Activity of a FIX-Padua Transgene Product in Commonly Used FIX:C One-Stage Clot and Chromogenic Assay Systems Following PF-06838435 (SPK-9001) Gene Delivery

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Background

PF-06838435 (SPK-9001), a gene therapy candidate containing a high specific-activity factor IX variant (R338L, FIX-Padua), is currently in phase 3 of clinical development for the treatment of Hemophilia B. Initial data with this vector is promising with significant reductions in bleeding episodes and FIX consumption[1]. To date, little is known about the activity of the expressed transgene product as measured in FIX:C one-stage and chromogenic assay systems commonly used to monitor FIX replacement therapy in patients with Hemophilia B.

Objective

To assess the FIX:C of the PF-06838435 expressed transgene product in plasma samples collected from participants in the Phase 1/2 trial using four commonly used FIX:C aPTT reagents and one FIX:C chromogenic assay.

Methods

- FIX:C in four samples collected from two patients who received PF-06838435 gene therapy were tested using four in vitro diagnostic (IVD) FIX:C one-stage clot assay systems and one FIX chromogenic assay system (Table 1).
- The FIX aPTT one-stage clot assay systems selected represent the most common aPTT reagents used in CAP accredited laboratories in the US in 2017 and represent the three main types of activator commonly used in FIX one-stage clot assays: silica (STA®-PTT Automate, HemosIL® SynthASil®), ellagic acid (Dade Actin® FSL) and kaolin (STA®-C.K. Prest®)[2] .
- For comparison, FIX:C was also determined in pooled congenital FIX deficient plasma spiked with purified rHFIX-Padua protein (provided by Dr. Samelson-Jones) and rHFIX-BeneFIX® product (provided by Sparks Therapeutics Inc.) in each of the five FIX:C assay systems.
- The rHFIX-Padua and the rHFIX-BeneFIX® spiked plasma samples were prepared as follows:
  - rHFIX-Padua protein at 20X protein concentrations of 5000, 3760 and 2500 ng/mL and rHFIX-BeneFIX® product at 20X FIX:C concentrations of 800, 600 and 400% in 20mM HEPES, 150 mM NaCl, 2 mM $CaCl_2$, 0.1% PEG-800 were diluted 1:20 into pooled congenital FIX deficient plasma on the day of testing to provide samples with approximate FIX:C concentrations of 40, 30 and 20%.
- The assay systems were calibrated and quality control, patient, rFIX-Padua and rHFIX-BeneFIX® samples were tested in accordance with manufacturer recommendations and/or existing laboratory SOPs . Patient samples were tested in singlicate. The rHFIX-Padua and rHFIX-BeneFIX® spiked plasma samples were tested in triplicate.

References

1. George LA et al, NEJM 2017; 377:2215-2217
2. 2017 College of American Pathologists (CAP) Proficiency Survey

Table 1: Commonly used FIX:C One-Stage Clot and Chromogenic Assay Systems

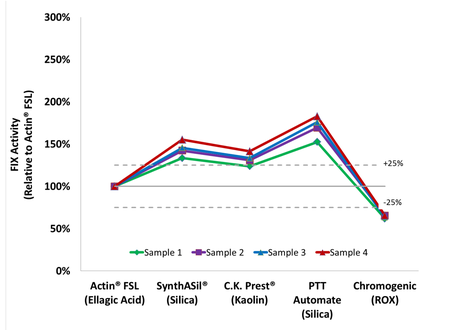
	FIX:C aPTT One-Stage Clot Assays				FIX:C Chromogenic Assay
Manufacturer	Siemens Healthcare Diagnostics	Instrumentation Laboratory	Diagnostica Stago, Inc.	Diagnostica Stago, Inc.	ROSSIX
Instrument	BCS®XP	ACL TOP®	STA-R Evolution®	STA-R Evolution®	BCS®XP
Reagent	Actin FSL	HemosIL® SynthASil	STA®- CK Prest®	STA®- PTT Automate	ROX Factor IX Kit
Activator	Ellagic Acid	Silica	Kaolin	Silica	NA
% CAP Accredited Laboratories (2017)	23%	26%	5%	34%	Not Applicable

Results

Table 2: FIX:C Measured in Plasma from Patients Receiving PF-06838435 Gene Therapy

	PF-0683843 Patient Plasma Sample			
Reagent	**1**	**2**	**3**	**4**
Actin® FSL	21 (100%)	26 (100%)	33 (100%)	29 (100%)
SynthASil®	28 (133%)	37 (142%)	48 (145%)	45 (155%)
C.K. Prest®	26 (124%)	34 (131%)	44 (133%)	41 (141%)
PTT Automate	32 (152%)	44 (169%)	58 (176%)	53 (183%)
Chromogenic	13 (62%)	17 (65%)	21 (64%)	19 (66%)

(FIX activity relative to FIX activity measured using Actin® FSL)

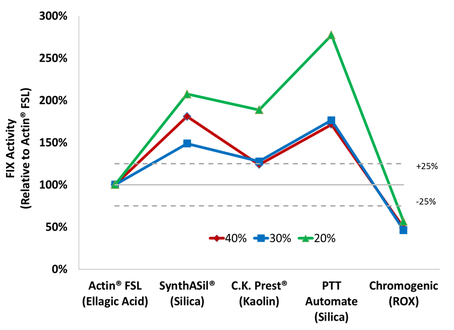


- When the measured FIX:C for the remaining aPTT FIX:C assays was normalized over values measured in the Actin® FSL FIX:C assay, a consistent pattern in recovery for patient samples containing PF-06838435 transgene product was observed.
- Compared to Actin FSL, FIX:C in the three remaining aPTT reagents was higher (25% or more), with PTT Automate providing the highest FIX:C values for all samples tested.
- In all cases the FIX chromogenic assay gave the lowest FIX:C values for the patient samples containing PF-06838435 transgene product.

Table 3: FIX:C Measured in FIX Deficient Plasma Spiked with rHFIX-Padua Protein

	rHFIX-Padua Protein Concentration		
Reagent	**40%**	**30%**	**20%**
Actin® FSL	21 (100%)	12 (100%)	5 (100%)
SynthASil®	38 (181%)	18 (149%)	11 (208%)
C.K. Prest®	26 (124%)	16 (128%)	10 (189%)
PTT Automate	36 (171%)	22 (176%)	15 (277%)
Chromogenic	10 (49%)	6 (46%)	3 (57%)

(FIX activity relative to FIX activity measured using Actin® FSL)

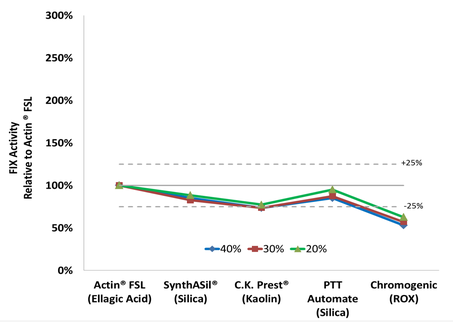


- When normalized against FIX:C measured in the Actin® FSL assay system, a similar FIX reagent dependent recovery pattern was observed for samples spiked with purified rHFIX-Padua at 40, 30 and 20% FIX:C, compared to FIX recovery measured for the transgene product in patient samples.
- Overall, rHFIX-Padua spiked samples under-recovered to varying degrees from target in all assay systems with the lowest FIX:C being observed in the FIX chromogenic assay.

Table 4: FIX:C Measured in FIX Deficient Plasma Spiked with rHFIX-BeneFIX® Product

	rHFIX-BeneFIX® Concentration		
Reagent	**40%**	**30%**	**20%**
Actin® FSL	49 (100%)	36 (100%)	21 (100%)
SynthASil®	42 (85%)	30 (83%)	18 (88%)
C.K. Prest®	36 (73%)	27 (74%)	16 (77%)
PTT Automate	42 (85%)	32 (87%)	20 (95%)
Chromogenic	26 (53%)	21 (57%)	13 (63%)

(FIX activity relative to FIX activity measured using Actin® FSL)



- In contrast to rHFIX-Padua, normalized FIX:C values for rHFIX-BeneFIX® spiked plasma samples showed a different aPTT assay reagent dependent pattern.
- FIX:C recovered within ±25% of target in all aPTT based FIX:C assays and only modestly under-recovered in the FIX chromogenic assay.

Conclusions

- This study reports aPTT reagent dependent FIX recovery obtained for a Padua FIX variant transgene product and rHFIX-Padua protein when tested in four commonly used IVD approved FIX:C assay systems .
- These differences suggest that FIX:C assay selection may be important when measuring FIX-Padua activity, which is particularly relevant when monitoring hemophilia gene therapy following FIX-Padua gene transfer.