Aims: To examine gene delivery efficiency of NP in vitro and regain FVIII function in HA mice using CRISPR/Cas9 in vivo.

Methods: NPs carrying p2X-GFP plasmid was examined for transfection efficiency in HUVEC cells by flow cytometry. Immunodeficient hemophilia A mice (NSG HA) with indel mutation in exon 1 of FVIII gene were used as an animal model. sgRNAs that target mouse FVIII gene (mF8sgRNA) or mutant FVIII gene in NSG HA mice (NSGHAsgRNA) were examined in vitro using T7E1 assay, respectively. Mice that were hydrodynamically injected with sgRNA and Ca9 protein expressing plasmids simultaneously. Blood was collected periodically to examine FVIII activity in plasma by aPTT assay.

Results: DNA electrophoresis showed that NPs can carry plasmid efficiently. Flow analysis suggested that NPs carrying p2X-GFP can efficiently transfect HUVEC cells compared to control. mF8sgRNA but not NSGHAsgRNA can specifically induce indel mutation in NIH3T3 cells. NSG HA mice challenged with hydrodynamic injection of mF8sgRNA or NSGHAsgRNA regained FVIII activity at day 7.

Conclusions: NPs can efficiently transfect endothelial cells as shown by GFP expression in HUVEC cells. Both mF8sgRNA and NSGHAsgRNA can induce indel mutation in frameshift site of NSG HA mice, leading to therapeutic levels of FVIII expression. Combining NP and gene editing technology has the potential to recover FVIII gene expression in HA patients and rescue them from daily infusion of recombinant proteins.

PB1157 | Design of a Novel Factor IX Albumin Fusion Protein with Enhanced Coagulant Activity and Pharmacokinetic Profile

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Background: Several approaches have been developed to prolong half-life of infused recombinant factor IX (FIX), such as genetic fusion with wild-type human albumin (HSA). However, to further widen the therapeutic window, rational engineering for improved binding to the neonatal Fc receptor (FcRn), which regulates HSA half-life, combined with the use of natural gain-of-function FIX variants, may result in products with more favourable features.

Aims: To develop a novel fusion protein with improved features conferred by the gain-of-function FIX-Padua variant and by an engineered HSA variant (QMP) with enhanced FcRn binding, and thus endowed with extended half-life.

Methods: FIX-Padua variant was fused to engineered albumin through an optimized cleavable linker. Wild-type (FIX-HSA) and improved (Padua-QMP) purified proteins were characterized for activity (chromogenic and aPTT-based assays), FcRn binding properties (SPR and ELISA-based assays) and *in vivo* plasma persistence in humanized transgenic mouse models.

Results: The hyperactive features of the FIX-Padua variant were completely preserved upon HSA fusion, with an 8-to-15-fold improved activity. The presence of the HSA QMP variant greatly enhanced FcRn binding of the engineered Padua-QMP fusion protein (K_d = 0.4 nM) in comparison with the wild-type (K_d = 200 nM). Noticeably, this translated into a more than 2-fold extended half-life of the Padua-QMP chimaera in human FcRn transgenic mice (2.5 days) compared to wild-type FIX-HSA (1.1 days), as well as to the commercially-available albutrepenonacog alfa (1.0 days) fusion proteins.

Conclusions: The combined improvements conferred by the FIX-Padua and QMP variants resulted in a novel engineered fusion protein with hyperactive features, enhanced FcRn binding and extended half-life in pre-clinical relevant human FcRn transgenic mouse models. This would translate into a widened therapeutic window and thus an amelioration of patients' quality of life.

PB1158 | Optimizing Outcomes in Hemophilia A Prophylaxis Using Recombinant Factor VIII Fc Fusion Protein (rFVIIIFc): Results from Three Portuguese Haemophilia Centers

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Background: In the last years, several extended half-life (EHL) products became available to prophylaxis in persons with Hemophilia A (pwHA) which increased *real-world* experience. EHL products might lead to higher trough levels without increasing infusion frequency or lead to reduce infusion frequency while maintaining through levels.

Aims: To evaluate outcomes after switching 35 pwHA from standard half-life (SHL) to EHL products from three Portuguese Haemophilia Centers, comparing bleeding rates (BR) and amount of FVIII used, in the same period of time before and after starting EHL.

Methods: Since august 2018, 35 males, mean age 23 yrs (range 2-66), all but one with severe HA, switched from different SHL products to rFVIIIFc. The mean period with ELH was 10.6 months (range 4-16) with 51% of patients with >12 months of observation. Comparisons were performed for BR, joint bleed rates (JBR) and amounts of clotting factor used, before and after switching. Half-lives (HL) of the SHL and EHL products (pharmacokinetics with WAPPS-Hemo platform) were also evaluated in twelve patients.