

## BIO\_11 - Characterization of anti-PD-1 biosimilar monoclonal antibody candidates using established methodologies

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**Introduction:** Anti-PD1 antibodies are effective treatments for several types of cancer. The innovative biological products currently available are expensive and biosimilars are a lower cost option that could increase population access. Bio-Manguinhos is currently developing two biosimilar candidates, named as candidates A and B (CA and CB), respectively. According to regulatory requirements, candidates must demonstrate quality, safety, efficacy and biosimilarity to the innovative biological product (RA and RB) through analytical methods for the structural characterization.

**Objectives:** Structural characterization through developed methods to demonstrate analytical similarity between candidates and their reference products.

**Methodology:** Scanning profiles by UV, secondary structures by Circular Dichroism, tertiary structures by Fluorescence, thermal stability by NanoDSF, homogeneity and relative molecular weight by size exclusion chromatography (SEC) were evaluated for both candidates. In addition, CB concentration was determined based on the experimentally obtained extinction coefficient from the reference product and *Ellman's* assay was used to quantify free thiol groups. Hydrophobic interaction (HIC) and ion exchange (CEX) chromatography were also employed for CB characterization. All assays were simultaneously carried out for both candidates and its respective innovative biological product.

**Results:** UV scanning of both candidates presented similar profiles compared to the reference product, as well as secondary structures with predominance of regular beta-sheet. Tertiary structures results were also comparable to the reference products and demonstrated maximum fluorescence intensity at 330nm for CA and RA and 346nm for CB and RB. Thermal kinetics results showed a melting temperature of 58.5°C and 63.7°C for CA and RA, respectively, while for CB, the obtained value was 68.2°C and 68.3°C for RB. The estimated molecular weight for both candidates and reference products was around 150kDa by SEC analysis. The amount of free sulfhydryl observed was 0.0114mM for CB and 0.0045mM for RB, the results corroborated with the expected. In addition, HIC and CEX methods were effective to distinguish potential molecular variants.

**Conclusion:** All methods were adequate for structural characterization of innovative products and Bio-Manguinhos biosimilars candidates showing their capability to be used as analytical tools to certify analytical similarity of both developed molecules.

**Keywords:** Biosimilars; Monoclonal antibody; Anti-PD-1