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fied, through a screening process, a panel of BE/PE able to rescued FVIII secretion for the p.R2166*, p.R2182H and p.R2228Q variants. Additional studies on stable clones expressing the p.R2166* and p.R2228Q mutations demonstrated that the BE and PE tools were able to revert the mutations at DNA levels and resulted in significant rescue (up to 20% of FVIII-WT) of secreted FVIII protein and activity levels. **Conclusions**: Overall, for the first time we applied BE and PE to frequent FVIII point mutations leading to severe Haemophilia A. Experimental data provided the proof-of-principle of efficacy in cellular models, which are currently under validation in HA blood-outgrowth endothelial cells (BOECs) and planned in mouse models.

OC067

RESCUE OF A HEMOPHILIA A-CAUSING FVIII SPLICING VARIANT VIA ENGINEERED U1SNRNAS

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Background and Aims: Hemophilia A (HA), the most common coagulation bleeding disorder (1:5000 males), is an X-linked hereditary disease caused by F8 gene mutations leading to deficiency of factor VIII (FVIII). Current HA therapies, albeit significantly improving HA treatment, are mainly aimed at replacing the missing factor and do not provide a definitive cure. Among all HA-causing mutations, those affecting splicing are relatively frequent, particularly in the severest forms. These mutations are generally associated with exon skipping and can be potentially rescued by RNA therapeutics. Among these, variants of the key U1snRNA spliceosomal component have been shown to efficiently rescue exon inclusion impaired by mutations located at the 3'splice site (ss), 5'ss or within the defective exon, in both cellular and animal models of human disease. This study aims to dissect the molecular mechanism underlying the moderate HA (FVIII:C<5%) phenotype identified in two brothers carrying the F8 c.1752+5G>C variant and to develop RNA therapeutics based on engineered U1snRNAs to restore proper exon 11 definition. **Methods**: Creation of expression vectors for the wild-type (pIVS11wt) and mutant (pIVS11+5G>C) F8 minigenes and for the engineered U1 snRNAs designed to base pairs to the mutated 5'ss (compensatory U1snRNA) or to less-conserved downstream intronic sequences (Exon Specific U1snRNA). Transient expression of F8 minigenes, either wild-type (pIVS11wt) or harbouring the variant c.1752+5G>C (pIVS11+5G>C), in different hepatoma cell lines (Huh7, HepG2, Hepa1-6) as well as HEK293T, followed by splicing pattern analysis. Rescue of exon 11 definition by co-transfection of mutant minigene with compensatory (n=1) or Exon Specific U1snRNA (n=3) followed by splicing pattern analysis with plasmid-specific amplicons. **Results**: Bioinformatic analysis did not predict aberrant splicing due to the F8 c.1752+5G>C mutation. However, splicing assays in different hepatoma cells demonstrated that exon 11 is well defined, as confirmed by the complete exon inclusion in the pIVS11wt context, and that the c.1752+5G>C change induces exon 11 skipping, to an extent that depends on the transfected cell lines. In the worst scenario, the mutation is associated with low levels of correctly spliced transcripts (~10%). Notably, co-transfection of different engineered U1snRNA significantly improves FVIII exon 11 definitions and thus inclusion, with the compensatory U1snRNA associated with exon 11 inclusion up to 92%. **Conclusions**: Overall, we provide experimental evidence that the F8 c.1752+5G>C change leads to exon skipping by impairing proper exon 11 definition and is associated with trace levels of correctly spliced transcripts, in accordance with the HA patients' phenotype. Moreover, the splicing outcome is cell-dependent, and further studies aimed at identifying the involved splicing factor will be conducted. Notably, F8 exon 11 inclusion can be efficiently restored by RNA therapeutics based on engineered U1snRNAs, which are currently under investigation through lentiviral-mediated delivery in Blood Outgrowth Endothelial Cells (BOEC) isolated from HA patients.

OC068

INFLUENCE OF EMICIZUMAB OF PROTEIN C-MEDIATED CLOTTING REGULATION

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Background and Aims: Emicizumab is a bispecific antibody which functions as FVIII-mimetic by simultaneously binding FIXa and FX. At variance with plasma FVIII, emicizumab is insensitive to degradation by activated protein C (APC) and may thus tilt the coagulation balance towards a prothrombotic state. We investigated the effect of emicizumab on PC-mediated inhibition of coagulation under *in vitro* conditions mimicking physiological and pathological clotting activation. **Methods**: Hemophilic plasma (<1% FVIII) was supplemented with emicizumab (50 or 100 µg/ml) or recombinant FVIII (Kovaltry, 1 IU/ml). Thrombin generation was assessed by CAT assay using as clotting trigger tissue factor (TF, 1 pM) or an intrinsic pathway activator (1/50 diluted aPTT reagent or 20 pM FXIa). The effect of the PC system was assessed by adding APC (0.2-0.8 µg/ml) or by activating plasma PC by thrombomodulin (TM, 4 nM) or endothelial cells (EA.hy926, 50,000/well). In some experiments, FXa generation was assessed by a chro-

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