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Towards Improved Antibody Therapeutics: Studying the Effect of Glycosylation on IgG2

Khalid Al-Kinani

Thomas J. Tolbert

University of Kansas, Department of Pharmaceutical Chemistry, Lawrence, KS 66047 USA

Monoclonal antibodies (mAb) and related products are the fastest growing class of human therapeutics and most of these mAb are of the IgG isotype. The human IgG2 is the second most abundant in human serum. Glycosylation of antibodies is a very important post-translational modification (PTM) that affects antibody yield, effector functions (through binding of the Fc region to Fcγ-receptors), pharmacokinetics, immunogenicity as well as antibody physical and chemical stability. Unlike other processes like transcription and translation, glycosylation is a non-templated process which ultimately leads to heterogeneity of the glycosylated protein. Therefore a consistent human glycoform profile has been and remains a considerable challenge to the biopharmaceutical industry and the FDA requires that a consistent glycosylation profile be maintained for mAbs.

For IgG2, there are two variants: a more common one with only one glycosylation site in each heavy chain (at Asn 297), and a less common one which has two glycosylation sites (at Asn 297 and Asn 392). The gene that codes for the Fc region of each variant was cloned into pPICZαA plasmids. Then, the recombinant DNA was transformed into glycoengineered Pichia pastoris to express IgG2 Fc with a defined high mannose glycan that can be further modified by in vitro chemo-enzymatic reactions in order to address the effect of different glycans on the abovementioned criteria. After expression, the proteins were purified by protein G affinity chromatography then hydrophobic interaction chromatography (HIC) was used to separate the different glycoforms of the expressed protein.

FcγRIIa receptor variants (both 131His and 131Arg variants) were also cloned into the pPICZαA plasmid and expressed recombinantly in Pichia pastoris. Some preliminary binding studies were done to see the effect of glycosylation on binding of IgG2 Fc to these receptors. Continuing studies will elucidate the effect of different glycans on IgG2 properties to ultimately optimize this important subclass of antibodies for better and improved biotherapeutics.