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DEVELOPMENT OF INNOVATIVE LONG-ACTING FACTOR IX AND FACTOR X VARIANTS VIA CONDITIONAL ALBUMIN FUSION

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Background and Aims. The management of Hemophilia A and B requires therapeutics that combine high hemostatic efficacy with reduced dosing frequency. While extending the half-life of clotting factors is a primary goal, fusion with high molecular weight partners often impairs biological activity. We describe the development of novel fusion proteins for Factor IX (FIX) and an engineered Factor X (FX) variant designed for bypassing activity. This work compares traditional full length QMP-albumin fusions with an innovative approach integrating minimized albumin domains into FIX and FX at strategic activation-sensitive sites. This conditional release mechanism aims to balance long-acting potential with uncompromised efficacy.

Methods. Five novel chimeric proteins were expressed in HEK293 cells: 1) Full length fusions comprising two for engineered bypassing FX variant and one for FIX-Padua variant fused with full-length QMP-albumin; 2) Minimized domain chimeras (one FIX-Padua and one bypassing FX) featuring albumin domains at activation-sensitive sites. Functional characterization included aPTT in FVIII and FIX-deficient plasmas and PT assays in FX-deficient plasma. Western Blot to characterize the proteolytic cleavage of the albumin domains. To predict half-life extension, FcRn binding affinity was evaluated via ELISA and Surface Plasmon Resonance (SPR) to determine kinetic constants.

Results. Preliminary data confirm the functional efficacy of all constructs. The engineered FX variants (full-length and minimized) demonstrated bypassing activity, significantly shortening aPTT in FVIII-deficient plasma compared to wild type controls. PT assays confirmed that albumin domain integration or full-length fusion did not impair native extrinsic FX activation. FIX variants successfully restored the intrinsic pathway in FIX-deficient plasma, with ongoing analyses intended to confirm conditional albumin cleavage upon activation. Finally, ELISA and SPR demonstrated that full-length QMP-albumin fusions retain high pH-dependent affinity for FcRn at pH 5.5 (KD 2-6 nM), comparable to unfused QMP-albumin and efficient release at physiological pH, that strongly supports the potential for an extended plasma half-life through the FcRn-mediated recycling pathway.

Conclusions. Fusing FIX-Padua and bypassing FX variant with albumin domains through cleavage-dependent sites is a promising strategy to enhance pharmacokinetics without compromising procoagulant function. The successful expression and functional performance of these minimized chimeras suggest a novel direction for long-acting hemostatic agents. Further characterization of cleavage efficiency and FcRn-binding kinetics will finalize the profile of these innovative therapeutic candidates.