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ORIGINAL ARTICLE

Evaluation of One-Stage Assays for the Monitoring of Recombinant Human Factor IX Padua Activity After Etranacogene Dezaparvovec Gene Therapy

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ABSTRACT

Introduction: Accurate and reproducible measures of factor activity are required to guide clinical decision-making following gene therapy for haemophilia B (HB). Highly significant discrepancies have been observed in measurements of various factor IX (FIX) concentrates that carry molecular modifications to extend their half-life, arguing for the need for careful analysis of new HB treatment modalities with respect to FIX assay performance.

Aim: To further characterise variability in FIX activity measured using different one-stage assays (OSAs) and chromogenic assays (CAs) in patients with HB receiving gene therapy utilising the FIX Padua variant and to assess whether assay differences were due to the FIX-Padua variant.

Methods: FIX activity was assessed centrally (OSA and CA) and locally (OSA only) using plasma samples collected from a phase 2b and phase 3 study of etranacogene dezaparvovec and in an in vitro study of wild-type (wt) recombinant human FIX (rhFIX) and rhFIX-Padua.

Results: Lower CA than OSA FIX activity for plasma samples from the phase 3 trial was observed (CA:OSA ratio: 0.408 [±0.049]–0.547 [±0.062]). Local OSA:central OSA FIX activity ratios were 0.789 (±0.314)–1.021 (±0.159). Local OSA:central OSA FIX activity ratios across methods and/or reagents were 0.81 (±0.02)–1.28 (±0.04) for rhFIX-wt-spiked samples and 0.67 (±0.02)–1.13 (±0.09) for rhFIX-Padua-spiked samples.

Conclusion: FIX activity differences between central and local OSAs were modest; similar differences were observed in vitro with rhFIX-wt versus rhFIX-Padua. Commonly available OSAs can be used to monitor patients post-etranacogene dezaparvovec administration; we recommend using the same assay platform throughout the post-treatment period.

1 | Introduction

Etranacogene dezaparvovec (HEMGENIX, AMT-061) was the first gene therapy to receive full US Food and Drug Administration approval for haemophilia B (HB). In addition, etranacogene dezaparvovec has received conditional approval from the European Medicines Agency and has been recommended for use in the National Health Service by the UK National Institute of Health and Care Excellence for the treatment of HB [1–3]. Etranacogene dezaparvovec comprises an adeno-associated virus of serotype 5 (AAV5) containing a genome that consists of a codon-optimised coding sequence for the Padua variant of human factor IX (FIX-Padua), and with transgene expression under control of a liver-specific promoter [4]. Due to its high specific activity, FIX Padua is the only FIX variant used for approved HB gene therapies to date [5–7].

In addition to establishing an initial diagnosis of HB, including its likely clinical severity, laboratory determination of FIX activity is also required for post-treatment monitoring to assess the degree of haemostatic effect and to inform clinical decision-making, and, in the case of gene therapy, to assess the durability of FIX expression [8, 9]. Two broad classes of FIX activity assays are suitable both for diagnosis and clinical monitoring of HB: the one-stage assay (OSA), a modified activated partial thromboplastin time (aPTT) assay; and the chromogenic substrate assay (CA), a two-stage assay using an amidolytic endpoint rather than fibrin formation to assess factor Xa (FXa) generation, which is proportional to the level of functional factor being investigated [10]. OSAs are commonly used to measure FIX activity in patients with HB in the EU and USA; CAs are less widely available and are perceived to have various limitations versus OSAs [8, 10, 11]. These include higher costs and fewer laboratory personnel with the requisite technical expertise, compared with OSAs [10, 11].

Defined by the Factor VIII, Factor IX and Rare Bleeding Disorders subcommittee of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis as being a ratio >2.0 or <0.5, discrepancies in FIX activity measurements across OSAs are principally due to differences among phospholipid and activating reagents used across the range of commonly available assays, as well as use of different instruments and calibration standards [8, 10, 12–15]. A ratio falling within these parameters is considered to be an acceptable and/or modest difference. Although differences between CAs and OSAs have not been observed for plasma-derived FIX, substantial discrepancies have been observed for both potency assignment and clinical monitoring of standard half-life recombinant human FIX (rhFIX), with CAs generally yielding lower FIX levels than OSAs [16, 17]. One study found that rhFIX levels measured by a CA were approximately 70% of the rhFIX levels measured by an OSA [17]. Another study found that the median FIX activity measured by CAs following rhFIX administration was significantly lower than FIX activity measured by OSAs (66.5 vs. 87.5 IU/dL; $p < 0.01$) [18]. These discrepancies appear to be related to the greater impact of FX in CAs compared with OSAs due to the timing of factor VIIIa (FVIIIa) generation [12, 19]. The absence or paucity of FVIIIa in the first stage of the CA means that the newly generated FIXa may form relatively unproductive complexes with FX, thus leading to a reduction in observed FIX activity. In OSAs, sufficient quantities of FVIIIa are generated early in the course of the assay to obviate this effect [12].

Clinically important differences between OSAs and CAs have been observed in the characterisation of non-severe HB patients with certain amino acid sequence mutations [20, 21].

Similar discrepancies in FIX activity measured with OSAs and CAs have been reported for recombinant human (rh) FIX Padua (rhFIX Padua) and gene therapy-derived FIX Padua [17, 22]. Moreover, differences in the effect of excess FX levels, which are more pronounced with wild-type FIX, on wild-type tenase formation relative to FIX Padua tenase formation, add further complexity to OSA/CA discrepancies in the setting of HB gene therapy [12, 19, 23].

OSAs generally yield FIX Padua activity levels that are approximately 1.6-fold higher than those obtained with CAs, with variability in gene therapy-derived FIX Padua of approximately 3-fold being reported between CAs and OSAs [8, 12, 13, 24]. Variability across OSAs has also been observed specifically with both rhFIX Padua and gene therapy-derived FIX Padua and may be due to the gain-of-function haemostatic effect induced by the hyperactive Padua sequence variant [13]. Uncertainty about the importance of differences in FIX activity results between CAs and OSAs in haemophilia gene therapy trials has been increased by a discrepancy that exists in gene therapy for haemophilia A (HA) but does not confound HB gene therapy. It is well known that recombinant B-domain-deleted FVIII therapies produce higher measurements with some CAs than with OSAs, while the B-domain-deleted transgenic protein secreted from hepatocytes after HA gene therapy yields higher FVIII activity values using an OSA, compared with a CA [8, 25]. This apparent 'inversion' of the relationship of the OSA:CA ratio is not seen when comparing recombinant FIX concentrate and HB gene therapies [8].

Therefore, to aid accurate and reliable monitoring of patients with HB receiving gene therapy utilising the FIX Padua variant, further investigation of the variability in FIX activity levels measured using a CA and an OSA is warranted. Here, we report the differences in FIX activity between a central CA and OSA, and also between a central OSA and local OSAs, using participant plasma samples from a phase 2b study (NCT03489291) and a phase 3 study (HOPE-B; NCT03569891) that assessed a single dose of etranacogene dezaparvovec in participants with HB [26–28]. In addition, to determine whether assay variability is an inherent property of the rhFIX Padua sequence variant, an in vitro analysis was performed to measure CA and OSA FIX activity levels for both rhFIX-Padua and rhFIX-wild type proteins.

2 | Methods

2.1 | FIX Activity in Clinical Samples

2.1.1 | Phase 2b Study

An initial analysis comparing assays used for the measurement of FIX activity was conducted using representative samples from the phase 2b study. Three test samples generated by pooling of blood samples taken from each of the three participants when FIX

Padua levels had reached a stable level following etranacogene dezaparvovec administration (weeks 10–18 post-dose, based on interim data), which generated one pooled sample per subject. The pooled samples were then analysed for FIX activity using the central laboratory CA and OSA as well as three separate testing laboratories using OSA platforms/reagents. Quality control (QC) samples (used as routine controls to assess assay validity, from healthy donor plasma representing a low, medium, and high level of FIX activity) were also supplied and tested.

2.1.2 | Phase 3 Study

In the phase 3 HOPE-B study (N = 54), FIX activity pre- and post-treatment was evaluated using an OSA performed at a central laboratory to monitor and report FIX activity after administration of a single dose of etranacogene dezaparvovec (2×10^{13} gc/kg) in adult males with severe-to-moderately-severe HB (FIX activity ≤2 IU/dL). FIX activity was measured by a CA in parallel on matching samples from the same time points pre- and post-treatment, with testing performed at a central laboratory. The samples were also analysed by over 30 local laboratories employing one of four OSA platforms, one of which was the same as that used by the central laboratory. FIX activity levels were evaluated in individual patient plasma samples collected over 24 months [26–28].

Further details regarding the assays used for both clinical studies are described below as well as in the Supporting information.

2.2 | FIX Activity In Vitro

FIX-deficient plasma was spiked with rhFIX-wt (identical in sequence to BeneFIX) or rhFIX Padua to achieve low (15 IU/dL), medium (30 IU/dL), and high (50 IU/dL) concentration samples and analysed for FIX activity by the same assay platforms as were used for the pooled samples from the phase 2b study. Please see Supporting information for more information about the in vitro methodology used.

2.3 | FIX OSA and CA Assay Details

2.3.1 | Central Laboratory CA and OSA Details

The central laboratory (Unilabs, Copenhagen) used an ACL TOP 300 and ACL TOP 500 platform to measure FIX activity by OSAs and CAs. The central OSA comprised HemosIL SynthASiL as the aPTT reagent, colloidal silica as the activator, and synthetic phospholipid. A Conformité Européenne-marked CA was used in the central laboratory. Further details are given in the Supporting information (Tables S1 and S2).

2.3.2 | Local OSA Platform Details for the Phase 3 Study

Over 30 local laboratories were involved in the phase 3 study and primarily used one of four different OSA platforms [reagents (manufacturer)]: HemosIL SynthASiL (Instrumentation Laboratory); aPTT Automate (Diagnostica Stago); Actin FS (Siemens Healthcare Diagnostics); and Actin FSL (Siemens Healthcare Diagnostics). Supporting information provides more details on the assay parameters, including Table S3.

2.3.3 | Local OSA Details for the Phase 2b and In Vitro Samples

For the representative pooled phase 2b samples and in vitro samples, three laboratories (laboratories A, B, and C) using different OSA platforms/reagents were used as well as the previously described central OSA. Please see Supporting information, including Table S2, for further details on these assays and the methodologies used.

3 | Results

3.1 | FIX Activity in Clinical Samples

3.1.1 | Phase 2b Study Pooled Samples

Phase 2b-derived test samples, consisting of pooled plasma from each of the three patients, and taken at a time post-dosing of etranacogene dezaparvovec when rhFIX Padua levels had reached a stable level, showed a mean (±SD) CA:OSA FIX activity ratio of 0.60 (±0.01). Comparison between the different OSA platforms/reagents showed mean (±SD) local OSA:central OSA FIX activity ratios ranging between 0.61 (±0.02) and 1.19 (±0.05) depending on the platform/reagents used (Table 1). The largest difference was observed with the Siemens platform using Actin-FSL reagent, where the activator is ellagic acid.

3.1.2 | Phase 3 Study

FIX activity levels measured by the central CA were consistently lower than those measured by the central OSA in clinical samples from the phase 3 HOPE-B study of etranacogene dezaparvovec, with the mean CA:OSA FIX activity ratio (SD) ranging from

TABLE 1 | Mean local OSA:central OSA[†] and the mean central CA:central OSA FIX activity ratios by platform/reagent in the phase 2b study at 24 months post-etranacogene dezaparvovec administration.

OSA reagent (manufacturer)	Mean (SD)
HemosIL SynthASiL (Instrumentation Laboratory)	—
Pathromtin SL (Siemens Healthcare Diagnostics)	0.90 (±0.03)
STA-CK-Prest (Diagnostica Stago)	1.19 (±0.05)
Actin FSL (Siemens Healthcare Diagnostics)	0.61 (±0.02)
CA reagent (manufacturer)	
BIOPHEN Factor IX Kit (Aniara Diagnostica)	0.60 (±0.01)

Abbreviations: FIX, factor IX; OSA, one-stage assay; SD, standard deviation.
[†]The central laboratory used the HemosIL SynthASiL OSA platform.

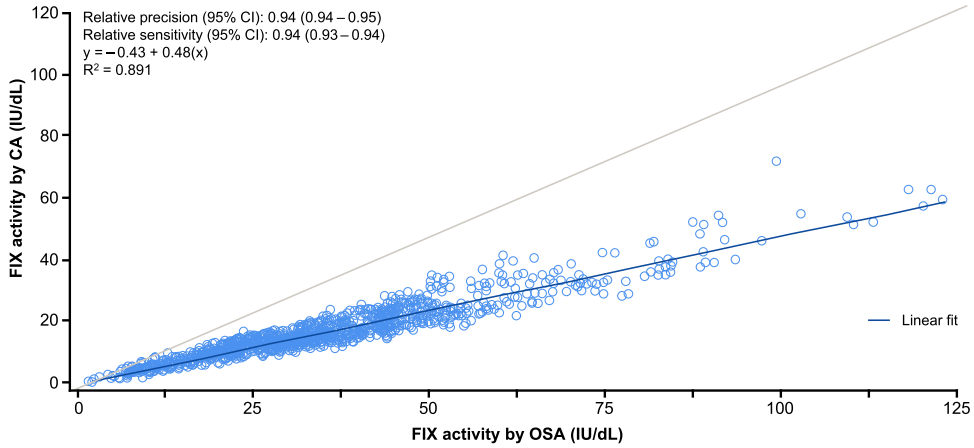

FIGURE 1 | Correlation of post-treatment uncontaminated FIX activity levels measured by CA and OSA in the phase 3 HOPE-B study central laboratory.

CA, chromogenic assay; CI, confidence interval; FIX, factor IX; OSA, one-stage assay.

TABLE 2 | Mean local OSA:central OSA[†] and the mean central CA:central OSA* FIX activity ratios by platform/reagent in the phase 3 HOPE-B study at 24 months post-etranacogene dezaparvovec administration.

OSA reagent (manufacturer)	Number of unique sites ($N = 28$ [%])	Number of samples tested overall	Mean (SD)
HemosIL SynthASiL (Instrumentation Laboratory)	12 (42.9)[†]	187	1.002 (±0.138)
STA-PTT Automate 5 (Diagnostica Stago)	5 (17.9)[†]	38	1.021 (±0.159)
Actin FS (Siemens Healthcare Diagnostics)	6 (21.4)[†]	107	0.886 (±0.137)
Actin FSL (Siemens Healthcare Diagnostics)	5 (17.9)[†]	66	0.789 (±0.314)
CA reagent (manufacturer)			
BIOPHEN Factor IX Kit (Aniara Diagnostica)*	1	187	0.547 (±0.062)

Abbreviations: FIX, factor IX; OSA, one-stage assay; SD, standard deviation.

[†]Site numbers and percentages are based on the number of unique sites. Sites that utilised multiple reagents were summarised based on the reagent predominantly used; *The central laboratory used the HemosIL SynthASiL OSA platform.

0.408 (±0.049) to 0.547 (±0.062) across multiple time points post-dose up to month 24 post-treatment. A strong correlation was observed between FIX activity levels measured by CA and by central OSA post-treatment over a wide range of measured FIX activity levels (R^2: 0.891; Figure 1). Although a subsequent Bland–Altman analysis demonstrated that OSA FIX activity measured consistently higher than CA FIX activity through to month 24 post-treatment (Figure S1), the intraclass correlation coefficients (ICCs) for the CA and OSA assays using all data from week 3 post-treatment onward indicated that both assays offer substantial reliability to measure longitudinal FIX activity (0.874 [95% CI: 0.829, 0.914] and 0.873 [95% CI: 0.827, 0.913], respectively).

FIX activity levels measured by local OSAs across >30 clinical sites were similar to those measured by the central OSA, with the mean local OSA:central OSA FIX activity ratios ranging from 0.789 to 1.021 (Table 2). A strong correlation between local and central FIX activity measurements was observed (R^2: 0.869; Figure 2). The closest correlation was observed between local laboratories using the same HemosIL SynthASiL reagent as that used by the central laboratory (mean [SD]: 1.002 [0.138]); this was also the most commonly used platform (12 [42.9%] sites) (Figures 2 and 3; Table 2). A separate Bland–Altman analysis comparing local to central laboratory measurements of OSA FIX activity showed stable differences up to 24 months post-treatment (Figure S2). In addition, the ICC showed excellent agreement between the local and central laboratories (median = 0.938; quartile 1 = 0.902, quartile 3 = 0.95).

3.2 | FIX Activity In Vitro

CA and OSA performance were further characterised with respect to measurement of wild-type FIX and FIX Padua. In vitro FIX-deficient plasma samples spiked with rhFIX-wt and rhFIX Padua showed directionally similar CA:OSA ratios (SD) for mean FIX activity (0.49 [±0.01] vs. 0.32 [±0.02], respectively; Table 3). In rhFIX-wt-spiked samples (FIX activity level

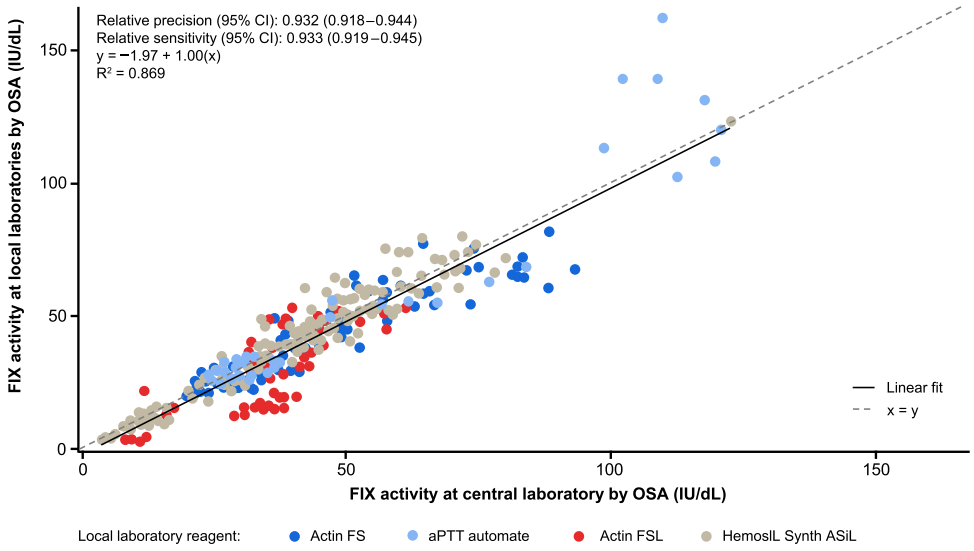

FIGURE 2 | Correlation of FIX activity levels measured by central and local laboratory OSAs in the phase 3 HOPE-B study. aPTT, activated partial thromboplastin time; CI, confidence interval; FIX, factor IX; OSA, one-stage assay.

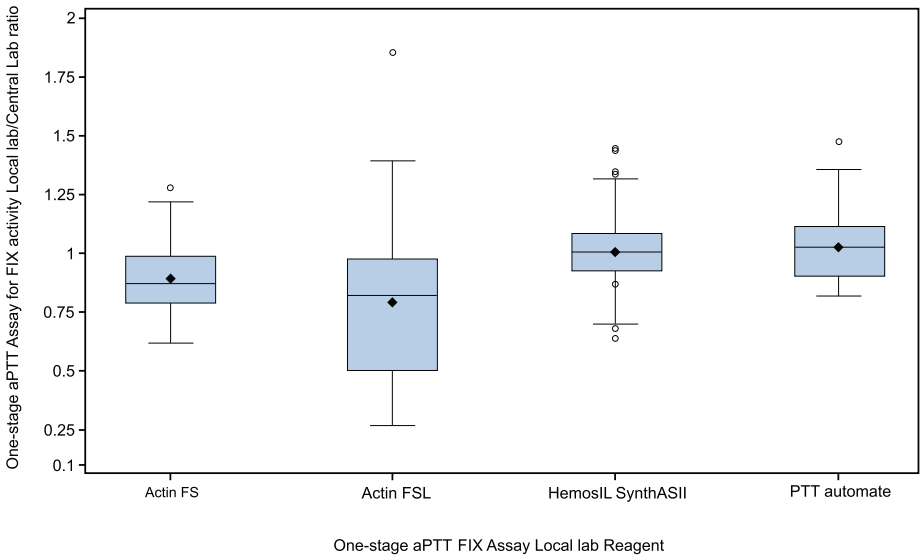

FIGURE 3 | Box/whisker plot of FIX activity ratios of local:central OSA ratios by reagent in the phase 3 HOPE-B study. aPTT, activated partial thromboplastin time; FIX, factor IX; lab, laboratory.

15–50 IU/dL), mean (SD) FIX activity laboratory A–C: central laboratory OSA ratios varied from 0.81 (±0.02) to 1.28 (±0.04) across methods and/or reagents (Table 3). Similarly, for rhFIX Padua-spiked samples (FIX activity level 15–50 IU/dL), mean (SD) FIX activity laboratory A–C: central laboratory OSA ratios varied from 0.67 (±0.02) to 1.13 (±0.09) across methods and/or reagents (Table 3). Similar effects were observed across QC samples.

4 | Discussion

In this analysis of FIX activity in a phase 3 study in patients with HB following etranacogene dezaparvovec administration, moderate differences in FIX activity were observed across various commonly used OSAs. The closest correlation of mean local OSA:central OSA FIX activity ratios was observed between local laboratories using the same HemosIL SynthASiL reagent as that used by the central laboratory (mean ratio of 1.002). However, even the highest and lowest mean correlates, which were 1.021 and 0.789 for the silica-based STA PTT Automate-5 and ellagic acid-based Actin FSL assay, respectively, fall within acceptable parameters for OSAs [14]. Similar results were observed in a study of FIX activity assessed by OSAs following HB gene therapy with fidanacogene elaparvovec, with the silica-based assay generating the highest FIX activity and the ellagic acid-based assay generating the lowest FIX activity [15].

The differences between the central CA and OSA were more pronounced than differences between commonly used OSAs.

TABLE 3 | FIX activity in vitro by platform and reagent.

	Method				
	Central laboratory OSA	**Central laboratory CA**	**Local laboratory OSA**	**Local laboratory OSA**	**Local laboratory OSA**
Reagent (manufacturer)	HemosIL SynthASiL (Instrumentation Laboratory)	XIa-thrombin-calcium-phospholipids (Hyphen Biomed)	Pathromtin SL (Siemans Healthcare Diagnostics)	STA-CK Prest (STAGO)	Actin FSL (Siemens)*
Sample (target FIX activity level)	**FIX activity as mean % of normal (% difference vs. central OSA)**				
LQC (3.1 IU/dL)[†]	3.1	1.7 (−45.2)	2.9 (−5.4)	4 (+40)	3.4 (10.8)
MQC (28.6 IU/dL)[†]	28.4	21.0 (−26.1)	28.1 (−0.9)	35 (+25)	28.3 (−0.1)
HQC (71 IU/dL)[†]	70.1	64.1 (−8.5)	65.5 (−6.6)	82 (+17)	69.7 (−0.5)
rhFIX-wt (15 IU/dL)	19.1	9.1 (−52.5)	15.1 (−21.1)	25 (+29)	21.6 (+13.3)
rhFIX-wt (30 IU/dL)	36.3	17.5 (−51.9)	28.8 (−20.8)	45 (+23)	43.1 (+18.7)
rhFIX-wt (50 IU/dL)	55.7	27.9 (−50.0)	46.0 (−17.5)	73 (+32)	67.0 (+20.3)
rhFIX Padua (15 IU/dL)	16.8	5.0 (−70.0)	11.1 (−34.1)	17 (+3)	11.9 (−29.2)
rhFIX Padua (30 IU/dL)	34.7	11.2 (−67.8)	24.0 (−30.7)	42 (+20)	23.4 (−32.4)
rhFIX Padua (50 IU/dL)	53.5	18.1 (−66.3)	35.9 (−33.0)	61 (+15)	35.4 (−33.9)

Abbreviations: aPTT, activated partial thromboplastin time; CA, chromogenic assay; FIX, factor IX; HQC, high-concentration quality control; LQC, low-concentration quality control; MQC, mid-concentration quality control; NA, not available; OSA, one-stage assay; rhFIX Padua, recombinant human factor IX Padua; rhFIX-wt, recombinant human wild-type factor IX.

[†]LQC, MQC, and HQC samples correspond to target FIX activities of 3.1%, 28.6%, and 71.0%, respectively; all were derived from healthy donor plasma; *This reagent was not able to be used in all instruments.

CA-assessed FIX activity was consistently lower than FIX activity measured using the OSA, with each assessed in the central laboratory. These data are in accordance with other studies, including results reported from a multicentre field study of CA and OSA discrepancies [15, 16, 29]. That FIX activity levels measured by CAs following gene therapy are lower than those with OSAs has been recognised previously by the World Federation of Hemophilia (WFH) guidelines [8]. These differences are largely due to fundamental differences in assay properties, such as the timing of FVIIIa generation [12, 15]. Furthermore, differences in FIX activity levels measured by CAs and OSAs depend on the assay platforms/reagents used as well as laboratory-specific methodologies that obviate the application of a consistent conversion factor [11, 30, 21].

A study of FIX activity levels post-administration of fidanacogene elaparvovec, another HB gene therapy utilising FIX Padua, as well as FIX activity levels following spiking with rhFIX Padua, also demonstrated consistently higher FIX values for OSAs versus CAs. These data confirm that assay discrepancies are similar for enzyme replacement and gene therapy approaches to supplementing FIX activity in plasma. For endogenous FIX, OSA FIX activity levels across all samples were 1.5–1.9-fold higher than the lowest FIX activity values observed, with the CA generating the lowest FIX activity values for all samples [13]. Similar, albeit less pronounced outcomes, were observed in a phase 1/2 study that assessed expression of wild-type FIX following gene therapy with AMT-060, the predecessor of etranacogene dezaparvovec, with CA FIX activity values being lower than OSA values (CA:OSA ratio of 0.73) (data not published).

Importantly, the discrepancy between OSAs and CAs creates challenges in providing accurate information about a patient's FIX activity, with one study showing that measuring FIX activity with a CA alone would have led to a diagnosis of a more severe haemophilia phenotype [21]. It is of note that the WFH guidelines recommend use of the OSA for the diagnosis of mild, moderate, or severe HB [8]. Similar considerations apply to the clinical management of patients with HB as the OSA tends to yield FIX activity values that are aligned with the WFH guidelines, as well as with a clinician's prior experience. This is due to OSAs being used much more widely than CAs both for diagnosis and post-treatment monitoring, as was shown in a review of proficiency testing programmes and field surveys [8, 22, 31]. For example, if one were to obtain a FIX activity value of 40 IU/dL using a CA, and the practice pattern guidance for a major haemorrhagic event or for surgical cover is a FIX activity of 60–80 IU/dL, an infusion of additional FIX may not be warranted due to the guidelines being based on OSA FIX activity values rather than CA FIX values.

Within the context of gene therapy, two principal purposes of FIX assessment are to monitor the durability of FIX transgene expression over time and to provide insights into haemostatic risk. Consequently, there is a need for accurate assessment of plasma FIX activity levels following any form of FIX replacement therapy, including gene therapy.

Deciphering the relationship between FIX activity levels and clinical outcomes is challenging given the low incidence of bleeding events at FIX activity above a certain level and is further confounded by errors associated with self-reporting of bleeds, the

patient's activity level, and the interaction of other components involved in the coagulation cascade. However, a recently conducted analysis of joint health in patients in HOPE-B showed a significant correlation between steady-state FIX activity level, as measured by the central OSA, and joint bleeding rate during months 7–36 post-etranacogene dezaparvovec administration, as well as a significant reduction in overall annualised bleeding rate during this period, compared with the lead-in period ($p < 0.001$) [32].

In the in vitro study we report here, spiking of deficient plasma with rhFIX-wt and rhFIX Padua showed discrepancies in FIX activity between CAs and OSAs for both FIX proteins, demonstrating that these differences are not unique to rhFIX Padua, with similar discrepancies being reported for AMT-060 in the aforementioned phase 1/2 study (data not published), and suggesting that the assay discrepancies are not related to hepatic transgene expression. This finding is also consistent with previously published findings from other FIX Padua gene therapy studies, further suggesting that theoretical differences in FIX protein structure resulting from this amino acid sequence variant do not materially contribute to the assay discrepancies [13, 33]. However, although directionally similar, the magnitude of the CA:OSA discrepancy in vitro was slightly more pronounced for rhFIX Padua than for rhFIX-wt (mean CA:OSA ratios of 0.49 and 0.32 for rhFIX-wt and rhFIX Padua, respectively) or for AMT-060 in the phase 1/2 study (data not published). As the Padua sequence variant increases the specific activity of FIX by enhancing its interaction with FVIIIa and is differentially affected by the presence of non-activated FX, it is reasonable to speculate that the discrepancy between the assay platforms might be somewhat enhanced. A limitation of this study was the lack of a direct comparison of OSA and CA discrepancies between gene therapy-based hepatic expression of FIX-Padua and rhFIX-wt, to further explore this hypothesis. The platforms/reagents and methodologies tested in our investigation covered 72% of the OSAs and 60% of the CAs used routinely by testing laboratories participating in the External Quality Control for Assays and Tests (ECAT) programme in 2024 [34]. Comparisons between the FIX activity data obtained with the central OSA and the FIX activity data from the local OSAs used in the HOPE-B study provide a surrogate for the expected real-world experience, with an acceptable degree of discordance reported across the local OSAs. In conclusion, with respect to the study reported here, the FIX Padua variant did not appear to impact FIX activity discrepancies observed between various OSAs and between an OSA and a CA when compared with rhFIX-wt in the in vitro analysis. Moreover, in the clinical studies, all OSA platforms/reagents used in the central and local laboratories demonstrated correlations within an acceptably narrow range, suggesting that no specific platform is preferable for the post-treatment monitoring of FIX activity following gene therapy for HB. However, the consistent use of one assay is recommended for longitudinal follow-up of individual patients. This recommendation appears to be especially important if switching between an ellagic acid- versus silica-based OSA is contemplated.

Author Contributions

J.A. interpreted the data, provided critical review of the manuscript. W.M. interpreted the data, provided critical review of the manuscript. M.C. interpreted the data, provided critical review of the manuscript. S.G. designed the research and provided critical review of the manuscript. J.T. designed the research, interpreted the data, and provided critical review of the manuscript. R.D. interpreted the data, provided critical review of the manuscript. S.V. provided critical review of the manuscript. P.E.M. designed the research, analysed the data and provided critical review of the manuscript and provided critical text for the manuscript drafts. B.M.E. analysed the data, provided critical review of the manuscript, provided critical text for the manuscript drafts. S.N. designed the research, provided critical review of the manuscript, provided critical text for the manuscript drafts. N.G. designed the research, generated figures, performed statistical analyses, provided critical review of the manuscript. G.Y. interpreted the data, provided critical review of the manuscript. All authors provided final approval of the version to be published as well as agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethics Statement

Both the phase 2b and HOPE-B trials used in this FIX assay analysis were performed according to ethics principles originating in the Declaration of Helsinki and conducted in accordance with International Council for Harmonisation Good Clinical Practice guidelines.

Conflicts of Interest

J.A.: Grant/research support from SOBI, Bayer, Takeda/Shire and CSL Behring; honorarium as member of advisory boards and speaker for BioMarin, Pfizer, Sparks, uniQure, CSL Behring, SOBI, Sanofi, Novo Nordisk, Bayer, Roche, Takeda/Shire and Octapharma. W.M.: Grant/research support from Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, Takeda/Shire; consultation/speaker fees from Bayer, Biomarin, Biotest, CSL Behring, Chugai, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, Takeda/Shire; and consultation fees from Bayer, Biomarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Regeneron, Roche, Sanofi, Sobi, Takeda/Shire, and uniQure. M.C.: has received financial support for research from Anthos, Bayer, CSL Behring, Novo Nordisk, and Hoffmann-La Roche; and honoraria for lecturing or consultancy from Alexion, CSL Behring, Daiichi Sankyo, Sanofi, Spark Therapeutics, Octapharma, Pfizer, Sobi, and Viatris. All funds were received by his institution. Non-financial conflicts of interest: member of the gene therapy working group of the European Association for Haemophilia and Allied Disorders (EAHAD); and member of the European Reference Network (ERN) EuroBloodNet. S.G., J.T., R.D., S.V.: Employees of uniQure biopharma B.V. at the time of this research; RD is now an employee of Tempuro Bio. P.E.M., B.M.E., S.N., N.G.: Employees of CSL Behring. G.Y.: has received consulting fees from ASC Biotherapeutics, BioMarin, Centessa, CSL Behring, Genentech/Roche, Hema Biologics/LFB, Novo Nordisk, Octapharma, Pfizer, Sanofi Genzyme, Spark, and Takeda, and funds for research support from Sanofi.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.