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ABSTRACT BOOK OF

BIC INTERNATIONAL CONFERENCE

ADVANCES IN **HAEMOSTASIS** AND **BLEEDING DISORDERS**

VENICE, ITALY 17-19 SEPTEMBER 2021

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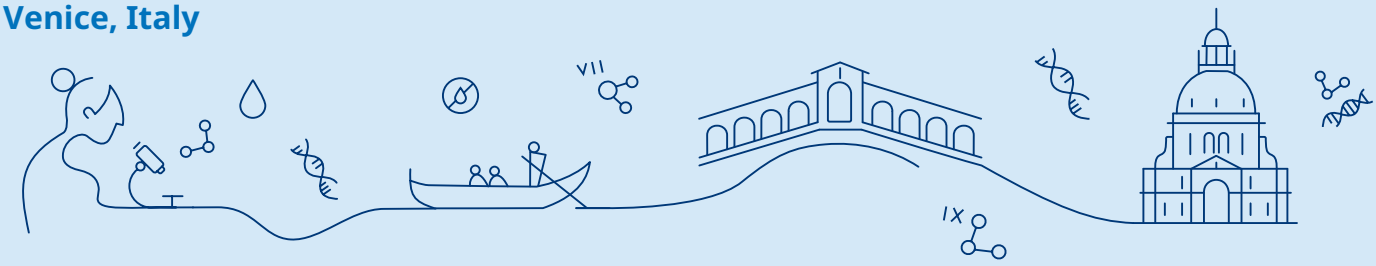
Continuing the commitment to patients with Haemophilia A: Clinical evidence of N8-GP (turoctocog alfa pegol) in PTPs and PUPs

BIC Symposium
18 September 2021
Venice, Italy



Agenda

12:00 – 12:10 **Opening & Welcome**
Prof. Pratima Chowdary

12:10 – 12:20 **Pathfinder Clinical Trial Programme: Efficacy and safety in adults PTPs**
Prof . Pratima Chowdary

12:20 – 12:30 **Pathfinder Clinical Trial Programme: Efficacy and Safety in children PTPs**
Prof. Gili Kenet

12:30 – 12:45 **Expanding experience into PUPs Efficacy and Safety results**
Dr. Christoph Königs

12:45 – 13:00 **Q&A**

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Example: Graça NAG, Ercig B, Kangro K. et al. N-glycan shielded rhADAMTS13 for treatment of immune thrombotic thrombocytopenic purpura. Haematologica. 2021; 106(S1): OC 01, 1. doi:10.3324/haematol.2021.s1

ORAL COMMUNICATIONS

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ABS 05

DEVELOPMENT OF A NEW ELISA METHOD FOR ANTI-FVIIA ANTIBODY MEASUREMENT

Valsecchi C.[1], Schiavone L.[1], Behrouz H.[2], Ahsani Z.[2], Saadatirad A.[2], Peyvandi F.[3*]

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Background: Recombinant FVIIa has been shown to be efficacious for treatment of bleeding in congenital and acquired hemophilia A with inhibitor and in congenital FVII deficiency as well as in Glanzmann's thrombasthenia. As for other recombinant therapeutic proteins, administration of rFVIIa may induce the development of anti-FVIIa antibody that could be associated with a decrease of the treatment efficacy. Very limited data are available on development of anti-FVIIa antibody. Surveillance of rFVIIa immunogenicity is then required.

Aim: To develop a method for measuring the anti-FVIIa antibody in patients treated with rFVIIa.

Method: An ELISA bridging method has been developed by using rFVIIa (NovoSeven®) for both coating and detection. The best experimental conditions were a high-density of immobilized antigen, acid dissociation and long incubation time with plasma samples.

Commercial (Affinity Biological) FVII deficient plasma and FVII Inhibitor plasma (4.5 and 67.1 BU) have been used as negative and positive controls with sheep anti-human FVII IgG used as a reference standard.

Results: Several experiments have been performed and a significant improvement in IgG recovery has been achieved with FVII depleted plasma containing 1 ug/mL rFVIIa and 1.25 or 0.6 ug/mL of anti-human FVII IgG, by the acid dissociation and the long incubation time (Figure 1).

By increasing the concentration of immobilized antigen from 1 to to 5ug/mL further improvement has been achieved in the IgG recovery.

Conclusion: Monitoring the patient immune-response to a therapeutic protein is important to assess the drug efficacy and its immunogenicity. However, the antibody detection could be complicated by the presence of the antigen in the plasma sample. Here we describe a novel method unaffected by the presence of rFVIIa that could detect the incidence of the anti-FVIIa antibodies during the treatment with recombinant FVIIa.

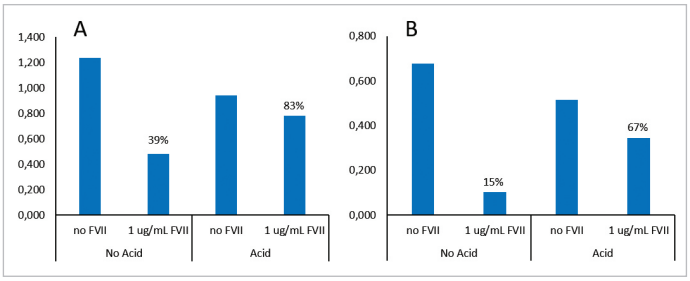

Figure 1. Improvement of IgG recovery obtained by acid dissociation with FVII depleted plasma containing 1 ug/mL of rFVIIa and 1.25 (A) or 0.6 ug/mL (B) of anti-human FVII IgG.

ABS 06

DEEP MOLECULAR MECHANISMS OF *F8* EXON 19 VARIANTS AND TRANSLATIONAL APPROACHES IN HEMOPHILIA A

Lombardi S.[1], Peretto L.[1], Merlin S.[2], Follenzi A.[2], McVey J.H.[3], Maestri I.[4], Bernardi F.[1], Pinotti M.[1], Balestra D.[1*]

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Background: A major aim of Hemophilia A (HA) studies is the definition of *F8* variants' causative role and the association with bleeding phenotypes. The pathogenic significance of missense variants is generally attributed to quantitative or qualitative changes in protein expression. Nevertheless, these changes may exert pleiotropic effects and also impair mRNA splicing due to the overlapping of the amino acid and splicing codes.

Aims: To combine and compare *in silico* and *in vitro* analyses to systematically characterize the pleiotropic effects of *F8* exon 19 variants on both protein biology and mRNA splicing.

Methods: Analysis of nucleotide variants through bioinformatic tools, combining results from multiple algorithms, and transient expression of minigenes and recombinant FVIII variants in cellular models, to evaluate their impact on splicing and protein features.

Results: Data on thirty variants provided evidence for higher prediction ability of *in vitro* assays. Whereas bioinformatics provided qualitative indications, recombinant expression provided better quantitative prediction (60% vs 90%), essential for relationships with the degree of bleeding severity. Importantly, the knowledge of the specific pathogenic molecular mechanisms led to the development of tailored correction approaches. In particular, a single engineered U1snRNA rescued mRNA splicing of nine different variants and the use of a chaperone-like drug resulted in improved factor VIII protein secretion for four missense variants.

Conclusions: We extensively characterized and provided molecular insights for a large panel of HA-causing variants by combining *in silico* and *in vitro* analysis, demonstrating the pleiotropic effects of several exonic changes. Our data suggest caution during variants classification based on nucleotide location or bioinformatic prediction and highlight the importance of experimental characterization to dissect the molecular mechanisms underlying HA, which might pave the way for the development of new individualized therapeutic strategies, also translatable to other genetic diseases.