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A new Lectin Glyco-Array approach – A perfect technology to characterize Glycol-Pattern of Biosimilars & Biologics

Major characteristics of a protein are determined by its amino acid sequence. The next levels are secondary, tertiary, and quaternary structures. Notably, protein features can be modified by a variety of posttranslational modifications including glycosylations. Approximately 50 - 60% of human proteins get some kind of glycosylation usually by the addition of N- or O-linked glycans. Glycosylation cannot only affect the structure of proteins, but also their biological activity, potency, serum half-life, pharmacokinetics, pharmacodynamics, and immunogenicity. Therapeutic proteins represent the fastest growing market of the pharmaceutical industry and there is a tremendous rush by many companies worldwide to develop Biosimilar versions of innovator products. The type of cell line used for the production of therapeutic proteins, and defined cell culture conditions have a considerable influence on the glycosylation pattern, which in turn can directly affect product quality, safety and efficacy. Carbohydrates like terminal galactose residues, bisecting GlcNAc and core fucose have a critical impact on mAb mediated effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC). Mannosylated glycans and sialic acid N-Acetylneuraminic Acid (NANA) can impact PK, and lower levels of galactose reduce Complement-Dependent Cytotoxicity (CDC) activity. For production of therapeutic proteins, analysis of glycosylation patterns is thus of utmost importance. As an example, rituximab makes it clear that a biosimilar should never be developed without knowing the status of the glycosylation pattern in comparison to the originator molecule. One biosimilar in development showed higher receptor affinity and higher ADCC activity, and therefore EMA has advised the applicant to adjust the manufacturing process. After thorough analysis, the primary amino acid sequence of the biosimilar was shown to be identical, and secondary and tertiary structures of the proteins were indistinguishable. However, proportions of some glycosylations were slightly different. A modified manufacturing process finally directed the oligosaccharide composition within the variability of the originator. Standard techniques for analysis of glycoproteins are based on high performance liquid chromatography, mass spectrometry, and capillary electrophoresis. The application of lectins as a class of molecules that can specifically bind carbohydrate-protein structures has evolved in the last years in combination with

microarrays as a promising additional tool. Lectin microarrays were first reported in 2005 and are prepared by immobilizing various lectins on a solid surface. The microarray procedure is based on an evanescent-field fluorescence-detection principle, which allows sensitive, real-time observation of multiple lectin-carbohydrate interactions under equilibrium conditions. Analytes including glycoproteins, whole cells, or bacteria are labelled with a fluorescent dye or antibody before loading onto a commercially available lectin microarray containing up to 45 specific lectins. The usefulness of lectin microarray technology is demonstrated with several examples. In one of these, the glycosylation pattern of the mAb MB311 expressed in SP2.0 cells was compared to the mAb MB314 expressed in a plant expression system to generate a de-fucosylated version of MB311. The fucose indicative lectin-binding patterns correlated with the respective ADCC activities. Such data and available literature show convincing evidence that lectin microarrays can be used for screening and evaluating different glycan patterns of therapeutic glycoproteins.

Biography

Besides serving as a Managing Director, Markus has also taken responsibilities as Head of Regulatory Affairs within the Quality Operations team. Additionally, he is in charge of business development items and client relations topics. He was previously Head Quality Control at Igeneon/Aphton Biopharma where he was responsible for all QC aspects of pre-clinical and clinical projects such as stability studies, specifications, method validation, and product release. Before that, he was Group Leader of Immunology and Product Development at Biomin gmbh, Head Biochemical Control at Baxter AG and Head Quality Operations at Octapharma gmbh. He has more than 20 years of experience in pharmaceutical industry, with focus in vaccines, recombinant proteins and immune therapeutics. He has profound expertise in regulatory aspects such as third party audits, inspections and GMP guidelines. He has a management consulting education and additionally owns a doctorate in chemistry/biochemistry from the Technical University of Graz, Austria.

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