STUDY OF AN UNUSUALLY HIGH LEVEL OF N-GLYCOLYLNEURAMINIC ACID (NGNA) SIALYLATION ON A MONOCLONAL ANTIBODY EXPRESSED IN CHINESE HAMSTER OVARY CELLS

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Sialic or neuraminic acids of recombinant therapeutic glycoproteins produced in mammalian cells, including monoclonal antibodies, have significant impact on the half-life, stability, and biological activity of these proteins (Hossler et al., 2009; Ghaderi et al., 2012). The predominant sialic acid *N*-acetylneuraminic acid (NANA or Neu5Ac) is added from precursor CMP-NANA to galactose residues of *N*-linked glycoproteins by sialytransferases. In most mammals CMP-NANA can also be modified to its hydroxylated derivative CMP-NGNA by CMP-*N*-acetylneuraminic acid hydroxylase (CMAH). NGNA can then be added from CMP-NGNA to galactose residues of the *N*-linked glycoproteins, also by sialytransferases. However, humans cannot make functional CMAH due to an inactivating exon deletion mutation in *CMAH* gene (Okerblom and Varki, 2017), and therefore cannot convert CMP-NANA to CMP-NGNA. Consequently, when injected into human patients, NGNA sialic acid containing mAbs or other recombinant glycoproteins may induce immune responses, which could negatively impact pharmacokinetics or efficacy. Therefore high levels of NGNA on therapeutic mAbs or other recombinant glycoproteins are an undesirable product quality attribute.

The level of total sialic acids of recombinant glycoproteins produced in Chinese hamster ovary (CHO) cells is dictated largely by the selected cell lines, upstream process, and to a lesser degree, downstream process. NGNA sialylation is generally rare in CHO cells (Könitzer et al., 2015). Hence, therapeutic glycoproteins manufactured in these cells are considered safe for human use. However, during a first-in-human (FIH) upstream process development for a novel mAb, an initially selected desirable cell line (A) was found to produce the mAb with an unexpectedly high level of the NGNA sialic acid (>30%). To the best of our knowledge such high level of NGNA sialylation on a mAb produced by CHO cells has not been reported. To mitigate potential risks associated with high NGNA in human patients, a new cell line (B) that produces the mAb with very low NGNA was selected as the manufacturing cell line for this project.

In order to understand the molecular mechanism causing the high NGNA content in cell line A, we initiated comprehensive genetic gap analyses using next-generation sequencing technologies and determined the differences in genomic, transcriptomic, and miRnomic profiles of the two cell lines. The results indicate spontaneous upregulation of CMAH mRNA expression, at least 10 fold higher in cell line A compared to cell line B. In this talk we will summarize the results of our studies of this unusual sialylation behavior in CHO cells.