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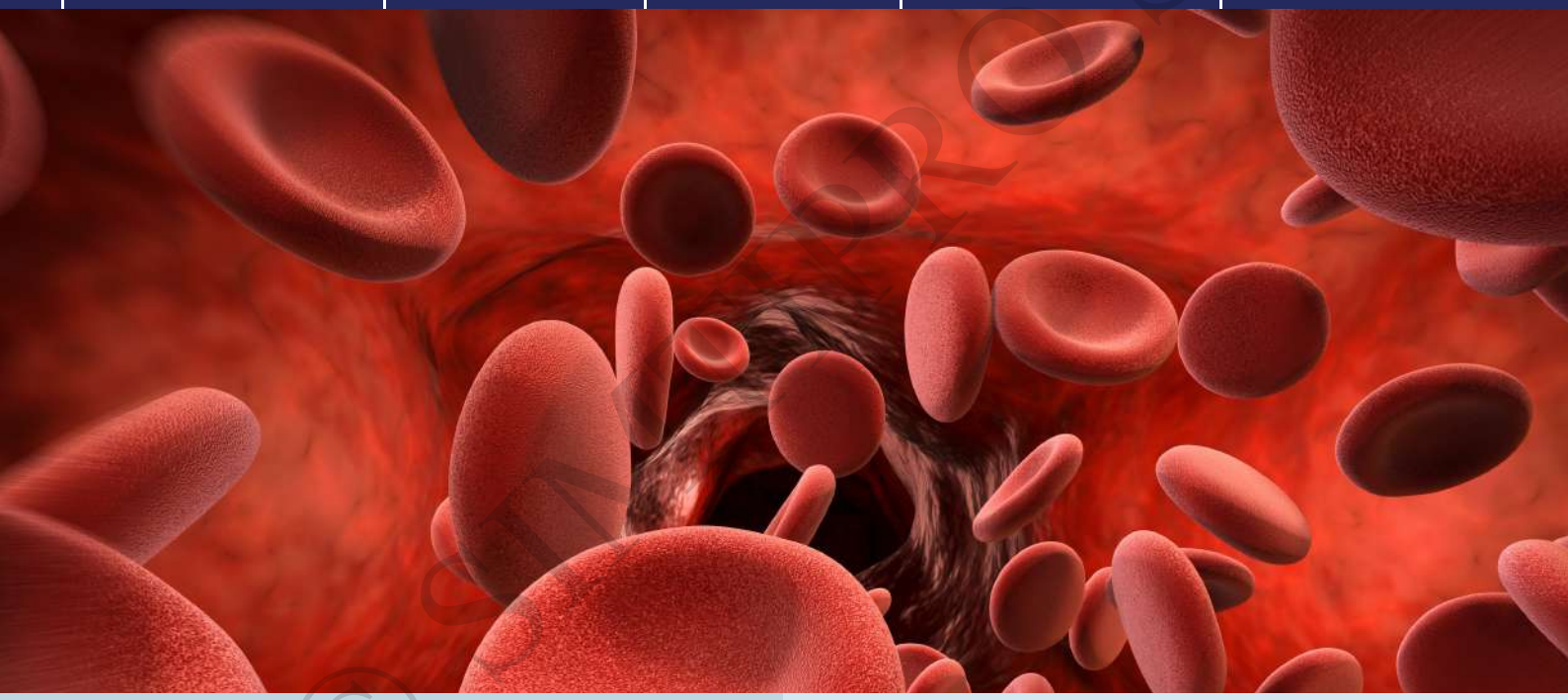
SIMTI Società Italiana di Medicina Trasfusionale e Immunoematologia

AICE Associazione Italiana dei Centri Emofilia

HDTM Hrvatsko Društvo za Transfuzijsku Medicinu

SETS Sociedad Española de Transfusión Sanguínea y Terapia Celular

SISET Società Italiana per lo Studio dell'Emostasi e della Trombosi



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ABSTRACT BOOK

XVII Convegno Triennale sui Problemi Clinici e Sociali dell'Emofilia e delle Malattie Emorragiche Congenite

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D-Dimer or a decreased level of physiological coagulation inhibitors, moreover no abnormal increase of coagulation factors was evidenced. The levels of the FVIII and FXIII, in terms of trough-level, also showed results corresponding to those expected. Patients underwent prophylactic therapy for a period ranging from 11 to 35 months, demonstrating good compliance with the prescribed therapy. No spontaneous hemorrhagic events occurred, except in one case of severe hemophilia A where we report an elbow hemarthrosis after intense sports activity, in a patient who had not complied with the normal prophylaxis protocol.

Conclusion. Our study showed that the administration of FVIII and rFXIII concentrate, in addition to being effective in preventing bleeding, does not modify clotting parameters in a prothrombotic state. Broader studies, which include PWHs with comorbidities, may confirm the usefulness of D-Dimers and physiological coagulation inhibitors as markers for the evaluation of coagulation concentrate factors in patients with congenital bleeding diseases.

2.04 - NOVEL THERAPEUTIC AGENTS

ABS26 - A next-generation rFVIIa fusion protein with enhanced half-life as a novel by-passing tool in hemophilia

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Background. Recombinant activated factor VII (rFVIIa) has represented an enormous therapeutic advancement, particularly for hemophilia A (HA) and B (HB) patients with inhibitors. However, rFVIIa short half-life still represents a major limitation posing the need for strategies improving its pharmacokinetics.

To this purpose several approaches have been developed, including human albumin (HSA) fusion, which relies on the acquired capacity of the fused molecule to undergo the recycling pathway mediated by the neonatal Fc receptor (FcRn). However, infused albumin-based therapeutics may suffer from the high abundance of endogenous albumin that may affect FcRn binding. Engineered variants with improved properties provide ideal tools to develop unique molecules for therapeutic purposes. In particular, tailored mutagenesis of albumin residues would translate into enhanced FcRn binding, and thus improved half-life.

The aim of this study was to develop a next-generation rFVIIa with superior half-life through fusion with a novel engineered HSA variant (HSA^{QMP}) with improved FcRn binding.

Methods. Wild-type (rFVIIa-HSA^{wt}) and engineered (rFVIIa-HSA^{QMP}) fusion proteins were expressed in HEK293 cells, purified and characterized in vitro through activity (PT-based, thrombin generation) and binding (SPR, ELISA) assays, as well as in vivo in state-of-the art mouse models.

Results. The engineered rFVIIa-HSA^{QMP} showed the same ability of commercial rFVIIa (Novoseven®) to restore coagulation in FVII-depleted plasma and, most importantly, to act as by-passing agent in HA patient

plasma with high-titer inhibitors. In vitro, binding affinity of rFVIIa-HSA^{QMP} to human (h)FcRn was significantly higher than that of rFVIIa-HSA^{WT}.

After injection in HB mice (expressing mouse FcRn), rFVIIa-HSA^{QMP} by-passing activity was detectable up to 72 hours, while activity of Novoseven® was negligible after 6 hours. Strikingly, rFVIIa-HSA^{QMP} showed a half-life of 2.9 days, compared to only 0.8 days of rFVIIa-HSA^{WT}, in transgenic mice expressing hFcRn.

Overall, these data demonstrate the therapeutic potential of the rFVIIa-HSA^{QMP} fusion protein as well as the strong half-life improvement conferred by the QMP albumin variant.

Conclusions. Fusion of the engineered QMP variant preserved rFVIIa by-passing activity both in vitro and in vivo, and strongly extended its half-life profile by 4-fold compared with the wild-type fusion. This supports the novel rFVIIa-HSA^{QMP} protein as a promising next-generation tool for hemophilia patients with inhibitors as well as the engineered albumin variant as an attractive carrier for half-life extension of other coagulation proteins.

ABS27 - Design of a novel factor IX variant with enhanced procoagulant activity and half-life

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Background. Several approaches have been developed to prolong half-life of coagulation factors, including factor IX (FIX), such as fusion with human albumin (HSA). This strategy relies on the acquired capacity of the fusion partner to undergo the recycling pathway mediated by the neonatal Fc receptor (FcRn). However, the improvement of biological properties of coagulation factors may be achieved either in terms of half-life or activity, or by a synergistic combination of these two features. In this view, rationally-engineered variants provide ideal tools to develop unique molecules to be exploited for therapy.

To this purpose, rational engineering aimed at improving FcRn binding combined with a natural gain-of-function FIX variant would result in strongly improved biological features, which would translate into a widen therapeutic window.

The aim of this study was to develop a novel fusion protein by combining the gain-of-function FIX Padua (FIX^{Padua}) with an engineered HSA variant (HSA^{QMP}) with enhanced FcRn binding, resulting in improved coagulant features and extended half-life.

Methods. The FIX^{Padua} variant was fused to the engineered HSA^{QMP} through an optimized cleavable linker. Wild-type (FIX^{wt}-HSA^{wt}) and improved (FIX^{Padua}-HSA^{QMP}) fusion proteins were expressed in HEK293 cells, purified and characterized for activity (chromogenic and aPTT-based assays), FcRn binding properties (SPR and ELISA-based assays), and half-life (state-of-the-art mouse models with different FcRn settings).

Results. Preliminary evaluation of the activity profile showed that the hyperactive features of the FIX^{Padua} were preserved after fusion with HSA in chromogenic and coagulant activity assays, as further confirmed after purification of fusion proteins. Binding assays to FcRn clearly indicated the extremely improved FcRn binding capacity of the FIX^{Padua}-HSA^{QMP} variant (K_D=0.4 nM) in comparison with that of FIX^{wt}-HSA^{wt} (K_D=200 nM).

Fusion proteins were pre-clinically characterized by *in vivo* studies in different mouse models, namely knock-out for FcRn (FcRn KO mice) or expressing human FcRn (Tg32 mice). In FcRn KO mice, the contribution of HSA to half-life was negligible, confirming the central role of FcRn binding for half-life prolongation *in vivo*. Noticeably, in Tg32 mice the half-life of the improved FIX^{Padua}-HSA^{QMP} fusion protein (2.5 days) was more than 2-fold extended than that of FIX^{wt}-HSA^{wt} (1.1 days), as well as of the commercial product Idelvion® (1.0 days) used as control.

Conclusions. The combined improvements conferred by FIX^{Padua} and HSA^{QMP} variants resulted in a novel fusion protein endowed of hyperactive features, enhanced FcRn binding and extended half-life in pre-clinical relevant mouse models. This would translate into a significantly widened therapeutic window, and thus a lower frequency of administration, which represent major goals to improve treatment, patient care and patients' quality of life.