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Charge Variants Analysis of Recombinant Monoclonal Antibodies

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ABSTRACT

Recombinant monoclonal antibodies are among the most structurally complex therapeutics with various potential molecular modifications during production processes. Rapid and efficient characterization of these heterogeneities and controlling the upstream and downstream processes for achieving a consistent product is absolutely necessary. In this minireview the importance of characterization of mAb heterogeneity and the current analytical methods for mAb variants will be discussed.

Introduction

Recombinant monoclonal antibodies (mAbs) are the most approved biopharmaceuticals, as new drugs or biosimilars, between 2015 and 2018 [1]. These magic bullets target biological molecules with crucial role in severe and chronic diseases such as cancer, autoimmune, cardiovascular, respiratory, hematology, and infectious diseases [2]. They have incredible therapeutic and commercial value which make them among the top 10 best sellers of pharmaceuticals for several past years [1]. Chinese Hamster Ovary (CHO) cell lines are the mostly used mammalian host for mAb production. These cell lines are well characterized and experienced by many manufacturers, so the knowledge about their production behavior from high expression capability to desirable post translational modifications made them almost irreplaceable by alternative cell lines [3]. The complexity of mAb with about 150 kDa molecular weight make the implementation of quality by design (QbD) as an unavoidable strategy for development and manufacturing of these molecules [4]. QbD defines the critical quality attributes and a control strategy to assure the stable and consistent quality during the manufacturing process. Different post translational modifications during upstream process such as amino or carboxy terminal processing and glycosylation or during downstream processes or storage, such as deamidation, oxidation and fragmentation will end to different variants which could affect the safety, quality and efficacy of mAbs [5].Therefore, characterization and quantification of mAbs charge variants are required for assessing a consistent product quality [6].

Methods of mAb Charge Variant Analysis

Analytical methods with capability to separate differently charged molecules are used to characterize the variants of mAbs. The using of fast, reliable, and quantitative analytical methods is necessary for this purpose [7]. Charge- based variants have been categorized as acidic, main, and basic species. Main species of mAbs usually contains three PTMs including N-pyroGlu of either or both of the light and heavy chains, heavy chain(s) Lys-C removal, and glycosylation of the Asn residue in the CH2 domain with neutral oligosaccharides [8]. Chromatographic and electrophoretic tools include ion exchange chromatography (IEX) and isoelectric focusing (IEF), are the most common and simple analytical methods for the analysis of mAbs charge heterogeneities [9-11]. IEF may help to visualize the charge isoforms, but chromatographic tools are more appropriate for precise quantification [12].

Cation exchange chromatography (CEX), has been a standard method for the characterization of mAbs charge heterogeneity and routinely used as a fingerprint of the distribution of posttranslational modifications present on the mAb, also CEX analysis is necessary for mAbs quality control analysis and requested by regulatory [13]. When higher resolution is required to resolve all protein variants,

improved techniques, such as capillary zone electrophoresis (CZE), capillary isoelectric focusing (cIEF), imaging isoelectric focusing (icIEF), micro liquid chromatography, and two-dimensional electrophoresis can be useful [14-16]. Two-dimensional liquid chromatography (2D-LC) coupled with mass spectrometry can produce an excellent resolution for identification of individual charge variants. The analytical methods include cIEF, CEX, LC –MS and tryptic peptide mapping can be used to analyze and identify C-terminal and N-terminal charge variants [14]. It was reported that LC –MS after trypsin digestion is the most useful and precise analytical method for C-terminal variant analysis in comparison to other methods.

Also, an orthogonal method such as MALDI-TOF (Matrix-assisted laser desorption/ionization time of flight), ESI-TOF (Electrospray ionization time of flight), or ion chromatography should be used to measure relative percentages of mAb isoforms including 0, 1 or 2 C-terminal lysines [17]. The gold standard for precise analysis of charge variants is peptide mapping followed by RP-HPLC-MS/MS. Huang et al. used protein sample digestion with trypsin and Glu-C sequentially, followed by RP-HPLC-MS/MS analysis to study the humanized IgG1 mAb [18]. There are different methods for evaluating the variant species of therapeutic mAbs. The depth of the knowledge prepared by these methods are different and they could be used in various stages of developing, establishment the production process, and releasing the formulated products.

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