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XIX Convegno Triennale sugli Aspetti Clinici e Sociali dell'Emofilia ABSTRACT N. CO-55

2.07 Novel therapeutic products and approaches (including gene therapy)

DEVELOPMENT OF ENGINEERED LONG-ACTING BYPASSING FX VIA ALBUMIN FUSION FOR HEMOPHILIA WITH INHIBITORS

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Background and Aims. The management of bleeding in patients with Hemophilia and inhibitors requires effective bypassing agents to restore hemostasis. We engineered a novel Factor X variant with bypassing activity (FXBP), featuring an activation peptide modified to be cleaved and activated by FXIa, triggering the thrombin production independently from the intrinsic tenase complex. To extend its plasma half-life, we fused FXBP with an albumin variant (QMP) characterized by enhanced affinity for neonatal Fc receptor (FcRn). This study evaluates and compares two albumin fusion strategies to balance prolonged circulation with optimal procoagulant efficacy.

Materials and Methods. The engineered FXBP variant was generated under two configurations: a direct C-terminal fusion and a conditional release construct via a cleavable linker. Both chimeras were expressed in Expi293 cells and purified via affinity chromatography and size-exclusion chromatography. Human FcRn binding affinity and kinetic constants were evaluated at different pH conditions via ELISA and Surface Plasmon Resonance (SPR). The functional profile of fusion proteins was evaluated i) in PT assays in FX-deficient plasma to assess extrinsic pathway function, ii) in aPTT assays in Hemophilia A and Hemophilia B plasma for bypassing activity, and iii) by FXIa-mediated activation.

Results. Both fusions demonstrated robust expression, purity, and efficacy. The in vitro assay with FXIa successfully validated specific proteolytic cleavage and conditional activation of FXBP. ELISA and SPR confirmed that both the direct fusion (FXBP-QMP direct) and the cleavable construct (FXBP-Linker-QMP) retained high pH-dependent affinity for human FcRn at pH 5.5, exhibiting an outstanding KD of 2.77 nM. PT assays showed that the albumin fusion strategy only slightly impaired native extrinsic FX activation compared to plasma-derived FX. Crucially, aPTT assays demonstrated bypassing activity for both constructs, which shortened clotting times in both Hemophilia A and B plasma compared to their wild-type counterparts. In particular, FXB variants showed higher bypassing activity in Hemophilia B plasma.

Conclusions. Fusing the engineered bypassing FX variant with QMP represents a highly successful strategy to optimize pharmacokinetics while preserving procoagulant function. The high-affinity FcRn binding kinetics strongly predict a prolonged plasma half-life through the receptor-mediated recycling pathway. Moreover, bypassing efficacy in hemophilic plasma confirms the therapeutic potential of these chimeras.