Application of Proteometer-L Kit in the Development of mAbs With Reduced FcγR Effector Function

NOVILYTIC

BACKGROUND AND INTRODUCTION

Blocking Fc effector functions of therapeutic monoclonal antibodies (mAbs) through Fc engineering is a preferred strategy for reducing unwanted inflammatory responses [1,2]. This approach is commonly used in the development of anti-cancer mAbs[3,4].

Throughout the product development lifetime, it is crucial to consistently monitor the quality of these engineered mAbs lacking Fc effector functions, particularly titer and aggregate content to ensure manufacturability and safety. This application note demonstrates how Novilytic's Proteometer-L Kit is utilized for measuring the titer and relative aggregate composition of a biosimilar of Tislelizumab, a therapeutic mAb that completely lacks Fc receptor function. We further establish the Proteometer-L Kit's capability to perform these measurements directly in clarified fermentation broth (CFB) without prior Protein A purification. The results show that the Proteometer-L Kit is an economical, time, and labor-saving analytical method that can be employed for the development of mAbs lacking some or all Fc effector functions.

PRINCIPLE

The Proteometer-L Kit utilizes the new Mobile Affinity Sorbent Chromatography (MASC™) Luminon technology[5]. Detection of the fractionated mAb aggregate and monomer peaks is achieved by utilizing a highly specific fluorescent molecular recognition agent that interacts with an unknown but conserved structure in the Fc domain of the mAb. As a result, non-mAb components of CFB do not interfere, allowing for direct measurement of these two mAb critical quality attributes in CFB.

RESULTS

Tislelizumab is an IgG4 subclass Fc-engineered anti-cancer mAb. It has six mutations spanning the CH2 and CH3 domains (E233P, F234V, L235A, D265A, L209V, R409K) to eliminate Fc effector functions. Tislelizumab biosimilar (research use only) was formulated at 0.3 mg/mL in CFB. Using the Proteometer-L Kit, duplicate samples of varying amounts were analyzed on an HPLC system equipped with a fluorescence detector (Figure 1). Despite the presence of host cell proteins in the CFB sample matrix, there was no interference in the mAb peaks from CFB components. The mAb monomer and aggregate content were therefore determined without any sample cleanup prior to analysis. The total area of monomer plus aggregate peaks is a measure of the mAb content in the injected sample and it is used to determine mAb titer. mAb titer exhibited excellent linearity for injected mAb amounts from 0.375 to 3 µg, for the Tislelizumab biosimilar (R^2 = 0.9996). Relative aggregate composition was estimated as the ratio of aggregate peak area to total area of mAb and aggregate peaks and expressed as a percentage. From the ten injections, the aggregate content was estimated as 2.48 ± 0.20 percent in the Tislelizumab biosimilar (CV, 7.9 %).

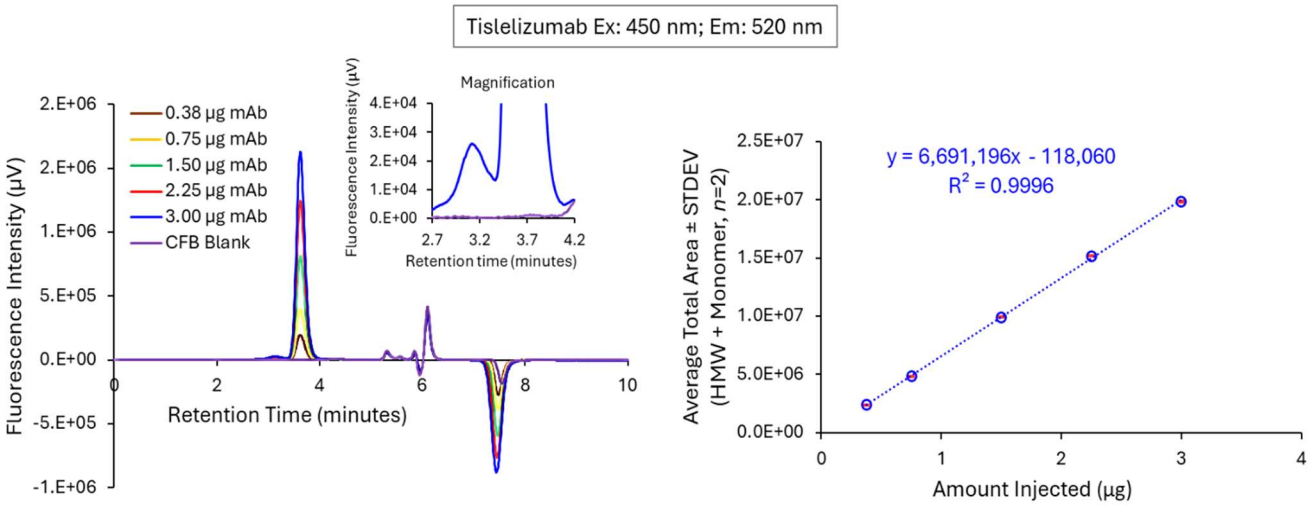

Figure. 1. Chromatograms and linear dynamic range of Tislelizumab in CFB.

The results clearly demonstrate that the Proteometer-L Kit can be used to monitor titer and relative aggregate content of mAbs that contain Fc mutations that abrogate Fc receptor functions. These results support and exptend previous findings that showed it could be used to analyze aglycosylated mAbs that also exhibit impaired Fc receptor functions[6].

REFERENCES

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