafael.Sande@solvias.com,
https://www.solvias.com/capabilities/services/biopharmaceutical-analysis/characterization.php

Success in today's complex world of biological products demands a well thought out drug development workflow. Strategies to mitigate risks and reduce development costs along the way are in the focus of drug developers. One major tool used for risk mitigation today is the performance of advanced analytics, using state of the art analytical technologies and methodologies, very early in the development process of biologics. Analytical biologics product characterization has become the essential basis for a successful biological drug development.

Regulatory considerations

As per ICH Guideline Q6B, the goal of a biological product characterization is the generation of in-depth knowledge of the product's physiochemical and immunochemical properties, biological activity, molecule variants (purity, impurities), contaminants as well as protein quantity. The data generated is typically used for establishment of specifications, the identification of critical product quality attributes, to facilitate process design optimizations, and to ensure that the product attains critical safety, purity and potency. The application of characterization analytics is usually emphasized in pre-clinical and clinical phase I development stage; however characterization and comparability assessments are also a major tool being applied when significant process changes are introduced. Furthermore at the stage of submission, the product should have been compared with an appropriate reference standard, if available. The characterization analytics applied for products in development are also of key use for initial reference standard characterization.

Complexity of biological products

There are many factors relevant for elaborating complexity of biologics products and the impact of these factors on product quality. Some key attributes that make these large drug molecules complex are related to the biological manufacturing process. The bulk products manufactured consist of multiple variants with a natural product heterogeneity which is introduced by the cellular expression system.

This requires intensive downstream processing to produce a product with high quantity and quality. At the early stages of drug development many quality attributes of the drugs are yet unknown and need to be built up based on analytical data. A comprehensive analytical characterization of the molecule is at this stage achieved by applying multiple orthogonal and complementary analytical methodologies and technologies. The combination of all individual results deriving of these assays represent the comprehensive characterization result.

The 4 pillars of early phase biologics characterization

Typical comprehensive characterization strategies focus on four primary areas. High quality characterization is achieved when multiple orthogonal and complimentary methods/technologies are applied.

1) ***Physicochemical and Structural characterization*** focusing on Liquid chromatography-mass spectrometry (LC-MS) based analysis (primary amino acid sequence confirmation, molecular weight distribution (glycosylated and de-glycosylated variants), Disulfide linkage analysis, single amino acid misincorporation, post-translational modifications mapping) and purity/charge profile analysis (HPLC CEX/AEX and/or Capillary electrophoresis-based CE-SDS or cIEF)

2) ***Higher Order structure (HoS) characterization*** focusing on Spectroscopy based analysis (Near/Far Circular dichroism, Intrinsic fluorescence, Fourier transformation infrared spectroscopy, second derivative Fourier transformation infrared spectroscopy, Differential scanning calorimetry) and LC-MS based protein structure and dynamics analysis (Hydrogen-Deuterium Exchange (HDX-) mass spectrometry).

3) **Glycosylation characterization** focusing on HPLC and LC-MS based analysis (N- and O-Glycosylation variants mapping, Glycosylation site and occupancy determination as well as sialic acid and monosaccharide composition)

4) **Aggregation and fragments characterization** focusing on HPLC and spectroscopy-based analysis (Size exclusion HPLC or Asymmetrical flow field-flow fractionation (AF4) with UV or multi angle light scattering (MALS) detection, dynamic light scattering (DLS), Analytical ultracentrifugation (AUC)) as well as microscopical analysis (Sub-visible particles analysis)

Why focus on characterization within the four areas at pre-clinical and clinical stage I?

Due to the natural product variability of biologics drugs, typically being produced with a cellular based expression system, alterations and heterogeneities in the product can result in decreased product efficacy, reduced stability, or increased immunogenicity. For example, the confirmation of the primary protein structure, specifically the correct amino acid sequence in the complimentary determining regions (CDR) involved in target antigen binding is key to ensure correct effector function of the drug. Misincorporation of single amino acids in the target amino acid sequence or post-translational modifications within the CDRs can affect target antigen binding. Structural changes may also lead to altered higher order structure of the protein product which may result decreased stability and/or decrease or loss of efficacy of the molecule. The structural integrity of the biologic should be closely monitored at early drug development stage to identify potential critical quality attributes.
Highly successful biologics products like Immunoglobulin G based monoclonal antibodies (mAB) consist of N-Glycosylation sites in the crystallizable (FC)-region. The host cell lines, applied fermentation conditions and the used growth cell media used for biologic product manufacturing can influence the extent and nature of the glycosylation variants distribution. Thus the overall heterogeneity of the product is influenced by the heterogeneity of the N-Glycosylation variants. Typical N-Glycosylation structures in mABs consist of sugars like fucose, mannose, galactose, sialic acids like N-acetylneuraminic acid and N-acetylglucosamine moieties. Depending on the content and N-glycan variants present, a possible implication can be potential immunogenicity effects or altered pharmacokinetic and pharmacodynamic properties.

Solvias Characterization Platform method approach

Solvias provides high quality comprehensive characterization services for biologics since over a decade. Successfully supporting Virtual Biotech and Pharma's, CMOs Big Pharma and Big Biotech Customers worldwide. We can support customers by either applying customer methods or by application of our in-house developed platform characterization methods which are optimized for biological products like mABs, Bispecific antibodies and nanobodies, Protein conjugates like PEGylated proteins, Recombinant proteins and Glycoproteins, Antibody-drug conjugates.

The application of our platform methods reduces turnaround time and costs for our customers. Typically we onboard new customer products by performance of a product specific feasibility analysis, performing a target molecule analysis on basis of our platform methods. Our platform methods have a proven track record as they have been successfully applied to support numerous biologic products over the last decade. Often, the product specific analysis method is ready for studies after completion of the feasibility analysis and additional method qualification and robustness verification without extensive method development needed.

Concluding

High quality comprehensive biologics product analytical characterization at early development stage is a key factor used in risk mitigation strategies for biologic drug development. State of the art technologies and analytical methodologies can support key milestones in early development like clone selections, development of optimized manufacturing and/or downstream process as well as characterization of engineering batches, bulk drug substance batches, reference standards. Characterization analytics support comparability/similarity studies, for example to assess changing processes or change of manufacturing sites as well as batch to batch, stability studies or biosimilar/originator comparability. The more you know about your molecule at earliest stage, the more risk mitigation measures can be applied at the critical decision points.

Solvias AG
Römerpark 2
4303 Kaiseraugst
Switzerland
Tel. +41 61 845 60 00
Fax +41 61 845 69 00
info@solvias.com