

RESEARCH LETTER

Evaluation of the interference of fitusiran and antithrombin lowering in plasma on routine coagulation assays

Hemophilia A and B result from deficiencies in coagulation factor (F) VIII and FIX, respectively, resulting in insufficient thrombin generation [1,2], leading to reduced clot stability and bleeding events [3].

Depending on baseline coagulation factor levels, hemophilia can be classified as severe (<1% factor), moderate (1%-5% factor), or mild (>5%-40% factor) disease [1]. For people with hemophilia A or B with a severe bleeding phenotype, the World Federation of Hemophilia recommends prophylaxis to prevent bleeds at all times [1]. Common coagulation assays used in routine clinical practice for the diagnosis and monitoring of people with hemophilia include prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (aPTT), one-stage clotting assays, and 2-stage chromogenic assays [4]. In people with hemophilia without inhibitors, hemostatic efficacy assessment of clotting factor concentrates relies on clinical response and specific factor assays that measure factor activity levels [4]. In people with hemophilia with inhibitors, there are currently no standardized laboratory assays to measure the efficacy of bypassing agents [4].

Nonfactor therapies differ in their mechanism of action from clotting factor concentrates/bypassing agents [1]. Rather than replacing the missing factor, nonfactor therapies mimic activated FVIII or inhibit natural anticoagulants (activated protein C, antithrombin [AT], and tissue factor protein inhibitor [TFPI]) [5-7]. Measurement of drug plasma concentration (TFPI-based) or anticoagulant activity levels (AT-based) can be used for dose optimization of these therapies. However, the suitability of coagulation assays for the monitoring of people with hemophilia receiving nonfactor therapies remains unclear. Therefore, there is a need to evaluate their impact on routine laboratory assays.

Emicizumab is an activated FVIII mimetic [8,9], which, unlike FVIII, does not require activation to function; thus, in coagulation assays, the behavior of emicizumab differs from that of FVIII [10]. Also, emicizumab interferes with aPTT-based and one-stage FVIII activity assays [10,11]. Anti-TFPI therapies, including concizumab or marstacimab, do not interfere with aPTT, PT, one-stage, or chromogenic assays [12]. As investigational antiactivated protein C therapies, such as SR604, are still in very early stages of development, it is not yet clear if they would interfere with coagulation assays [13]. Fitusiran is a subcutaneous,

investigational AT-lowering therapeutic that increases thrombin generation to restore hemostasis in people with hemophilia A/B, regardless of inhibitor status [14]. This study was designed to evaluate the extent of interference of AT lowering or fitusiran in routine laboratory coagulation assays using commercially available plasma samples from people with hemophilia.

Coagulation assays were performed at 2 AT activity levels: 15% and 100% (inhibitor-negative control plasma). To obtain plasma with low-level AT activity (15%), control plasma was diluted using AT-immunodepleted plasma. This level corresponds to the lower threshold of the 15% to 35% AT range targeted by the AT-based dose regimen in the fitusiran phase 3 program [15-18]. Plasma AT concentrations were verified using the Biophen AT Assay Kit (on the Siemens Healthcare model BCS XP System).

Varying concentrations of recombinant full-length FVIII or FIX (at 0%, 5%, 20%, 50%, and 100%) were obtained by mixing ADVATE or BeneFIX, respectively, with factor-deficient plasma. These solutions were spiked into the control plasma (AT: 100%) and the immunodepleted test plasma (AT: 15%). Spiked-in plasma samples were tested with 19 validated assays, as performed in a College of American Pathologists or Clinical Laboratory Improvement Amendments-qualified laboratory (Supplementary Methods).

The impact of AT lowering on PT assays ($n = 2$), FVIII chromogenic assays ($n = 2$), FVIII or FIX aPTT assays ($n = 4$), TT assay ($n = 1$), FVIII or FIX one-stage activity assays ($n = 4$), FIX chromogenic assays ($n = 2$), and an AT activity (control) assay ($n = 1$) was assessed (Table 1). Additionally, FVIII or FIX Nijmegen-modified inhibitor assays ($n = 3$) were evaluated to confirm the absence of false positives in the inhibitor-negative plasma. All assays, where applicable, were performed at 2 AT levels: low (15%) and "normal" AT levels (100%). The impact of AT lowering was defined as "none" if variability in assays was within $\pm 20\%$, per standard practice for the respective clinical assays, between the 100% and 15% AT plasma levels. Each sample was tested in triplicate for all assays.

No impact of AT lowering ($\pm 20\%$ variability) was found for the FVIII or FIX PT, TT, aPTT, chromogenic, or one-stage assays (Table 1). While a reading of $> 20\%$ variability was recorded for the FVIII one-stage PTTA (silica) on STA-R Evolution analyzer (Diagnostica Stago)

TABLE 1 Assays performed at 15% and 100% antithrombin levels, and impact of antithrombin lowering.

Assays performed											
PT assay, s											
Dade Innovin on BCS XP System (Siemens Healthcare)	Target FVIII level/target AT level (%)	0/15	0/100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	11.8 (0.1)	11.6 (0.1)	11.9 (0.2)	11.6 (0.1)	11.8 (0.0)	11.6 (0.0)	11.8 (0.1)	11.7 (0.1)	11.8 (0.1)	11.7 (0.1)
	% change	2.0		2.6		1.7		0.9		0.3	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	12.0 (0.1)	11.9 (0.2)	11.8 (0.1)	11.6 (0.2)	11.5 (0.1)	11.5 (0.1)	11.1 (0.2)	11.2 (0.1)	10.7 (0.3)	10.9 (0.2)
	% change	0.8		2.0		0.3		-0.6		-2.4	
HemosIL RecombiPlasTin 2G on ACL Top 700 (Instrumentation Laboratory)	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	13.1 (0.3)	12.6 (0.2)	13.2 (0.3)	12.7 (0.1)	13.1 (0.1)	12.7 (0.1)	13.3 (0.1)	12.7 (0.1)	13.1 (0.2)	12.5 (0.3)
	% change	4.0		4.2		3.4		4.5		4.8	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	13.7 (0.1)	13.5 (0.6)	13.5 (0.3)	13.2 (0.2)	13.1 (0.4)	12.9 (0.1)	12.8 (0.4)	12.5 (0.22)	12.4 (0.4)	12.4 (0.11)
	% change	1.0		2.3		1.3		2.4		0.0	
TT assay, s											
BC Thrombin Reagent on BCS XP System	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	19.1 (1.1)	18.6 (0.1)	18.4 (0.3)	18.7 (0.2)	18.4 (0.2)	18.6 (0.2)	18.5 (0.2)	19.0 (0.2)	18.4 (0.5)	19.0 (0.5)
	% change	2.5		-1.4		-1.2		-2.9		-3.5	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	16.8 (0.2)	17.1 (0.1)	16.8 (0.6)	17.0 (0.5)	16.6 (0.3)	16.9 (0.3)	16.7 (0.5)	16.8 (0.1)	16.7 (0.3)	17.0 (0.2)
	% change	-1.8		-1.4		-1.8		-0.8		-2.1	
aPTT assays, s											
PTTA (silica) on STA-R Evolution (Diagnostica Stago)	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	108.4 (1.7)	101.3 (0.4)	55.0 (5.3)	51.6 (0.4)	38.7 (0.1)	39.9 (0.3)	33.8 (0.1)	35.0 (0.2)	30.0 (0.1)	31.3 (1.0)
	% change	7.0		6.5		-3.1		-3.3		-4.1	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100

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TABLE 1 (Continued)

Assays performed											
	Mean (SD)	95.4 (0.6)	99.1 (1.5)	42.4 (0.9)	42.1 (0.6)	33.9 (0.6)	34.3 (0.1)	30.1 (0.4)	30.3 (0.6)	27.9 (0.2)	28.7 (0.1)
	% change	-3.7		0.8		-1.1		-0.7		-2.6	
Dade Actin FSL (ellagic acid) on BCS XP System	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	67.3 (2.2)	69.5 (1.7)	42.1 (2.7)	44.2 (0.8)	35.1 (2.2)	36.1 (0.4)	29.8 (0.7)	31.8 (0.1)	27.0 (0.2)	28.4 (0.4)
	% change	-3.1		-4.7		-2.8		-6.1		-5.1	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	66.3 (1.7)	71.8 (2.8)	35.1 (0.6)	35.3 (0.5)	20.4 (0.7)	30.2 (0.4)	27.9 (0.6)	27.8 (0.4)	26.2 (0.3)	26.2 (0.5)
	% change	-7.7		-0.5		0.6		0.3		-0.2	
HemosIL SynthASil (silica) on ACL	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
TOP 700 (Instrumentation Laboratory)	Mean (SD)	123.1 (2.0)	130.9 (1.8)	51.7 (1.1)	55.3 (0.2)	41.3 (0.5)	43.2 (0.2)	35.2 (0.4)	37.3 (0.4)	31.5 (0.2)	32.9 (0.4)
	% change	-6.0		-6.5		-4.3		-5.6		-4.3	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	88.9 (10.6)	98.2 (16.7)	43.6 (0.4)	44.6 (0.2)	36.2 (0.4)	36.9 (0.3)	32.4 (0.3)	33.2 (0.2)	30.4 (0.1)	30.9 (0.6)
	% change	-9.4		-2.3		-1.8		-2.4		-1.5	
CK PREST (kaolin) on STA-R Evolution (Diagnostica Stago)	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	77.6 (0.2)	84.1 (1.8)	44.1 (0.3)	46.9 (0.5)	36.5 (0.2)	37.5 (0.3)	31.2 (0.1)	32.5 (0.3)	27.8 (0.1)	28.6 (0.3)
	% change	-7.7		-5.8		-2.6		-3.9		-2.6	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	82.7 (1.8)	88.5 (4.2)	39.7 (0.3)	40.3 (0.2)	33.0 (0.1)	33.6 (0.3)	29.6 (0.2)	29.8 (0.5)	27.2 (0.3)	27.8 (0.1)
	% change	-6.6		-1.5		-1.7		-0.6		-1.9	
FVIII/FIX one-stage activity assays, %											
Dade Actin FSL (ellagic acid) on BCS XP System	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	6.3 (0.6)	6.0 (1.0)	23.3 (2.5)	24.3 (0.6)	58.3 (2.1)	54.0 (1.7)	119.0 (0.0)	109.7 (6.1)
	% change	NI		5.0		-4.1		8.0		8.5	

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TABLE 1 (Continued)

Assays performed											
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	5.0 (1.0)	5.0 (1.0)	24.0 (0.0)	24.0 (0.0)	59.3 (2.5)	58.0 (0.0)	112.3 (2.9)	108.7 (3.2)
	% change	NI		0.0		0.0		2.2		3.3	
HemosIL SynthASil (silica) on ACL TOP 700	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	6.1 (0.3)	5.2 (0.3)	20.5 (1.0)	18.3 (0.6)	47.2 (1.8)	40.8 (1.3)	91.3 (3.4)	84.4 (3.2)
	% change	NI		17.4		12.0		15.6		8.2	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	8.3 (0.4)	8.2 (0.1)	27.5 (0.7)	25.5 (0.7)	64.7 (1.3)	65.0 (1.0)	122.8 (1.7)	109.9 (1.1)
	% change	NI		1.2		7.9		-0.5		11.8	
PTTA (silica) on STA-R Evolution	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	6.8 (0.5)	5.6 (0.1)	23.1 (0.8)	22.1 (0.8)	50.9 (1.5)	48.2 (1.7)	103.7 (1.9)	91.6 (3.3)
	% change	NI		22.6		4.2		5.7		13.2	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	10.2 (0.3)	10.8 (0.4)	36.4 (0.4)	35.2 (0.7)	70.0 (1.2)	65.7 (2.5)	129.6 (4.0)	108.6 (3.6)
	% change	NI		-5.8		3.5		6.5		19.3	
CK PREST (kaolin) on STA-R Evolution	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	6.1 (0.8)	5.2 (0.3)	20.9 (0.8)	21.3 (0.8)	49.2 (1.3)	42.9 (0.9)	95.2 (2.5)	87.2 (3.0)
	% change	NI		16.6		-1.7		12.2		9.1	
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	6.5 (0.1)	6.8 (0.3)	24.2 (0.8)	23.7 (0.5)	57.5 (0.4)	54.6 (2.2)	113.5 (3.2)	98.0 (3.4)
	% change	NI		1.5		3.1		0.7		2.8	
FVIII chromogenic assays											
FVIII Chromogenic Assay on BCS XP System, % (Siemens Healthineers)	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	23.6 (3.0)	22.4 (1.7)	57.9 (0.3)	52.5 (3.4)	110.6 (4.8)	99.2 (4.3)
	% change	NI		NI		5.6		10.3		11.5	

(Continues)

TABLE 1 (Continued)

Assays performed											
Biophen FVIII Chromogenic on the BCS XP System, IU/mL (Siemens Healthineers)	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	0.05 (0.001)	0.05 (0.001)	0.2 (0.003)	0.2 (0.003)	0.5 (0.001)	0.4 (0.02)	1.0 (0.02)	0.9 (0.008)
	% change	NI		14.1		5.9		5.8		7.5	
FIX chromogenic assays											
ROX FIX Chromogenic Assay (Rossix) on the BCS XP System, IU/mL	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	0.04 (0.002)	0.04 (0.004)	0.2 (0.007)	0.2 (0.01)	0.4 (0.02)	0.4 (0.06)	0.8 (0.05)	0.7 (0.05)
	% change	NI		11.6		2.7		5.5		14.9	
Biophen FIX Chromogenic Assay (Hyphen BioMed) on the BCS XP System, %	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	NI (NI)	NI (NI)	3.6 (0.1)	3.2 (0.1)	13.5 (0.2)	11.5 (0.2)	34.9 (0.9)	29.6 (0.3)	66.7 (1.3)	56.8 (1.5)
	% change	NI		12.6		17.7		17.8		17.4	
Nijmegen inhibitor assays, BU/mL											
FVIII Dade Actin FSL (ellagic acid) on the BCS XP System	Target FVIII level/target AT level (%)	0.0/15	0.0/100	0.1/15	0.1/100	0.2/15	0.2/100	0.5/15	0.5/100	1.0/15	1.0/100
	Mean (SD)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)
FIX Dade Actin FSL (ellagic acid) on the BCS XP System	Target FIX level/target AT level (%)	0.0/15	0.0/100	0.1/15	0.1/100	0.2/15	0.2/100	0.5/15	0.5/100	1.0/15	1.0/100
	Mean (SD)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)
Siemens FVIII Chromogenic on the BCS XP System	Target FVIII level/target AT level (%)	0.0/15	0.0/100	0.1/15	0.1/100	0.2/15	0.2/100	0.5/15	0.5/100	1.0/15	1.0/100
	Mean (SD)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)	NI (NI)
AT activity (control) assay, %											
Biophen AT on BCS XP System for FVIII and FIX	Target FVIII level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	12.8 (1.2)	96.1 (3.9)	13.5 (1.6)	92.7 (1.1)	12.0 (0.8)	91.7 (3.7)	13.5 (0.6)	93.9 (1.3)	14.0 (0.2)	90.6 (2.9)
	Target FIX level/target AT level (%)	0/15	0 /100	5/15	5/100	20/15	20/100	50/15	50/100	100/15	100/100
	Mean (SD)	12.3 (1.6)	89.8 (1.4)	13.2 (1.0)	87.5 (0.7)	12.0 (1.8)	92.6 (2.3)	11.9 (2.3)	89.3 (0.3)	12.2 (0.5)	91.7 (1.5)

The target FVIII or FIX levels (at 0%, 5%, 20%, 50%, and 100%) were achieved by mixing ADVATE or BeneFIX, respectively, with factor-deficient plasma. The impact of AT lowering was defined as “none” if there was $\pm 20\%$ variability between the 100% and 15% AT plasma levels. For the control assay, AT was measured at $<15\%$ variability. All assays were performed in triplicate.

aPTT, activated partial thromboplastin time; AT, antithrombin; BU, Bethesda Units; FIX/FVIII, factor IX/VIII; NI, no interference; PT, prothrombin time; SD, standard deviation; TT, thrombin time.

TABLE 2 Assays performed at 15% and 100% AT levels and impact of fitusiran.

Assays performed	Impact of fitusiran	FVIII				FIX			
PT assay using Dade Innovin on the BCS XP System, s	Target AT (%) / fitusiran (ng/mL) concentration	15/0	15/1000	100/0	100/1000	15/0	15/1000	100/0	100/1000
	Mean (SD)	12.4 (0.1)	12.4 (0.1)	12.4 (0.1)	12.4 (0.1)	11.7 (0.0)	11.7 (0.1)	10.8 (0.0)	10.8 (0.1)
	% change	0.0		-0.2		0.3		0.3	
aPTT assay using Dade Actin FSL on the BCS XP System, s	Target AT (%) / fitusiran (ng/mL) concentration	15/0	15/1000	100/0	100/1000	15/0	15/1000	100/0	100/1000
	Mean (SD)	61.1 (0.9)	60.6 (0.4)	56.0 (0.3)	56.1 (0.3)	62.9 (1.0)	63.9 (0.2)	62.3 (0.2)	63.2 (1.3)
	% change	-0.9		0.2		1.6		1.4	
One-stage clot assay using Dade Actin FSL on the BCS XP System, %	Target AT (%) / fitusiran (ng/mL) concentration	15/0	15/1000	100/0	100/1000	15/0	15/1000	100/0	100/1000
	Mean (SD)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)	NI	NI	NI	NI
	% change	-10.0		0.0		NI		NI	
Chromogenic assays									
FVIII chromogenic assay using Coatest SP4 (Chromogenix) on the BCS XP System, IU/mL	Target AT (%) / fitusiran (ng/mL) concentration	15/0	15/1000	100/0	100/1000	-	-	-	-
	Mean (SD)	NI	NI	NI	NI	-	-	-	-
	% change	NI		NI		-	-	-	-
FIX chromogenic assay using Hyphen BioMed BIOPHEN FIX Kit on the BCS XP System, %	Target AT (%) concentration	-	-	-	-	15/0	15/1000	100/15	100/1000
	Mean (SD)	-	-	-	-	NI	NI	NI	NI
	% change	-	-	-	-	NI		NI	
Nijmegen inhibitor assays									
FVIII one-stage clot Nijmegen Bethesda assay using Dade Actin FSL on the BCS XP System, BU/mL	Target AT (%) / fitusiran (ng/mL) concentration	15/0	15/1000	100/0	100/1000	15/0	15/1000	100/0	100/1000
	Mean (SD)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.4 (0.1)	0.3 (0.0)	0.3 (0.1)	0.3 (0.1)
	% change	-50.0 ^a		0.0		-18.9		-10.0	
FIX one-stage clot Nijmegen Bethesda assay using Dade Actin FSL on the BCS XP System, BU/mL	Target AT (%) / fitusiran (ng/mL) concentration	15/0	15/1000	100/0	100/1000	15/0	15/1000	100/0	100/1000
	Mean (SD)	-	-	-	-	0.4 (0.1)	0.3 (0.0)	0.3 (0.1)	0.3 (0.1)
	% change	-		-		-18.9		-10.0	
FVIII chromogenic Nijmegen Bethesda assay using Siemens FVIII Chromogenic Assay Kit on the BCS XP System, BU/mL	Target AT (%) concentration	15/0	15/1000	100/0	100/1000	-	-	-	-
	Mean (SD)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	-	-	-	-
	% change	0.0		0.0		-	-	-	-

The impact of fitusiran was defined as "none" if there was $\pm 15\%$ variability between the samples with and without fitusiran. For the Nijmegen inhibitor assays, the acceptable variability was $\pm 20\%$. All assays were performed in triplicate. The "-" symbol indicates unavailable data, as the assay is specific to either FVIII or FIX.

aPTT, activated partial thromboplastin time; AT, antithrombin; BU, Bethesda Units; FIX/FVIII, factor IX/VIII; NI, no interference; PT, prothrombin time; SD, standard deviation.

^aAs all results were < 0.6 BU/mL, it was deemed that there was no impact by fitusiran.

at 5% factor level, there was no overall trend suggesting an impact of AT lowering on any of the assays evaluated. The FVIII and FIX Nijmegen-modified inhibitor assays confirmed there were no false positives in the inhibitor-negative plasma. AT level was measured with <15% variability in the AT activity (control) assay (Table 1).

This analysis suggests that plasma containing different levels of recombinant FVIII or FIX displayed similar clotting potential at low (15%) and “normal” (100%) AT levels. While the readings at 15% AT in several assays were slightly higher than those at 100% AT, the difference in variability across the tested assays was within the acceptable margins of $\pm 20\%$, with no specific trend toward change.

The impact of fitusiran was also tested on several coagulation assays using FVIII- and FIX-deficient plasma samples containing either no fitusiran (0 ng/mL) or ~ 1000 ng/mL fitusiran at low and “normal” AT levels (Table 2). The impact of fitusiran was defined as “none” if there was $\pm 15\%$ variability between the samples with and without fitusiran. Compared with the previous coagulation assays, acceptable variability was more stringent to ensure accuracy in the presence or absence of negatively charged fitusiran. In line with the AT-lowering assessments, FVIII and FIX Nijmegen-modified inhibitor assays confirmed there were no false positives in the inhibitor-negative plasma (titers of <0.6 BU/mL) at low and “normal” AT levels, with or without fitusiran. Overall, up to 10 times therapeutic concentration of fitusiran (~ 1000 ng/mL) did not interfere with any of the tested assays at low or “normal” AT levels (Table 2).

Given its nature as a confirmatory study, this analysis may be limited by potential confirmation bias and difficulty generalizing results beyond the specific data sets and methods used. To enhance the robustness of the data within the experimental setup, 19 assays, each tested in triplicate, allowed for the generation of extensive data for this type of study. Additional limitations include the use of immunodepleted plasma instead of plasma from patients receiving fitusiran prophylaxis and the variability of the assays themselves. Notwithstanding, the results of this study suggest that AT lowering or fitusiran itself may not cause any major interference in routine coagulation assays, in line with a previous report [19]. As the impact of AT lowering was evaluated at the lower threshold of the 15% to 35% AT range targeted by the AT-based dose regimen, fitusiran prophylaxis may not interfere with routine coagulation assays at this range. However, further analysis is required to confirm these findings in samples of patients receiving fitusiran prophylaxis. In addition to the previously reported bleed protection, improvements in quality of life, and reduced treatment and disease burden with fitusiran prophylaxis, fitusiran could be administered subcutaneously [15–18], with potentially no impact on the routine clinical management and monitoring of people with hemophilia. Unlike other nonfactor therapies where dose optimization is based on measurement of drug plasma concentrations [20,21], fitusiran dosing follows a pharmacodynamic biomarker (ie, AT)-guided approach [15].

ACKNOWLEDGMENTS

Medical writing support was provided by Emily Evans, BMedSc, Zofia Zgrundo, MSc, and Kaleighshandra Isaac, MBio, of Ashfield MedComms, an Inizio company, and funded by Sanofi.

FUNDING

This study was funded by Sanofi.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception and design of the work related to this document, and/or data acquisition for the work related to this document, and/or data analysis or interpretation of data for the work related to this document. All authors have drafted the work or revised it critically for important intellectual content.

RELATIONSHIP DISCLOSURE

E.S.C. and M.L. are employees of Sanofi and may be shareholders.

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SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at <https://doi.org/10.1016/j.rpth.2025.102989>