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Therapeutic Fc-Fusion Proteins

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This new, comprehensive text published by Wiley Blackwell provides a much needed re-write on the earlier publication of 15 y ago,[1] published just after the approval of the first Fc-fusion protein, etanercept (Enbrel®). The field of therapeutic proteins, dominated by antibodies and Fc-fusion proteins, has advanced dramatically over this period, with multiple new structures and successful therapeutics being widely used to treat patients for a broad array of afflictions.

The authors tackle this large field systematically in 13 chapters, beginning with an introductory chapter on basic principles of antibody structure and the unique aspects of the Fc portion of the IgG antibody structure. They then go more in-depth with six chapters on expression, production, purification, formulation, quality by design, and analytics of Fc-fusion proteins. Finally, the text concludes with an overview chapter on Fc-fusion proteins followed by detailed chapters on five of the most significant Fc-fusion proteins, including alefacept (Amevive®), etanercept (Enbrel®), abatacept (Orencia®) and belatacept (Nulogix®), and aflibercept (Eylea® and Zaltrap®).

As a bioprocessor, I especially appreciated the book's coverage of the pragmatic aspects of the production, purification, analytical testing, and formulation of these designer proteins. While IgG antibodies are amenable to well-established, platform processes,[2] Fc-fusion proteins are at best only partially amenable to such approaches. There are multiple reasons for this difference, including that fact that Fc-fusion proteins are artificial constructs not evolved for desirable elements such as cellular production, solubility, and stability. Furthermore, properties of the fusion partner to the Fc can sometimes interfere with standard IgG-based purification strategies such as Protein A affinity chromatography.[3]

Therapeutic Fc-Fusion Proteins does an excellent job at describing the multiple functions associated with the Fc portion of the IgG molecule. The authors clarify the arcane world of Fc receptors, how they interact with different sections of the Fc, and how this differs both between species and subtypes of IgGs. One of the most valuable aspects of the Fc structure is its ability to prolong dramatically the circulatory half-life of IgGs and Fc-fusion proteins. Instead of the expected less than 1 week seen with similar, non-Fc containing proteins, the authors note that IgGs and Fc-fusion proteins can demonstrate half-lives of up to 3 weeks. This phenomenon is due to the unique recycling of Fc-containing structures via the neonatal Fc receptor (FcRn) found in hepatocytes. Fc-fusion proteins are able to exploit this property of IgG antibodies to provide a long plasma half-life, which allows reduced dosing and dosing frequency.

The authors note the importance of proper attention to post-translational modifications, especially N- and O-linked glycosylation, in the Fc partner. The Fc itself is glycosylated at only at one N-linked location, and the glycans are largely surrounded by the protein region of the CH2 domain. The effects of Fc glycosylation on half-life are thus minimal at best. In contrast, many Fc partners have significant, exposed glycosylation at multiple locations. The authors provide examples showing that, in spite of the Fc portion's ability to prolong plasma half-life, glycoform-mediated clearance due to the Fc partner in a fusion protein can result in an unexpectedly rapid clearance. They note the accelerated clearance resulted from specific receptors for incompletely formed, complex glycoforms, such as those with terminal N-acetylglucosamine (GlcNAc) or galactose, on the Fc partner. Control of bioreactor processes can reduce the extent of such incomplete glycoforms, as can downstream processing.

Other elements of complexity in the production of Fc-fusion proteins, such as their well-known tendency to aggregate, as well as to misfold in structure and mispair disulfides, are described in the chapters on production and purification. Specific control strategies are described, and a wide-ranging reference list for more in-depth reading is provided. A full chapter is devoted to formulation, drug product and delivery of these often challenging proteins.

The extensive analytical testing required for protein structures as complex as Fc-fusion proteins is well covered by a separate chapter. Building on the assays typically needed for IgG proteins, the authors systematically describe both in-common as well as unique assays for Fc-fusion proteins. These include not only specific potency assays, including cell-based ones, but also detailed structural characterization via powerful techniques such as mass spectroscopy (LC-MS) and various types of HPLC/UPLC analyses, including peptide mapping. The chapter properly notes that

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the additional glycosylation often seen in Fc-fusion proteins requires greater attention to glycosylation testing than for a typical IgG antibody.

I especially enjoyed the chapters dedicated to specific Fc-fusion proteins, each of which covered the structure's history, design, development, and therapeutic rationale. Helpful structural diagrams with color-coding of specific areas of the fusion protein are included in each of these chapters. Specific disease indications, which can be numerous for a drug such as etanercept (Enbrel®), are also detailed, showing the widespread applicability of several of the Fc-fusion protein therapeutics.

In conclusion, I found *Therapeutic Fc-Fusion Proteins* to be thorough, timely and well written. The editors have chosen leading subject-matter experts to author each chapter. Each chapter is extensively referenced for those seeking additional information. This book would be of interest to a wide biopharmaceutical audience, from scientists in discovery through development and analytics, as well as to manufacturing personnel and clinicians. I recommend it both as an informative, comprehensive review of the field and a valuable reference text.

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