
Preface

Therapeutic properties of monoclonal antibodies (mAbs) and other glycoproteins strongly depend on the composition of their glycans. Most of the currently approved biopharmaceuticals are produced in mammalian cell lines, which yield mixtures of different glycoforms that are close to those of humans but not fully identical. Glyco-engineering is being developed as a method to control the composition of carbohydrates and to enhance the pharmacological properties of mAbs and other proteins. The approval, on April 30, 2012, in Japan, of mogamulizumab, the first glyco-engineered antibody to reach the market, was a landmark in the field of engineered biopharmaceuticals. Mogamulizumab is a humanized mAb with enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity linked to optimized a-fucosylated glycoforms and an illustration of the therapeutic importance of a tailored glycosylation. The antibody is indicated for patients with relapsed or refractory CCR4-positive adult T-cell leukemia-lymphoma.

The aim of this present volume of *Methods in Molecular Biology* is to provide readers with production and characterization protocols of glycoproteins and glyco-engineered biopharmaceuticals with a focus on mAbs. The volume is divided in four complementary parts dealing with Glyco-engineering of therapeutic proteins (Part I), Glycoanalytics (Part II), Glycoprotein complexes characterization (Part III), and PK/PD assays for therapeutic antibodies (Part IV).

The first two chapters deal with recombinant glycoproteins produced in Chinese Hamster Ovary (CHO) cells, the most frequently used cell line to produce biopharmaceuticals. J. Holgersson (University of Gothenburg, SE) and colleagues report methods to engineer therapeutic and diagnostic O-glycans on recombinant mucin-type immunoglobulin fusion proteins. C. Ronin et al. (Siamed'Xpress, FR) follow up with protocols to engineer human-like glycosylation of therapeutic glycoproteins based on 6-linked sialylation. The next three chapters discuss the use of non-mammalian cell line to produce glycoproteins including yeasts (*Pichia pastoris* and *Saccharomyces cerevisiae*) and insect cells infected with baculoviruses. First, D. Zha (Merck-GlycoFi, US) describes the production of glyco-engineered *Pichia*-based expression of Monoclonal Antibodies. Then C. Javaud (Glycode, FR) presents the humanization of N-glycosylation of antibodies produced in *S. cerevisiae*. Finally, M. C erutti (CNRS, FR) and colleagues report methods to engineer the baculovirus genome to produce galactosylated antibodies.

To assess the structure of glycoprotein and glyco-engineered biopharmaceuticals, state-of-the-art orthogonal analytical methods are needed. E. Wagner-Rousset (CIPF, FR), C. Schaeffer-Reiss (CNRS-LSMBO), and colleagues describe nanoLC-chips-MS/MS methods for the characterization on N-glycopeptides generated from trypsin digestion of monoclonal antibodies. Alternatively, M.C-Janin Bussat, L. Tonini, and colleagues (CIPF, FR) propose the use of IdeS proteolytic digestion and electrospray ionization—time-of-flight mass spectrometry for antibody fast differential glycoprofiling of cetuximab Fab and Fc glycans. Then, A. Delobel, G. Van Vyncht, et al. (Quality Assistance, BE) report a panel of analytical methods that are used to characterize therapeutic antibody glycosylation for batch release or comparability support, tacking the case of trastuzumab. To have a complete

picture, mass spectrometric analysis of *O*-linked oligosaccharides from various recombinant expression systems are described by J. Holgersson, N.G. Karlsson, and colleagues (University of Gothenburg, SE). In complement to mass spectrometry, glycoprofiling can also be performed by liquid chromatography and by electrophoresis based methods. This is illustrated by T.S. Raju (Janssen R&D, US) who reports the assessment of Fc Glycan heterogeneity of therapeutic recombinant mAbs by Normal Phase—HPLC, and by R.R. Rustandi and colleagues (Merck) who report two different Capillary Electrophoresis systems. Alternatively glycoprofiling may be performed based on lectins as illustrated by L. Landemarre (GLYcodiag, FR) and E. Duverger (Université d'Orléans, FR) for recombinant therapeutic Interleukin-7. Glycosylation also has a deep impact on glycoprotein solubility and limits the propensity to aggregate. This can be assessed either by Hydrophobic Interaction Chromatography, as illustrated by R.R. Rustandi (Merck, US), or by Sedimentation Velocity Analytical Ultracentrifugation as reported by W.B. Stine (Abbott, US).

To go a step forward, glycoprotein complexes can be characterized by emerging mass spectrometry methods. S. Sanglier-Cianferani (CNRS-LSMBO), E. Wagner-Rousset (CIPF, FR), and colleagues report the use of non-covalent mass spectrometry for the characterization of antibody/antigen complexes. In addition, conformational analysis of recombinant mAbs can be performed by Hydrogen/Deuterium Exchange Mass Spectrometry as reported by D. Houde (BiogenIdec, US) and J.R. Engen (Northeastern University, Boston, MA). To gain insights on the interaction of antibodies with their target antigens, epitope and paratope can be mapped by different Mass Spectroscopy methods as described by Victor Obungu and colleagues (Lilly, US).

Last, but not least, PK/PD assays for therapeutic antibodies are mandatory to explore the impact of glycosylation or glyco-engineering on pharmacokinetics and potency. For this purpose, M. Broussas, L. Goetsch, and L. Broyer (CIPF, FR) describe a method for the evaluation of antibody-dependent cell cytotoxicity (ADCC) using Lactate Dehydrogenase Measurement and a method for complement-dependent cytotoxicity (CDC) determination using ATP measurement and C1q/C4b binding. As a surrogate in vitro assay, the capture of the human IgG1 antibodies by protein A for the kinetic study of h-IgG/Fcγ₁R interaction using SPR-based biosensor technology is reported by T. Champion (CIPF, FR). To evaluate the antibody clearance, a mass spectrometry protocol for the absolute quantification of a mAbs in serum with immuno-purification is described by F. Becher and colleagues (CEA, FR).

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The book is dedicated to my wife Nathalie, to my daughters Juliette and Louise, and to my parents Paulette and Norbert. Thanks also to Claire Catry for her help in some logistics aspect concerning this book.

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