P-M-057

Evaluation of the Thrombin Generation Potential of a Recombinant Factor IX Fc Fusion Protein (rFIXFc) in a Phase 3 Multinational Clinical Trial

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World Federation of Hemophilia (WFH) 2014 World Congress 11-15 May 2014 Melbourne, Australia

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INTRODUCTION

- Recombinant factor IX Fc fusion protein (rFIXFc) consists of a single molecule of human factor IX (FIX) covalently linked to the dimeric Fc domain of human immunoglobulin G_1 (IgG_1), with no intervening linker.
- rFIXFc utilizes the FcRn-mediated immunoglobulin recycling pathway to extend its plasma half-life.[1]
- In a global, multicenter, phase 3 clinical study (B-LONG), rFIXFc demonstrated safe and effective prevention and control of bleeding episodes, with prophylactic dosing intervals ranging from 7 to 21 days.[2]
- Traditionally, the coagulation activity of plasma samples in hemophilia replacement therapy is evaluated by activated partial thromboplastin time (aPTT) to correlate clotting time with in vivo activity. The tissue factor-triggered thrombin generation assay (TGA), as a global test of coagulation, may be useful to obtain information on an individual's coagulation potential and discern the clinical severity of bleeding phenotypes in hemophilia.

OBJECTIVE

- To compare the ex vivo thrombin generation potential of rFIXFc to that of BeneFIX®, in post-infusion subject plasma samples collected in a phase 3, multinational, clinical trial (B-LONG).

METHODS

Sample collection

- A total of 813 samples from 50 subjects enrolled in the study were obtained at 18 clinical sites for a standardized TGA analysis. The TGA analysis was performed at the same time points as the pharmacokinetics (PK) assessment.
- Blood was drawn into special tubes containing 3.2% citrate and 50 µg/mL corn trypsin inhibitor (CTI). Within 1 hour, CTI tubes were centrifuged at 2800 g for 15 minutes. Plasma was transferred to 15 mL conical tubes for a second centrifugation at 2800 g for 10 minutes. Samples were stored immediately in a –70°C freezer until shipment.

Assay setup

- In order to minimize the preanalytical variables, the TGA was performed at a central laboratory. Generally, each assay contained 80 µL plasma sample + 20 µL initiator + 20 µL fluorogenic substrate with calcium (Flu·Ca).
- Thrombin generation was triggered with 20 µL initiator composed of a 1:6000 dilution of tissue factor (Innovin®) and 4 µM synthetic phospholipids as the final concentration in the reaction mix.
- Plasma samples were thawed in a 37°C water bath, visually inspected for sample quality, centrifuged in a tabletop microcentrifuge at 17,000 g for 10 minutes, added to a 96-well assay plate (80 µL) with initiator solution (20 µL), and preheated at 37°C for 10 minutes.
- Flu·Ca substrate (20 µL) at 37°C was then added, mixed at medium intensity for 5 seconds; readings were obtained at 30-second intervals for 1 hour.

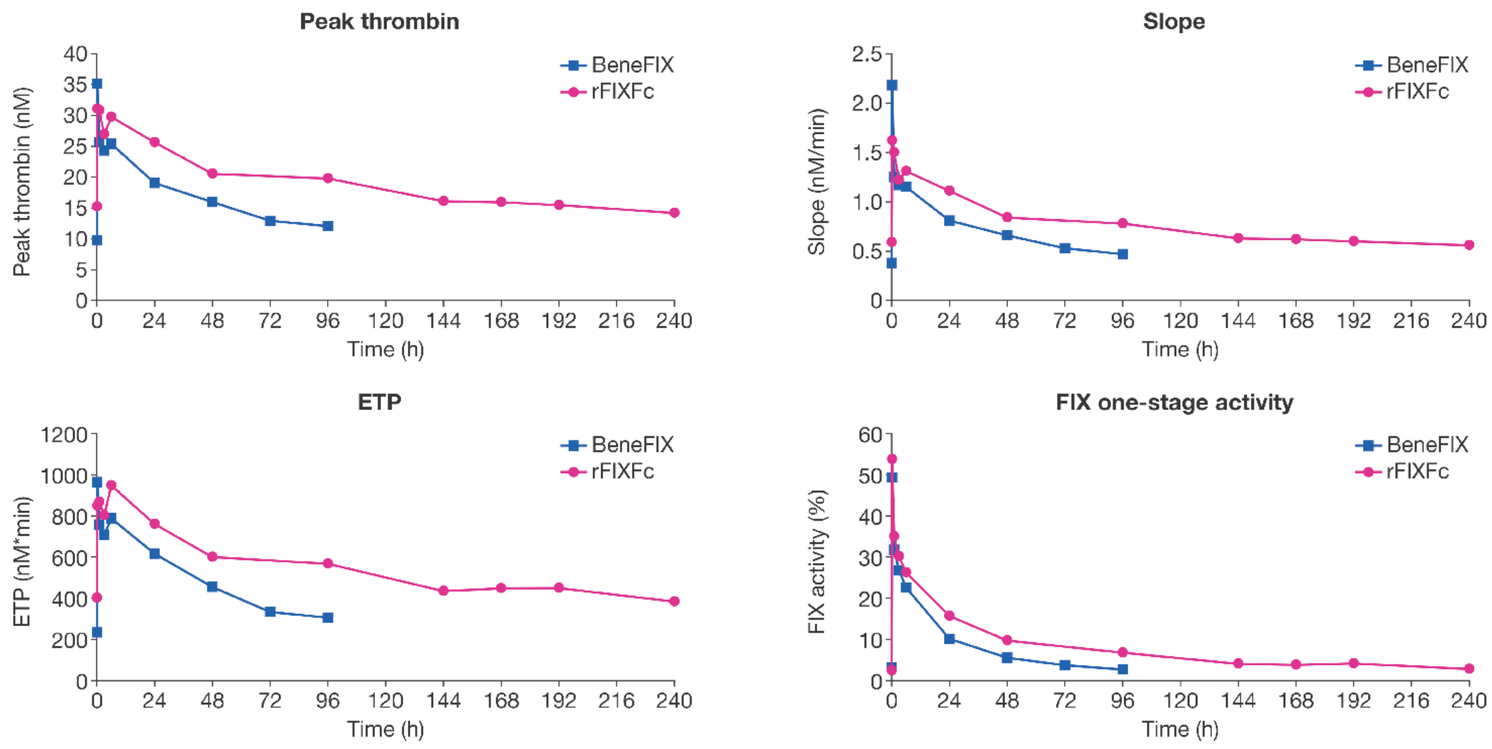
RESULTS

- Results of 571 samples (18 subjects), from the sequential PK subgroup of B-LONG,[2] for both rFIXFc and BeneFIX were included for this report.

TGA time profile correlates to one-stage FIX activity time profile

- To evaluate the thrombin generation response of PK samples after rFIXFc or BeneFIX dosing, the mean peak thrombin, mean endogenous thrombin potential (ETP), and mean slope values were plotted versus time pre- or post-dosing, along with the one-stage activity data (Figure 1). All 3 major TGA parameter time profiles were similar to that of the one-stage FIX activity time profile.
- At early time points post-dosing, rFIXFc demonstrated thrombin generation responses comparable to those of BeneFIX. The decay of thrombin generation activity was markedly slower for rFIXFc than for BeneFIX, indicating good protection at later time points post-dosing.
- The average thrombin generation profile versus time after dosing observed for rFIXFc and BeneFIX correlated well with the plasma PK profiles of these products determined by the one-stage clotting assay.

Figure 1. TGA responses (peak thrombin, slope, and ETP) and one-stage clotting assay results of rFIXFc and BeneFIX versus time



TGA, thrombin generation assay; ETP, endogenous thrombin potential; rFIXFc, recombinant factor IX Fc fusion protein; FIX, factor IX.

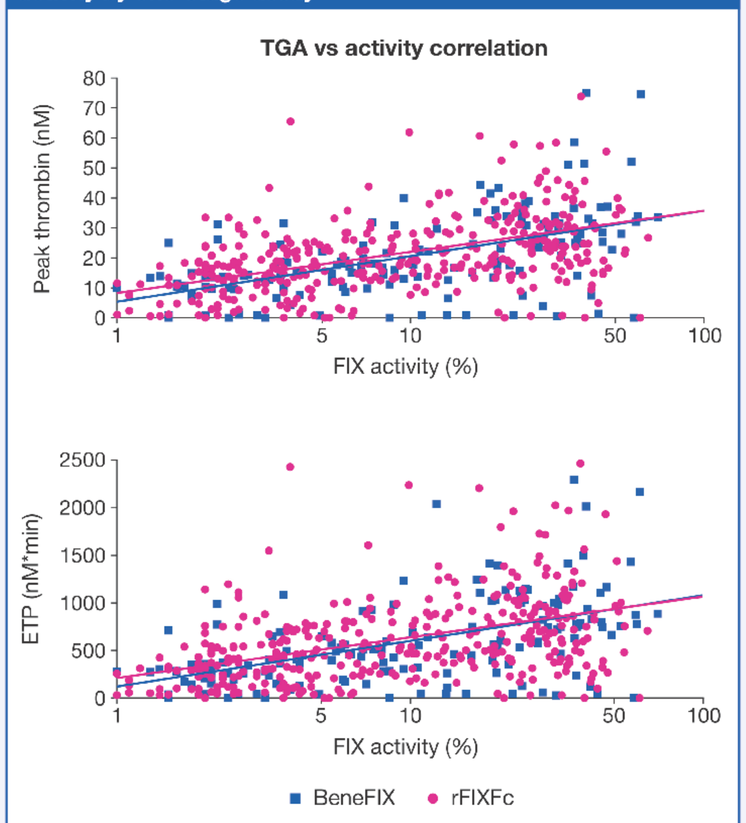
Comparable thrombin generation potential of rFIXFc and BeneFIX at equivalent FIX activities

- To assess the correlation of thrombin generation potential to FIX activity for rFIXFc and BeneFIX, the peak thrombin and ETP responses were plotted against the log [FIX] activity by one-stage activity, if measurable (≥0.5% FIX).
- The scatter plots suggest a positive correlation between the peak thrombin/ETP and FIX activity for both rFIXFc and BeneFIX (Figure 2). The log-linear trend lines for rFIXFc and BeneFIX were nearly parallel, or overlapped with each other by one-stage activity. The results suggest that rFIXFc and BeneFIX possess comparable thrombin generation potential at equivalent FIX activities.

Thrombin generation potential of rFIXFc and BeneFIX are comparable in individual subjects

- To further compare the thrombin generation capacity of rFIXFc and BeneFIX at equal FIX activity within each subject, we took advantage of the relationship between the peak thrombin or ETP and the log [FIX activity (%)], observed in Figure 2, by performing the following data transformation: For each subject, at each time point collected, peak thrombin or ETP was divided by log [FIX one-stage activity] to generate an index.

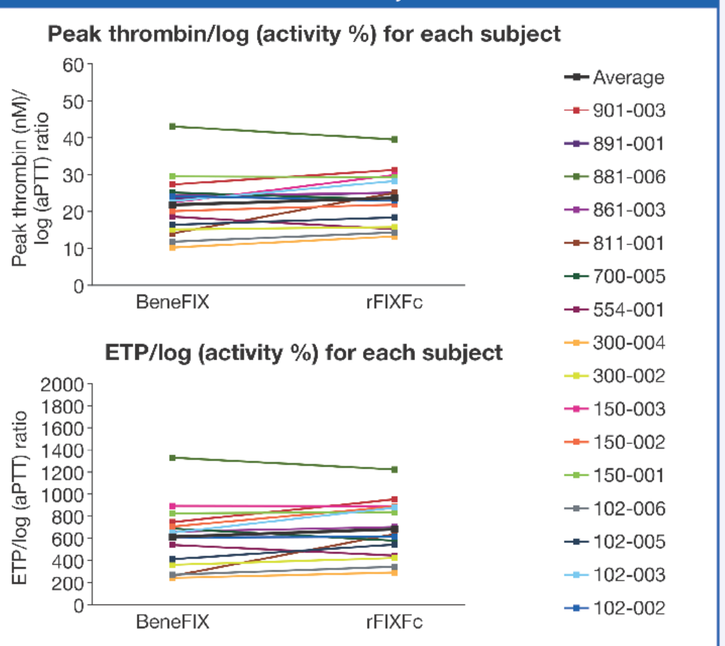
Figure 2. Scatter plots comparing peak thrombin/ETP and FIX activity by one-stage assay



ETP, endogenous thrombin potential; FIX, factor IX; TGA, thrombin generation assay; rFIXFc, recombinant factor IX Fc fusion protein.

- For each subject, the indices composed of all time points used for PK assessment of either rFIXFc or BeneFIX were averaged for comparison where the FIX activity was >2% (Figure 3). The wide ranges of the indices were manifestations of intersubject variances in thrombin generation potential.
- For a majority of subjects, their individual level of thrombin generation potential remained relatively constant between rFIXFc and BeneFIX. Thus, overall, rFIXFc and BeneFIX possessed similar thrombin generation potential by this evaluation.

Figure 3. Comparison of peak thrombin and ETP indices for rFIXFc and BeneFIX in individual subjects



ETP, endogenous thrombin potential; rFIXFc, recombinant factor IX Fc fusion protein; aPTT, activated partial thromboplastin time.

CONCLUSIONS

- This is a large scale, global, clinical evaluation of rFIXFc by TGA using a standardized sample collection procedure and an optimized and validated assay, performed in a central lab at Biogen Idec. Despite inherent intersubject differences in thrombin generation potential, our results suggest that:
  1. The TGA results confirmed the longer duration of activity of rFIXFc relative to BeneFIX, as was observed by PK assessment using the one-stage clotting assay. TGA appears to be a sensitive indicator of prolonged hemostasis potential in rFIXFc-treated subjects.
  2. The integrated TGA data showed, on average, a comparable thrombin generation response to rFIXFc and BeneFIX at equivalent FIX activities, and the individual thrombin generation potential in each subject was largely the same for both products.
  3. The comparable ex vivo thrombin generation potential of rFIXFc and BeneFIX observed in this study supports our method for rFIXFc potency assignment against the World Health Organization (WHO) FIX concentrate standard.

DISCLOSURES

All authors are employees of and hold equity interest in Biogen Idec.
This research was funded by Biogen Idec.

ACKNOWLEDGEMENTS

The authors would like to thank the investigators, study centers, patients, and families, and the staff at Biogen Idec. The development of this poster was supported by Biogen Idec. Copyedit assistance was provided by Shannon O'Sullivan, of MedErgy, and was funded by Biogen Idec.

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DOI: 10.3252/pso.eu.WFH2014.2014