

Fc-fusion proteins: therapeutic relevance and quality assessment

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Introduction

Fc-fusion proteins are bioengineered polypeptides that combine a biologically active protein with the crystallizable fragment (Fc) domain of an IgG to produce a molecule with unique properties and therapeutic potential (Linderholm & Chamow, 2014). Owing to its interaction with the salvage neonatal Fc-receptor, Fc domain substantially increases plasma half-life *in vivo* and reduces the clearance of Fc-fusion proteins, which prolongs their therapeutic activity (Czajkowsky et al., 2012). In addition, Fc fragment improves the solubility and stability of the fusion protein (Carter, 2011). Fc fusion proteins are very successful class of medicines with already 13 Fc-fusion proteins approved in the EU and in the USA (EMA, 2022; U.S. FDA, 2022). Currently four therapeutic Fc-fusion proteins including aflibercept, dulaglutide, etanercept and abatacept are among the 50 globally best-selling medicines (Buntz, 2022).

Classification of Fc-fusion proteins

Based on the ligand binding domain Fc-fusion proteins are classified in four main categories.

Receptor-ECD-based Fc-fusion proteins

This category includes TNFR-FC fusion protein, etanercept, and CTLA4-Fc fusion proteins (abatacept and belatacept). Etanercept is homodimeric fusion protein consisting of ECD of the human p75 TNF receptor linked to a human Fc-IgG1 domain. Etanercept is used for the treatment of inflammatory rheumatic disorders and psoriasis. Abatacept consists of ECD of human CTLA4 linked to the modified Fc. Abatacept is used for the

treatment of inflammatory rheumatic disorders. Belatacept is indicated for the prophylaxis of graft rejection (EMA, 2022).

Cytokine traps

This class of Fc-fusion proteins consist of multiple ligand-binding domains from different receptors linked to a human Fc domain. This group includes aflibercept consisting of portions of human VEGF receptor 1 and 2 ECDs fused to the Fc portion of human IgG1. Aflibercept is used for the treatment of macular degeneration (MD) and metastatic colorectal cancer. Conbercept is VEGF inhibitor fusion protein, used for the treatment of MD. The third representative, riloncept is designed by fusing the C-terminus of the IL1 receptor accessory protein ligand-binding region to IL1R1 ECD, then this hybrid construct is fused to human IgG1 Fc. Riloncept is used for the treatment of cryopyrin-associated periodic syndromes (EMA, 2022).

Peptide-Fc (peptibodies)

Romiplostim is a dimeric peptibody consisting of the IgG1 Fc-domain fused with two polypeptide mimetic sequences of thrombopoietin attached to each Fc chain. Romiplostim is used for the treatment of ITP. Dulaglutide is a glucagon-like peptide (GLP)-1 receptor agonist in which a modified GLP-1 peptide is fused to the IgG4 Fc fragment.. Dulaglutide is used for the treatment of type 2 diabetes (EMA, 2022; U.S. FDA, 2022).

Recombinant enzymes

Eftrenonacog alfa is a fusion protein consisting of human coagulation factor IX linked to the Fc fragment of

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human IgG1. It replaces the missing factor IX, which plays a central role in blood clotting and is indicated for the treatment of hemophilia B. Efmoroctocog alfa is a fusion protein composed of human, B-domain-deleted, coagulation factor VIII linked to the Fc domain of human IgG1. This drug is indicated for the treatment of hemophilia B. The third representative of this class is asfotase alfa, used for the treatment of hypophosphatasia. Asfotase alfa is a human recombinant tissue-nonspecific alkaline phosphatase-Fc-deca-aspartate (EMA, 2022).

Biosimilar Fc-fusion proteins

Biosimilar is a highly similar version of an already authorized innovator biological medicine known as reference product. Originator of etanercept, Amgen/Pfizer's Enbrel was among the best-selling medicines and in 2021 its sales reached about 4.5 billion USD. The patent expiry in Europe and the USA led to development of biosimilar versions. Currently three biosimilars of etanercept are authorized in EU and USA. Till 2026, it is expected that the global market value of the biosimilars will rise by 25% compared to the value in 2020 (Ratih et al., 2021).

Quality assessment of Fc-fusion proteins

Fc-fusion proteins are characterized with inherent heterogeneity and complex structure. The complexity of their manufacturing process is one of the key factors affecting consistency of the product quality. Small structural alterations could possibly initiate substantial changes in drug stability, immunogenicity and efficacy. Therefore, the analytical strategies for the characterization of Fc-fusion proteins should be product specific and involve state of the art analytical techniques. A wide range of highly selective analytical techniques are applied for assessment of the critical quality attributes (CQAs) of the Fc-fusion proteins. Analytical comparability of the CQAs between different production batches is performed for the assessment of the consistency of the product quality. In addition, comparability studies of the CQAs between originator and biosimilar are inevitable for the biosimilarity assessment. High standards of the quality, safety and efficacy are required for the biosimilars. Considering the diversity of the CQAs of this class of drugs (such as physicochemical characterization, primary structure, post-translatory modifications, purity, charge and size variants, etc.), a set of analytical techniques are required for the quality assessment. The method of choice for confirmation of the primary structure of the Fc-fusion proteins (identification and quantification of protein variations) is LC-MS/MS. Size-exclusion

chromatography is gold standard method for size variants analysis (Duivelshof et al., 2021). Two-dimensional gel electrophoresis is a suitable method for the assessment of identity, purity, structural integrity, charge heterogeneity and post-translational glycosylation of the Fc-fusion protein (Nebija et al., 2015). In addition, ion exchange chromatography and capillary zone electrophoresis are used for homogeneity assessment (Beck et al., 2013).

Concluding remarks

The unique functional properties of the Fc-fusion proteins, such as half-life extension and great therapeutic potential, place these medicines in the front line of drug research and development. The diversity of the Fc-fusion proteins, along with the rapid growth of their biosimilars, impose the need for implementation of specific and highly sensitive chromatographic and electrophoretic techniques for the quality assessment of the CQAs of the Fc-fusion proteins.

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