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Quality Assurance Using Mass Spectrometry to Analyze Structural Fidelity of Monoclonal Antibodies in HIV-1 Therapeutics

Troy Wood 230 - Quality Assurance Using Mass Spectrometry to Analyze Structural Fidelity of Monoclonal Antibodies in HIV-1 Therapeutics, Meeting Room 105, August 23, 2024, 12:14 - 12:33

The development of broadly neutralizing antibodies (bNAbs) for the prevention and treatment of HIV-1 infection has gained attention as an alternative to conventional antiretroviral therapy because of their potential for passive immunotherapy and long half-lives. A number of bNAbs targeting the HIV-1 viral envelope are under investigation for their effects on viral suppression in combination. While methods to quantify bNAbs using mass spectrometry are one element of quality assurance, it is also important to validate the structural fidelity of therapeutic antibodies; this includes not only validation of the primary amino acid sequence, but also the presence and location of posttranslational modifications (PTMs), many of which are essential for optimal efficacy of the antibody.

Methods

A bottom-up proteomics workflow was developed using the NIST mAb IgG1k reference material, used frequently in development of antibody-based proteomics workflows. The approach uses RapiGest to unfold the antibody, followed by reduction and alkylation steps prior to enzymatic digestion. This approach was then applied to the quality assurance of the primary structure of the therapeutic bNAb PGT 121.414.LS, which is currently being evaluated for efficacy of viral suppression in clinical trials in combination therapies. Enzymatic digests of antibodies were analyzed using Sequest node through Proteome Discoverer ver. 2.3; MS/MS using CID was performed to confirm amino acid sequence and locations of PTMs. Glycan sites on PGT 121 antibodies are essential for recognition of HIV-1 envelope glycoprotein are of particular interest.

Novel Aspect

Application of proteomic workflows to assess structural characteristics of a broadly neutralizing antibody used in treatment and prevention of HIV-1.

Preliminary Data or Plenary Speaker Abstract

Initial tryptic peptide maps of NIST RM 8671 revealed 30 peptides from the heavy chain and 14 from the light chain, accounting for 84% sequence coverage for the light chain and 80% for the heavy chain. Numerous PTMs were identified including oxidation, deamidation and glycosylation throughout the antibody. A number of glycans were determined to be present on Asn-300 in the consensus sequence EEQYNSTR coinciding with glycopeptides previously reported. The two highest abundance glycans present correspond to the glycans HexNAc(4)Hex(3)Fuc(1) and HexNAc(4)Hex(4)Fuc(1). The tryptic peptide mapping method developed for NIST RM 8671 was then applied to PGT121.414.LS. Initial peptide mapping results indicated 97% sequence coverage of the light chain and 80% of the heavy chain. 41

peptides were identified from the heavy chain and 21 peptides were identified from the light chain. Full coverage of the LCDR2, LCDR3, HCDR2 were obtained along with partial coverage of the LCDR1. No tryptic peptides derived from the HCDR1 or HCDR3 regions were detected. Additional digestion utilizing another protease is necessary for mapping these two regions. Numerous PTMs were identified for PGT 121.414.LS including oxidation of methionine and deamidation of asparagine and glutamine. Peptides derived from the complementary determining regions were closely investigated for the presence of PTMs. Peptides identified from the complementary determining region did contain PTMs. The glycosylation profile of PGT 121.414.LS revealed a number of glycopeptides for the backbone sequence EEQYNSTR. The identified glycoforms present included HexNAc(4)Hex(3)Fuc(1) and HexNAc(4)Hex(4)Fuc(1). Additionally, glycopeptides with masses corresponding to HexNAc(4)Hex(5)Fuc(1), HexNAc(3)Hex(3)Fuc(1), and HexNAc(2)Hex(3)Fuc(1) were identified. The bottom-up results indicate numerous glycoforms present in the sample of PGT 121.414.LS.