BIO_12 - Characterization methodologies establishment for an anti PD-1 biosimilar monoclonal antibody

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Introduction: Biosimilars are safe and effective treatment options for many illnesses. They increase access to lifesaving medications with potentially lower costs. This product must have the same safety, quality and efficacy of the original biological product. In accordance to the regulatory requirements, characterization assays and clinical studies should be done to ensure equivalence between the biosimilar and the reference drug. Besides that, the evaluation of the production process and quality control are also required.

Objectives: To develop the characterization methods for the quality attributes analysis of anti-PD-1 monoclonal antibodies.

Methodology: The reference monoclonal antibody (mAb) was evaluated for homogeneity by SDS-PAGE and UV-scanning, and structure by fluorescence. The wavelength of maximum absorbance was determined and a standard curve was built to calculate the molar absorptivity coefficient using the Lambert-Beer Law. Secondary structure evaluation was carried out by circular dichroism (CD). Microscale thermophoresis was used to evaluate the interaction between mAb and its PD-1 receptor. Determination of free thiol groups was performed using the *Ellman*'s assay. Hydrophobic interaction, ion exchange and molecular size exclusion chromatography (SEC) were used to access the homogeneity, relative molecular weight, and characterization of potential variants. Established chromatography methods were tested using mAbs forced degradation with the conditions determined by thermokinetic analysis. The biosimilar anti-PD1 mAb produced by Bio-manguinhos was purified by affinity chromatography and submitted to established methodologies.

Results: The coefficient of molar absorptivity obtained was 293.996 L/mol-1cm-1, with a coefficient of variation of 2.5%. Secondary structure analysis showed a profile of regular beta-sheet predominance and random structure. Interaction was observed with the PD-1 receptor (Kd=1.73 uM) determined by the second binding event. The amount of free sulfhydryl (0.603mM) corroborated with that expected by the theoretical calculations. The estimate molecular weight was 160.45 kDa. Chromatographic methods were effective for the detection of molecular variants obtained by forced degradation (60°C). Results obtained for the biossimilar mAb ensure the established methodologies and are in accordance with literature data.

Conclusion: The established methodologies suggested to be efficient for the characterization of the reference product such as the biosimilar monoclonal antibody produced by Bio-manguinhos.

Keywords: monoclonal, Antibody, biossimilar