aPTT and ELISA were used to detect the FVIII expression. NOD-*scid IL2rg*^{null} (NSG) mice consistently treated with FVIII LNPs were used to examine the routine therapeutic paradigm.

Results: Mice intravenously infused with the Luc LNPs showed luminescence signals 4 hours after injection and luciferase expression distributed mainly in the liver, indicating liver specificity. Furthermore, a highest-expressing FVIII variant LNP was identified. Single dose and dose response studies in mice established an optimal regimen that achieved supratherapeutic levels of FVIII activity (200-300%) at day 1 post LNP delivery and the expression slowly declined to about 5.6% after 7 days. Repeated delivery of FVIII LNPs into NSG mice every 5 days showed continued FVIII expression for two months (experimental duration).

Conclusions: Compared with FVIII protein replacement therapy, FVIII LNPs treatment produced rapid and longer duration of FVIII expression that can be applied to both on-demand and prophylactic treatment. Our study shows potential for a safe and effective platform of new mRNA therapies for hemophilia A.

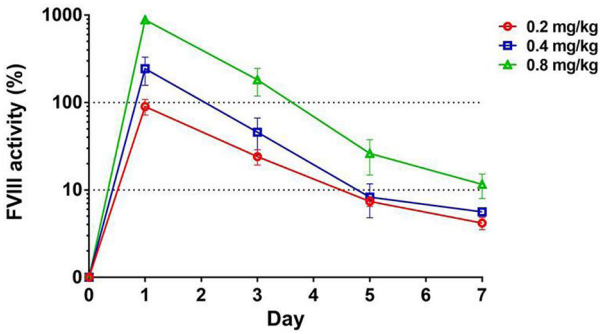

FIGURE 1 Injection of FVIII LNPs restored clotting activity in hemophilia A mice

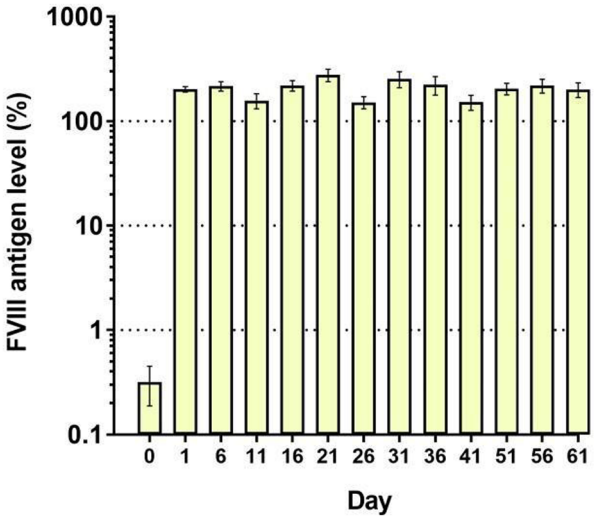

FIGURE 2 Routine injections of FVIII LNPs in NSG mice can stably express FVIII

PB0319 | Management of Surgery in Hemophilia A Patients with Inhibitors during Emicizumab Prophylaxis

C. Biron-Andreani[1,2]; I. Diaz-Cau[2]; R. Navarro[1]; C. Garcia-Gournay[1,2]; A. Theron[1]; E. Santagostino[3]; J.-F. Schved[1]

[1]University Hospital Montpellier, Hemophilia Treatment Center, Montpellier, France, [2]University Hospital Montpellier, Département of Hemostasis, Montpellier, France, [3]Foundation IRCCS Cà Granda, Maggiore Hospital Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy

Background: Emicizumab is a humanized monoclonal antibody that showed high efficacy in preventing bleeds in patients with hemophilia A and inhibitors. Surgery represents a challenge in such patients due to the risk of perioperative bleeding.

Aims: Management of major elective orthopedic surgery in two patients with severe hemophilia A and high-responding inhibitors treated by emicizumab at 1.5 mg/kg throughout the peri- and post-operative period.

Methods:: - Patient 1: 60 years, Inhibitor titer before the surgery 1 UB/mL (historical: 223 UB). Total hip replacement was performed under continuous infusion of pFVIII 100 UI/kg carried on from D0 to D5. FVIII levels were monitored peri- and post-operatively by chronometric/chromogenic. Anti-FVIII inhibitors were measured by chromogenic assay using bovine substrates.

- Patient 2: 57 years, inhibitor titer before the surgery 43 UB/mL (historical: 96 UB). For knee prosthesis, rFVIIa was given at 90µg/kg prior to skin incision, repeated as a bolus injection at 3-hour intervals for 48 hours after which the dosing interval was decreased to 2 hours because of a moderate swelling. At D7, rFVIIa was stopped.

Results: Surgery was uneventful for both patients. In patient1 the inhibitor titer raised to 2.5 at D7 and 23 UB/mL at D14. In patient 2, swelling decreased rapidly without invasive procedures. None of the patients received thromboprophylaxis.

Conclusions: In these high-responding patients receiving emicizumab prophylaxis, pFVIII or rFVIIa was used for major orthopedic surgery during 5 two 7 days, without major bleeding nor thrombosis.

PB0320 | Next Generation Factor VIIa with Enhanced Half-Life

M. Ferrarese[1,2]; J. Nilsen[3,4]; M. Pinotti[1]; M. Bern[3,4]; R.J. Davidson[5]; R.M. Camire[5,6]; H.E. Lode[3,4,7]; D.C. Roopenian[8]; I. Sandlie[4,9]; S. Lombardi[1]; G. Castaman[10]; J.T. Andersen[3,4]; A. Branchini[1]

[1]University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Italy, [2]University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy, [3]University of Oslo, Institute of Clinical Medicine and Department of Pharmacology, Oslo, Norway, [4]Oslo University Hospital Rikshospitalet and University of Oslo, Centre for Immune Regulation (CIR) and Department of Immunology, Oslo, Norway, [5]Children's Hospital of Philadelphia, Research Institute, Philadelphia, United States, [6]University of Pennsylvania, Department of

Pediatrics, Philadelphia, United States, [7]Oslo University Hospital Rikshospitalet and University of Oslo, Department of Ophthalmology, Oslo, Norway, [8]The Jackson Laboratory, Bar Harbor, United States, [9]University of Oslo, CIR and Department of Biosciences, Oslo, Norway, [10]Careggi University Hospital, Center for Bleeding Disorders, Florence, Italy

Background: Recombinant coagulation factor VIIa (rFVIIa) is used to control bleeding episodes in hemophilia patients with inhibitors. However, its therapeutic efficacy is hampered by an extremely short *in vivo* plasma half-life. Although genetic fusion of rFVIIa to wild-type human albumin prolongs its half-life, it is still very short. As the neonatal Fc receptor (FcRn) is a a key regulator of albumin homeostasis, engineered albumin with improved FcRn binding properties may extends the half-life beyond that of wild-type albumin.

Aims: To develop the next-generation rFVIIa with superior plasma half-life by taking advantage of a novel engineered human albumin variant with tailored FcRn binding.

Methods: Wild-type and the engineered (QMP) rFVIIa albumin fusions were expressed in HEK293E cells, purified and characterized *in vitro* through PT-based and thrombin generation assays, surface plasmon resonance and ELISA, followed by studies in state-of-the-art mouse models.

Results: The designed rFVIIa-QMP fusion efficiently restored coagulation in FVII-depleted plasma and, most importantly, showed a by-passing activity similar to that of commercial rFVIIa in plasma from hemophilia A patients with high-titer inhibitors. *In vitro*, rFVIIa-QMP bound human FcRn much more strongly compared to the wild-type fusion. After injection in hemophilia B mice (expressing the mouse FcRn), the by-passing activity of rFVIIa-QMP in plasma was still detectable after 48-73 hours, whereas the activity in plasma from mice given rFVIIa was undetectable at 3-6 hours. Strikingly, in human FcRn transgenic mice, rFVIIa-QMP showed a half-life of 2.9 days, compared to only 0.8 days for the wild-type fusion.

Conclusions: Fusion of engineered albumin to rFVIIa preserved the by-passing activity both *in vitro* and *in vivo*, and extended the plasma half-life by impressively 4-fold compared with the wild-type fusion. Thus, the novel engineered albumin should be an attractive carrier for half-life extension of other coagulation proteins.

PB0321 | Rescue of Multiple Haemophilia A - Causing Mutations by a Single ExSpeU1: The Importance of the Genomic Context

D. Balestra; S. Lombardi; G. Leo; I. Calanchi; F. Bernardi; M. Pinotti

University of Ferrara, Department of Life Sciences and Biotechnologies, Ferrara, Italy

Background: Therapies based on RNA splicing modulation are attracting interest for many disorders.. Variants of the spliceosomal U1snRNA have been successfully exploited to rescue defective exons in cellular and mouse models but no attempts have been done on Hemophilia A, the commonest coagulation disorder.

Aims: To explore U1snRNA variants targeting intronic sequences downstreaom of the defective exon (exon-specific U1snRNA; ExSpeU1) to correct *F8* exon 5 mutations leading to hemophilia A (HA).

Methods: Expression of ExSpeU1s and *F8* minigenes harboring the c.602-32A>G, c.602-10T>G, c.602G>A, c.655G>A, c.667G>A, c.669A>G, c.669A>T, c.670G>T, c.670+1G>T, c.670+1G>A, c.670+2T>G, c.670+5G>A and c.670+6T>C mutations in HEK293T cells and evaluation of *F8* mRNA splicing (RT-PCR).

Results: Expression studies demonstrated that all mutations, both intronic and exonic, occurring within the 5′ splice site (5′ss) induced aberrant transcripts, with the usage of two cryptic intronic 5′ss at positions c.670+64 and c.670+176. Some changes were also associated to trace level of correct transcripts (~10%) and missense changes had no effect on splicing. In co-transfection experiments, we identified an ExSpeU1 (U1sh7), designed to minimize potential off-target effects, able to properly restore splicing. We showed *in vitro* that the ExSpeU1 is able to strengthen or restore (~80%) proper 5′ss usage for all splicing mutations, including changes at +1 and +2 positions of 5′ss, commonly considered not rescuable. However, deep investigation of rescued transcripts from +1 and +2 variants revealed the usage of adjacent subtle cryptic 5′ss, leading to frameshift.

Conclusions: These data further support the therapeutic potential of the ExSpeU1 RNA, where a single therapeutic RNA can rescue multiple mutations. However, they suggest careful inspection of the genomic context and evaluation of transcripts to avoid over-interpretations.

PB0322 | Bispecific Antibodies with Light Chain Specificity for Factor IXa and X Improve Thrombin Generation in Hemophilia A Plasma

S. Knappe[1]; N. Fischer[2]; R. Hartmann[1]; P. Douillard[1]; U. Ravn[2]; G. Magistrelli[2]; F. Gueneau[2]; K. Masternak[2]; M. Kosco-Vilbois[2]; F. Scheiflinger[3]; M. Dockal[3]

[1]Baxalta Innovations GmbH, a Member of the Takeda Group of Companies, Orth a. d. Donau, Austria, [2]NovImmune SA, Geneva, Switzerland, [3]Baxalta Innovations GmbH, a Member of the Takeda Group of Companies, Vienna, Austria

Background: Bispecific antibodies (bsAbs) partially mimic the function of activated factor (F)VIII by bridging FIXa and FX. They facilitate FX activation and improve hemostatic potential under hemophilic conditions. The κλ body platform enables the development of bsAbs in which the light chains drive specific target binding. The native human IgG architecture of κλ bodies is suitable for long-term therapy.

Aims: To identify bsAbs targeting FIXa and FX that significantly increase thrombin generation in hemophilia A patient plasma.