MS#ADV-2020-002731-R1

Online supplement

Online supplement – Materials and Methods

F8 genotyping

An inverse PCR protocol was used to screen for intron 1 and intron 22 inversions as described.[1] If a patient was negative for both inversions, the *F8* gene was Sanger sequenced for all 26 exons, 5`UTR and 3`UTR, using an ABI3130xl Genetic Analyzer (ThermoFisher Scientific). Findings were confirmed using either repeated sequencing or restriction fragment length polymorphism if a restriction site could be identified.
If no gene variation was found with any of the methods above, Multiplex Ligation-dependent Probe Amplification was used to test for potential copy number variations. For this purpose, an ABI3130xl Genetic Analyzer and Coffalyzer data analysis software (Coffalyser.Net - MRC Holland) were used.
The overall sensitivity of the methodology to detect gene variations in the *F8* gene is about 97%.

1. Rossetti LC, Radic CP, Larripa IB, De Brasi CD. Developing a new generation of tests for genotyping hemophilia-causative rearrangements involving int22h and int1h hotspots in the factor VIII gene. *J Thromb Haemost.* 2008;6(5):830-6.

Blood sample schedule – additional information:

In the event of periods of multiple infusions for bleeding episodes that spanned several days (5 or more), the sampling schedule was modified to collect on the 5^{th} continuous day of treatment and 5 days after the last infusion. Following such episodes, the normal post-infusion blood draw schedule resumed. In the event that not all samples for the 1^{st}, 5^{th}, 10^{th}, 20^{th}, 30^{th}, 40^{th}, or 50^{th} post-FVIII exposure visit could be obtained, a scheduled blood draw was performed after the subsequent FVIII exposure following the same visit window of 5 days (± 2 days) after exposure to FVIII. All samples were drawn on the same schedule.

Online supplement – Table 1. Investigators and clinical centers participating in HIPS

Investigator	Center	Location
Deborah Brown *Principal Investigator*	University of Texas Health Science Center	Houston, USA
Elena Santagostino *Co-Principal Investigator*	Foundation IRCCS Ca' Granda, Maggiore Hospital Policlinico of Milan	Milano, Italy
Jan Blatny	Department of Paediatric Haematology, University Hospital Brno, Masaryk University	Brno, Czech Republic
Karin Fijnvandraat	Amsterdam Haemophilia Treatment Centre	Amsterdam, The Netherlands
Eric Mullins	Cincinnati Children's Hospital Medical Center and University of Cincinnati	Cincinnati, USA
Jenny Klintman	Clinical Coagulation Research Unit, Lund University	Malmö, Sweden
Christoph Male	Department of Paediatrics, Medical University of Vienna	Vienna, Austria
Catherine McGuinn	Weill Cornell Medicine	New York, USA
Shannon Meeks	Aflac Cancer and Blood Disorders Center, Emory University, Children's Healthcare of Atlanta	Atlanta, USA
Vlad Radulescu	University of Kentucky	Lexington, USA
Margaret Ragni	University of Pittsburgh Medical Center	Pittsburgh, USA
Michael Recht	Oregon Health & Science University	Portland, USA
Amy Shapiro	Indiana Hemophilia and Thrombosis Center	Indianapolis, USA
Janice Staber	Carver College of Medicine and Stead Family Department of Pediatrics, University of Iowa	Iowa City, USA
Hassan Yaish	University of Utah School of Medicine	Salt Lake City, USA
Donald Yee	Baylor College of Medicine	Houston, USA

HIPS: Hemophilia Inhibitor in Previously Untreated Patients Study

Online supplement – Figure 1.

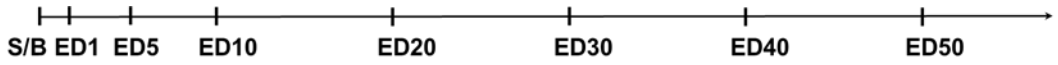

Figure 1. Sample collection schedule for the HIPS study
This figure provides the sample collection schedule which was applied throughout the HIPS study.
S/B: Screening (S) and Base line (B) samples were taken prior to the first exposure to any FVIII containing product
ED1-ED50: Exposure day (ED) to recombinant full-length FVIII (Advate^{TM})
ED1: sample taken at 7-9 days after the first exposure to FVIII
ED5-ED50: samples taken at 5 (±2) days after exposure to FVIII
In the event of periods of multiple consecutive infusions (≥5), samples were taken on the 5th day of treatment and, in addition, 5 days after the last FVIII infusion.

In the event that not all samples for the 1st, 5th, 10th, 20th, 30th, 40th, or 50th post-FVIII exposure visit could be obtained, a scheduled blood draw was performed after the subsequent FVIII exposure following the same visit window of 5 days (± 2 days) after exposure to FVIII.

Online supplement – Figure 2.

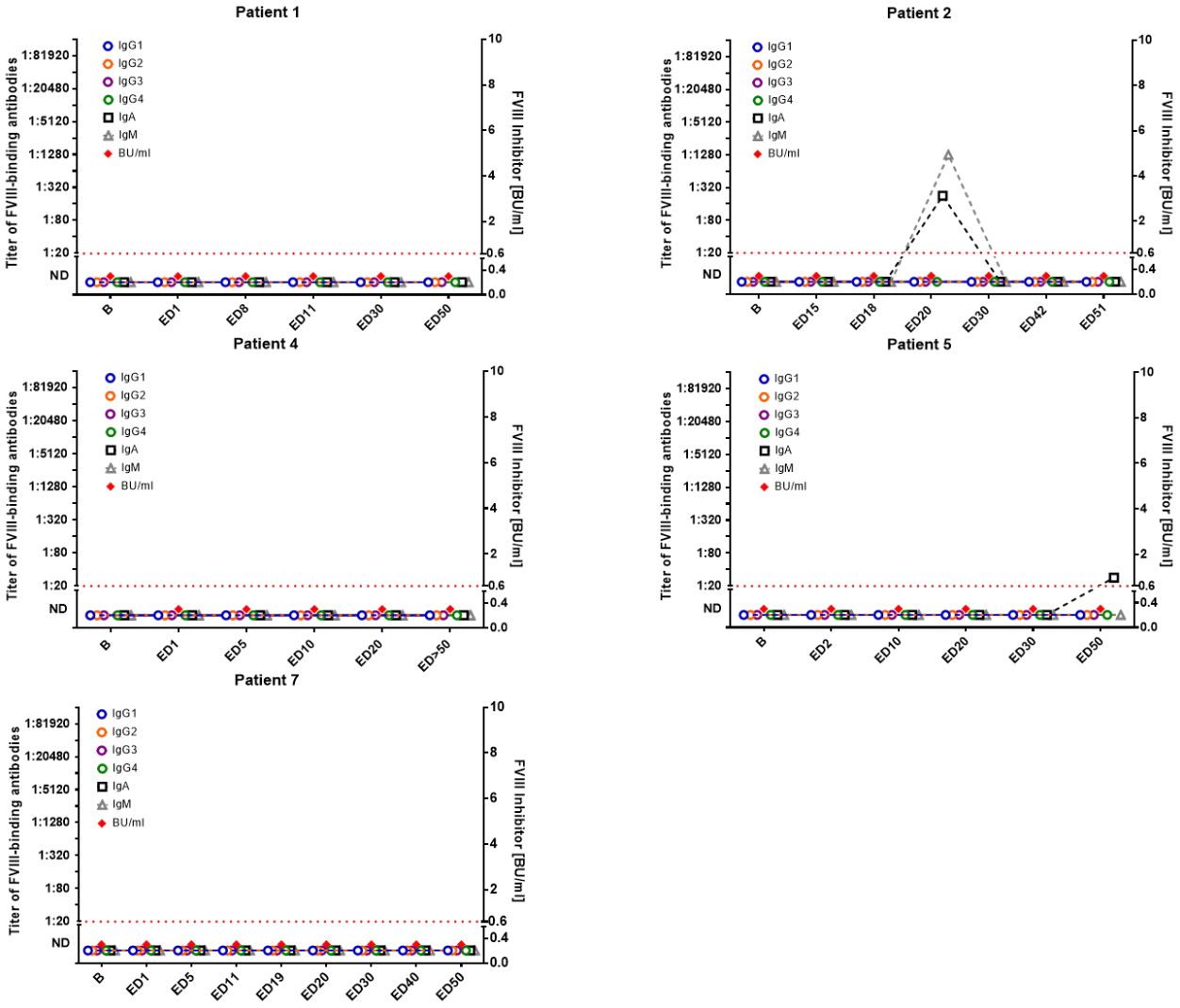

Figure 2: Longitudinal monitoring of FVIII-binding antibodies and FVIII inhibitors in 5 patients of subgroup 1

Presented are the results for the analysis of FVIII-binding antibodies (IgG1, IgG2, IgG3, IgG4, IgA, IgM, as indicated) and FVIII inhibitors in five patients of subgroup 1 who did not develop FVIII inhibitors throughout the study period. The red dotted lines represent the limit for positive evaluation of FVIII inhibitors (0.6 BU/mL).

ND: not detectable (below the detection limit of 1:20 for FVIII-binding antibodies)
B: Baseline; ED: Exposure day; BU/mL: Bethesda Units/milliliter; Ig: Immunoglobulin

The data for the remaining 2 patients of subgroup 1 are shown in Figure 1 of the main manuscript.

Online supplement – Figure 3.

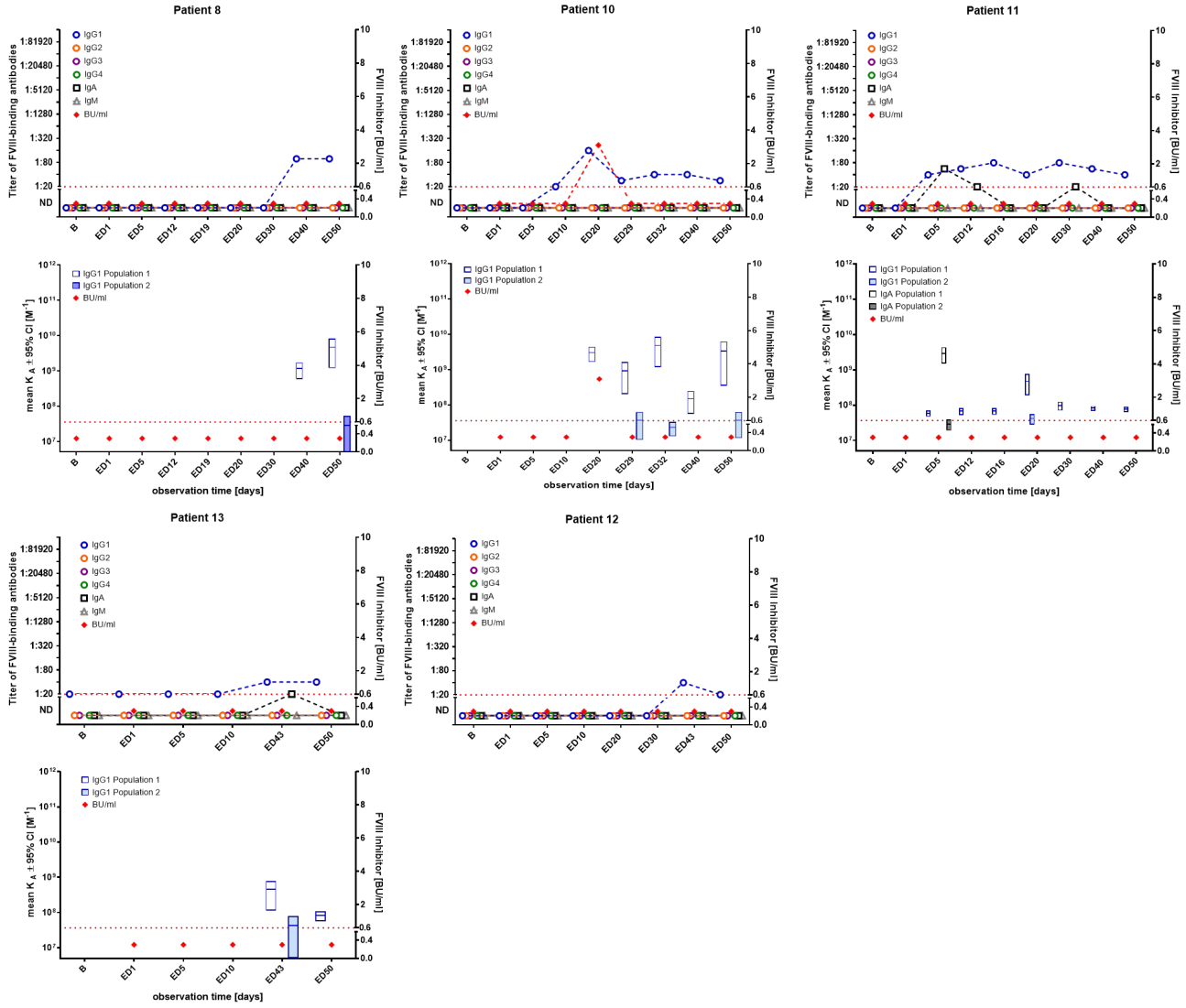

Figure 3: Longitudinal monitoring of FVIII-binding antibodies and FVIII inhibitors in 5 patients of subgroup 2

Upper panel for each patient: Presented are the results for the analysis of FVIII-binding antibodies (IgG1, IgG2, IgG3, IgG4, IgA, IgM as indicated) and FVIII inhibitors (BU/mL) for 5 patients who developed FVIII-binding antibodies (IgG1 or IgG1 and IgA) but did not develop FVIII inhibitors throughout the study period. The red dotted lines represent the limit for positive evaluation of FVIII inhibitors (0.6 BU/mL).
ND: not detectable (below the detection limit of 1:20 for FVIII-binding antibodies)
B: Baseline; ED: Exposure day; BU/mL: Bethesda Units/milliliter; Ig: Immunoglobulin

Lower panel for each patient: Presented are the results for the apparent affinity constants of FVIII-binding IgG1 and IgA antibodies (mean K_A) and for FVIII inhibitors (BU/mL). Data for apparent affinity constants include the 95% confidence intervals (CI) for up to 2 IgG1 and IgA affinity clusters (open blue/black bars, IgG1/IgA population 1; closed blue/black bars, IgG1/IgA population 2). The red dotted lines represent the limit for positive evaluation of FVIII inhibitors (0.6 BU/mL). There is no lower panel for patient 12 because the titer of FVIII-binding IgG1 in this patient was too low to be assessed for apparent affinity.

B: Baseline; ED: Exposure day; Ig: Immunoglobulin; BU/mL: Bethesda Units/milliliter; CI: Confidence interval; K_A: Apparent affinity constant

The data for the remaining 2 patients of subgroup 2 are shown in Figure 2 of the main manuscript.

Online supplement – Figure 4.

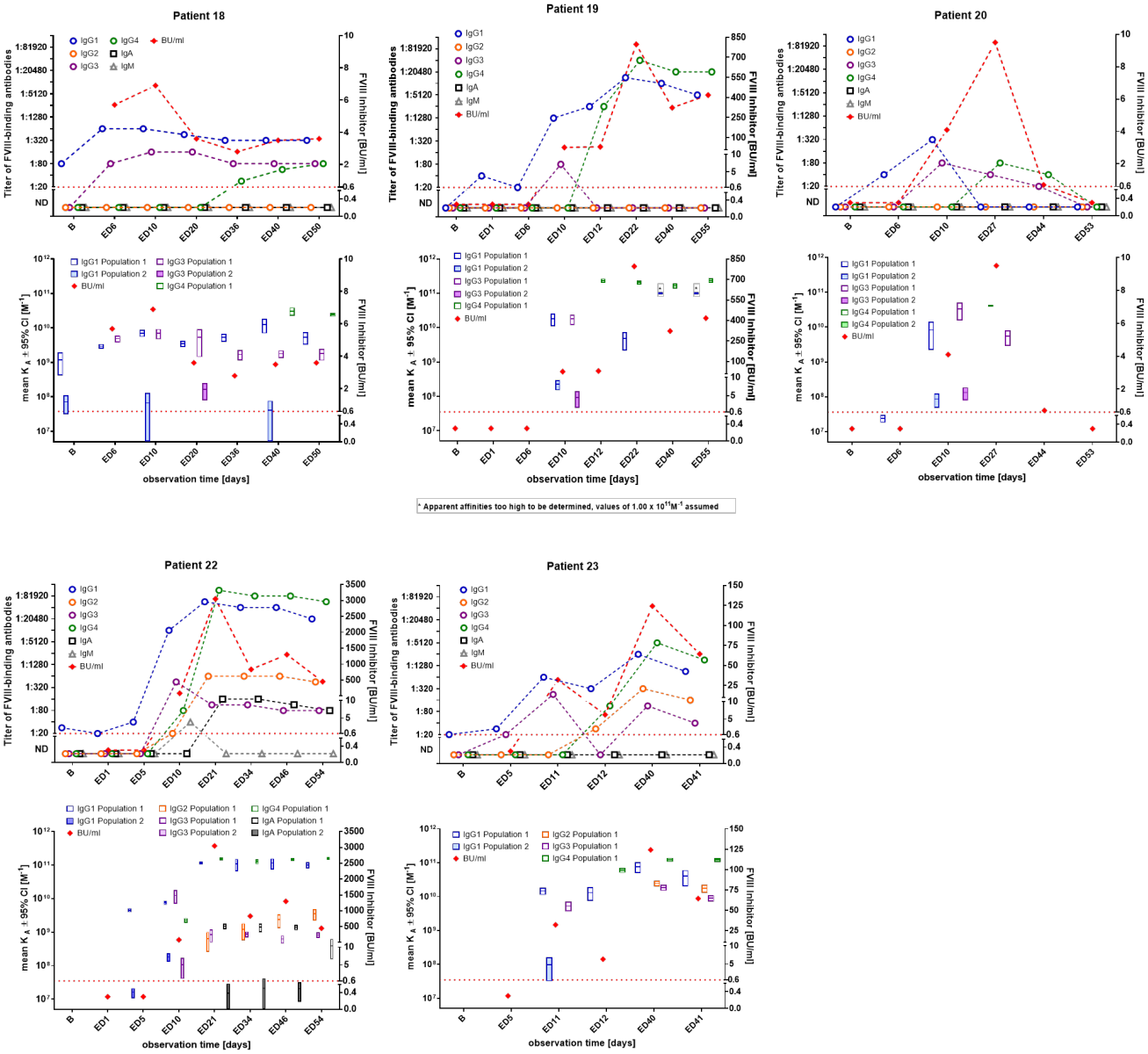

Figure 4: Longitudinal monitoring of FVIII-binding antibodies and FVIII inhibitors in 5 patients in subgroup 4

Upper panels: Presented are the results for the analysis of FVIII-binding antibodies (IgG1, IgG2, IgG3, IgG4, IgA, IgM as indicated) and FVIII inhibitors (BU/mL) for 5 patients in subgroup 4 who developed FVIII-binding IgG subclass-switched antibodies and persistent FVIII inhibitors. The red dotted lines represent the limit for positive evaluation of FVIII inhibitors (0.6 BU/mL).

ND: not detectable (below the detection limit of 1:20 for FVIII-binding antibodies)

B: Baseline; ED: Exposure day; Ig: Immunoglobulin; BU/mL: Bethesda Units/milliliter

Lower panels: Presented are the results for the apparent affinity constants of FVIII-binding antibodies (mean K_A), differentiated for individual IgG subclasses and Ig isotypes, and for FVIII inhibitors (BU/mL). Data for apparent affinity constants include the 95% confidence intervals (CI) for up to 2 affinity clusters for each IgG subclass or IgA (open bars: population 1; closed bars: population 2). The red dotted lines represent the limit for positive evaluation of FVIII inhibitors (0.6 BU/mL).

B: Baseline; ED: Exposure day; Ig: Immunoglobulin; BU/mL: Bethesda Units/milliliter; CI: Confidence interval; K_A: Apparent affinity constant

The data for the remaining 2 patients of subgroup 4 are shown in figure 4 of the main manuscript.