**Supplemental Material**

*Part 1 study design*

Part 1 was a 2-cohort, open-label, randomized, 2-sequence, 2-treatment, 2-way crossover study that established the punch biopsy procedure and determined the sensitivity and variability of BD and BV following single doses of edoxaban (**Data Supplement Figure I**). Two cohorts were enrolled. The first cohort was used for study center training on the punch biopsy procedure. Cohort 2 was used to determine the sensitivity, variability, reproducibility, and effect size for BD and BV following single doses of edoxaban 60 and 180 mg.

Sample size in part 1 was not determined based on statistical consideration. In part 1, the correlation between BD and BV with each biomarker was assessed at the 5% level, as well as the correlation between BD, BV, biomarkers, and plasma edoxaban peak exposure.

Screening occurred within 30 days of study initiation. In period 1, following screening and safety assessments, subjects underwent a baseline punch biopsy. After an overnight fast, subjects were randomized to receive a single oral dose of either edoxaban 60 mg or a supratherapeutic dose of edoxaban 180 mg. A punch biopsy was taken 2.75 hours after dosing and BV and BD were measured. After a 1-week washout period, subjects received the alternate dose of edoxaban in period 2 and assessments were repeated. Safety follow-up was conducted approximately 12 days after discharge.

*Bioanalytical methods*

Edoxaban plasma concentrations were analyzed using a validated liquid chromatography-mass spectrometry method, with upper and lower limits of quantification of 0.074 ng/ml and 382 ng/ml, respectively. FII, FVII, and FX were determined by one-stage

clotting assay using Thromborel® S as activator reagent, FIX was determined by a one-stage clotting assay using Pathromtin SL as the activator reagent, and protein C was measured by chromogenic assay Berichrom® Protein C (all Siemens Healthcare Diagnostics, Marburg, Germany), all using the BCSXP Analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany). Protein S was determined using a commercially available enzyme immunoassay (Affinity BiologicalsINC, Ontario, Canada) on the BEP® III Processor (Siemens Healthcare Diagnostics, Eschborn, Germany).

*Part 1 results*

Both edoxaban doses of 60 and 180 mg significantly increased both BD (60 mg: 9.7 min predose vs 14.1 min postdose, 95% CI 100.91 to 211.10, *P* =0.05; 180 mg: 9.79 min vs 17.38 min, 95% CI 128.0 to 246.3, *P* =0.005) and BV (60 mg: 1.9 mL predose vs 2.4 mL postdose, 95% CI 84.5 to 185.7, *P*=0.22; 180 mg: 1.5 mL vs 2.9 mL, 95% CI 122.5 to 300.4, *P*=0.01) relative to baseline. Irrespective of treatment, a period effect was also present, with increased baseline BD in period 2 relative to period 1 (17.2 minutes vs 8.45 minutes, respectively, for 60-mg edoxaban and 15.8 minutes vs 6.62 minutes, respectively, for 180-mg edoxaban).

Pharmacokinetic results for edoxaban treatment were consistent with those reported previously (**Data Supplement Table III**).[1,2] There were dose-dependent increases in BD and BV with edoxaban doses of 60 and 180 mg. Intrasubject variability associated with BD (35% for edoxaban 60 mg and 26% for edoxaban 180 mg) was lower than for BV (37.5% for edoxaban 60 mg and 35.7% for edoxaban 180 mg), therefore BD was selected as the primary endpoint in Part 2.

*Correlations*

Thrombin generation lag was significantly correlated with BD ($P$=0.04) and there was a trend towards a correlation between ETP (as a measure of thrombin generation) and BD ($P$=0.07). No other parameters of thrombin generation were significant at the 5% or 10% level. PT and aPTT were not significantly correlated with BD, but showed a trend towards significance at the 5% level ($P$ <0.1). Neither factor Xa ($P$=0.64) nor intrinsic factor Xa ($P$=0.07) were shown to correlate with BD. There was no significant correlation between edoxaban $C_{max}$ and BD or BV.

**Supplemental References**

1. Ogata K, Mendell-Harary J, Tachibana M, Masumoto H, Oguma T, Kojima M and Kunitada S. Clinical safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel factor Xa inhibitor edoxaban in healthy volunteers. J Clin Pharmacol. 2010;50:743-53.

**Data Supplement Table I:** Blood draw schedule

| | Day -1 | Day 1 (hours) | | | | | | | | | | | | Day 2 | Day 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre-dose | 0.5 | 1 | 1.75 | 2 | 2.25 | 2.5 | 2.75 | 3 | 4 | 6 | 10 | 14 | 24 | 48 |
| **Part 1** | | | | | | | | | | | | | | | | |
| Edoxaban plasma concentrations | | X | X | X | | X | | | | X | X | X | X | X | X | X |
| Biomarker assessment | X | X | | X | | X | | | X | | X | X | | X | X | X |
| **Part 2** | | | | | | | | | | | | | | | | |
| Edoxaban plasma concentrations | | X | X | X | | X | | | | X | X | X | X | X | X | X |
| PT/ETP | | X | | X | X | | X | X | X | X | X | | | X | X | X |
| F1+2, D-dimer | | X | | | | | | | X | | X | | | X | X | |

ETP, endogenous thrombin potential; F1+2, prothrombin fragment 1 + 2; PT, prothrombin time.

**Data Supplement Table II:** Subject demographics

| | **Overall (N = 110)** | **Part 1 (n = 17)** | **Part 2 (n = 93)** |
|---|---|---|---|
| **Sex, n (%)** | | | |
| Male | 77 (70.0) | 13 (76.5) | 64 (68.8) |
| Female | 33 (30.0) | 4 (23.5) | 29 (31.2) |
| **Race, n (%)** | | | |
| White | 64 (58.2) | 11 (64.7) | 53 (57.0) |
| Black/African American | 41 (37.3) | | |
| American Indian/Alaskan Native | 2 (1.8) | | |
| Asian | 1 (0.9) | | |
| Native Hawaiian/ Other Pacific Islander | 1 (0.9) | | |
| Other | 1 (0.9) | | |
| **Ethnicity, n (%)** | | | |
| Hispanic/Latino | 7(6.4) | | |
| Non-Hispanic/Latino | 103 (93.6) | | |
| **Age (years)** | | | |
| Mean ± SD | 30.4 ± 7.8 | 29.0 | 30.6 |
| **Weight (kg)** | | | |
| Mean ± SD | 75.9 ± 12.1 | 76.2 | 75.8 |
| **Body Mass Index (kg/m$^2$)** | | | |
| Mean ± SD | 25.0 ± 3.1 | 24.7 | 25.02 |

SD = standard deviation

**Data Supplement Table III:** Pharmacokinetic parameters of edoxaban

| | $C_{max}$, ng/ml | $t_{max}$, h | $AUC_{(0-24)}$, ng*h/mL | $AUC_{(0-last)}$, ng*h/mL |
|---|---|---|---|---|
| **Part 1** | | | | |
| Edoxaban 60 mg (n =10) | 294 (116) | 1.70 (0.83) | 1590 (323) | 1670 (337) |
| Edoxaban 180 mg (n = 12) | 554 (182) | 1.58 (0.76) | 3140 (1120) | 3480 (1140) |
| **Part 2** | | | | |
| Edoxaban 60 mg + Placebo (n = 31) | 234 (93.1) | 1.3 (0.7) | 1330 (336) | 1420 (343) |
| Edoxaban 60 mg + 50 IU/kg 4F-PCC (n = 33) | 209 (88.3) | 1.3 (1.0) | 1230 (287) | 1340 (304) |
| Edoxaban 60 mg + Placebo (n = 28) | 249 (90.8) | 1.6 (1.1) | 1390 (376) | 1460 (383) |
| Edoxaban 60 mg + 25 IU/kg 4F-PCC (n = 28) | 251 (94.2) | 1.3 (0.9) | 1310 (343) | 1400 (347) |
| Edoxaban 60 mg + Placebo (n = 30) | 318 (111) | 1.2 (0.8) | 1610 (370) | 1700 (385) |
| Edoxaban 60 mg + 10 IU/kg 4F-PCC (n = 30) | 315 (100) | 1.4 (1.1) | 1630 (363) | 1720 (390) |

All values presented as arithmetic mean (standard deviation).

AUC, area under the plasma concentration vs time curve; $AUC_{(0-24)}$, AUC from time 0 to 24 hours; $AUC_{(0-last)}$, AUC from time 0 to the last quantifiable concentration; $C_{max}$, maximum observed plasma drug concentration; 4F-PCC, 4-factor prothrombin complex concentrate; $t_{max}$, time to reach maximum plasma concentration.

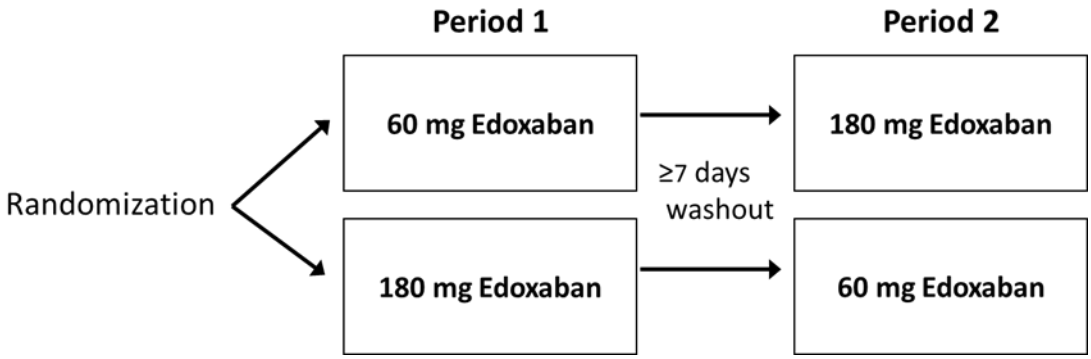
**Supplemental Table IV:** Pharmacodynamic parameters of endogenous thrombin potential in Part 2.

| | Cohort 1 | | Cohort 2 | | Cohort 3 | |
|---|---|---|---|---|---|---|
| | **Edoxaban 60 mg + Placebo n = 31** | **Edoxaban 60 mg + 50 IU/kg 4F-PCC n = 33** | **Edoxaban 60 mg + Placebo n = 28** | **Edoxaban 60 mg + 25 IU/kg 4F-PCC n = 28** | **Edoxaban 60 mg + Placebo n = 30** | **Edoxaban 60 mg + 10 IU/kg 4F-PCC n = 30** |
| **$A_{min}$ (nM*min)** | 1395 (818) | 1750 (1000) | 1230 (729) | 1500 (969) | 1280 (700) | 1560 (76.3) |
| **$A_{max}$ (nM*min)** | 2930 (720) | 5900 (720) | 3910 (576) | 5020 (609) | 3990 (621) | 4630 (747) |
| **$T_{max}$ (h)** | 19.7 (5.95) | 16.1 (6.96) | 17.1 (5.78) | 17.6 (4.88) | 19.0 (5.09) | 17.70 (4.89) |
| **$\Delta A_{min}$ (%)** | -64.4 (19.0) | -56.4 (22.4) | -68.5 (16.0) | -61.3 (23.4) | -66.8 (17.7) | -61.6 (16.7) |
| **$\%\Delta A_{min}$ (%)** | 3.15 (11.3) | 50.3 (10.2) | 3.48 (7.32) | 35.8 (15.8) | 5.21 (9.14) | 16.8 (11.2) |

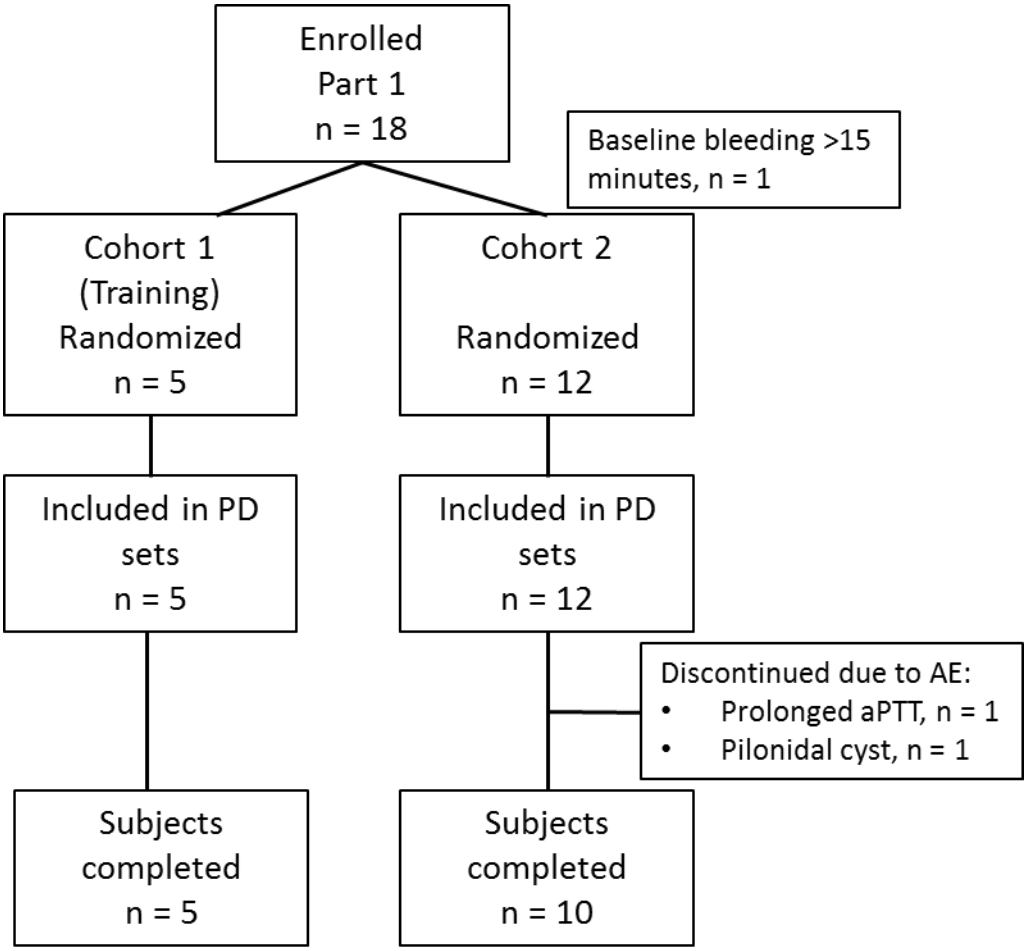
All values presented as arithmetic mean (standard deviation).

$\%\Delta A_{min}$, percent change in the minimum observed activity value; 4F-PCC, 4-factor prothrombin complex concentrate; $A_{min}$, the minimum observed activity value; $A_{max}$, the maximum observed activity value; $\Delta A_{min}$, change in the minimum observed activity value; $T_{max}$, the time of the maximum observed activity.

**Data Supplement Figure I:** Study design for part 1

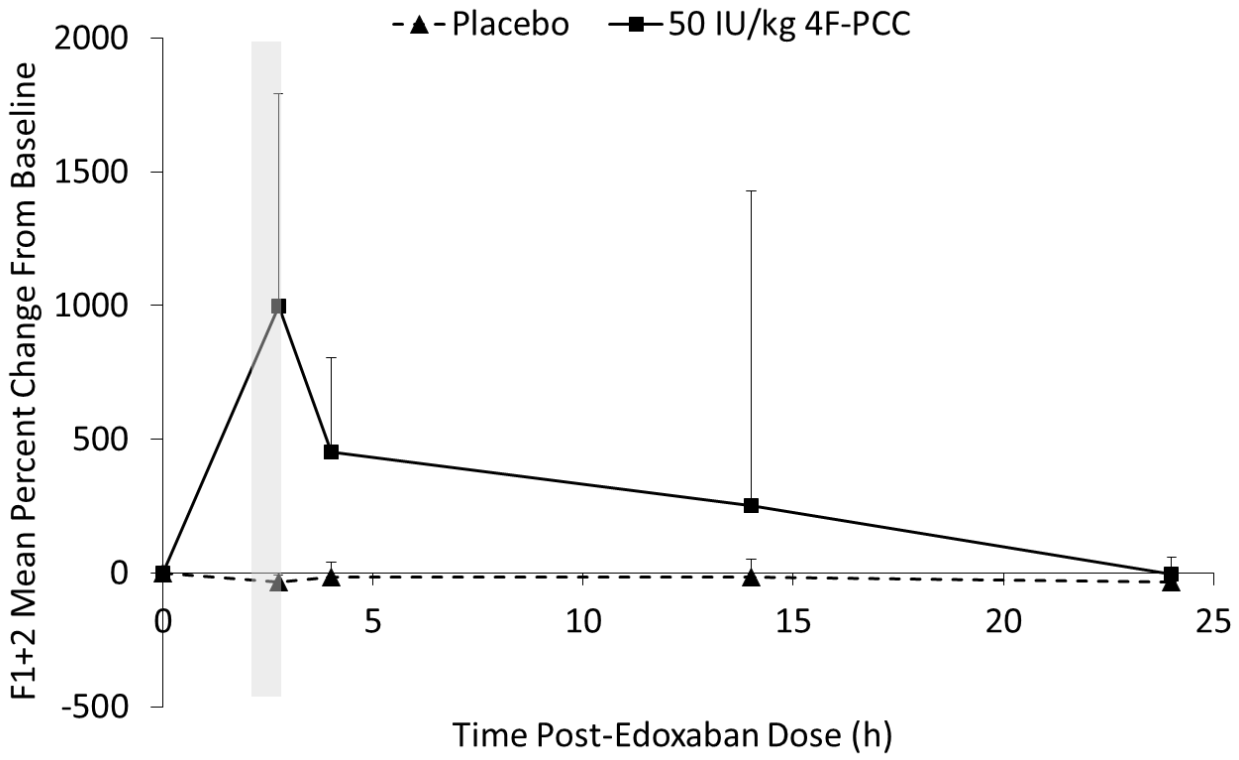


**Data Supplement Figure II:** CONSORT diagram, part 1. AE, adverse event; aPTT, activated partial thromboplastin time; PD, pharmacodynamics; PK, pharmacokinetic.

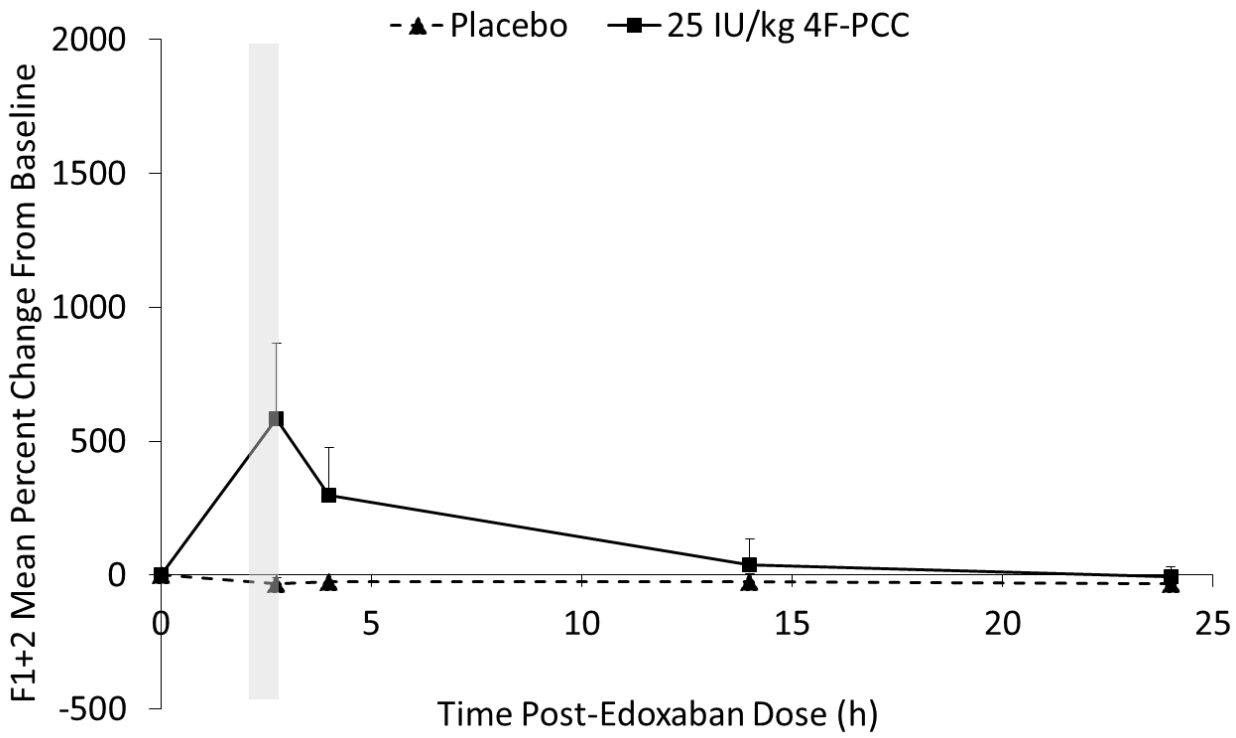


**Data Supplement Figure III:** Time course of F1+2 mean percent change from baseline for treatment with **(A)** 50 IU/kg, **(B)** 25 IU/kg, or **(C)** 10 IU/kg 4F-PCC or placebo. Error bars represent standard deviation. 4F-PCC, 4-factor prothrombin complex concentrate; F1+2, prothrombin fragment 1 + 2.

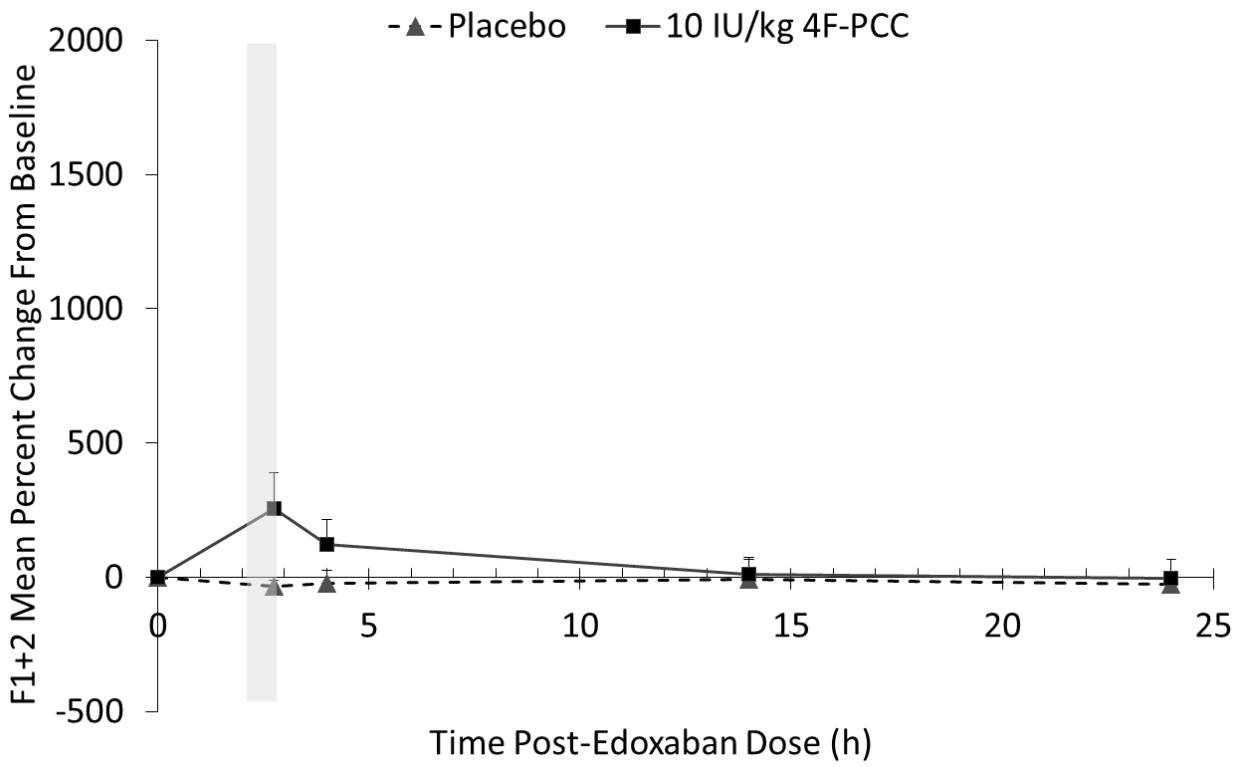
**A**



**B**

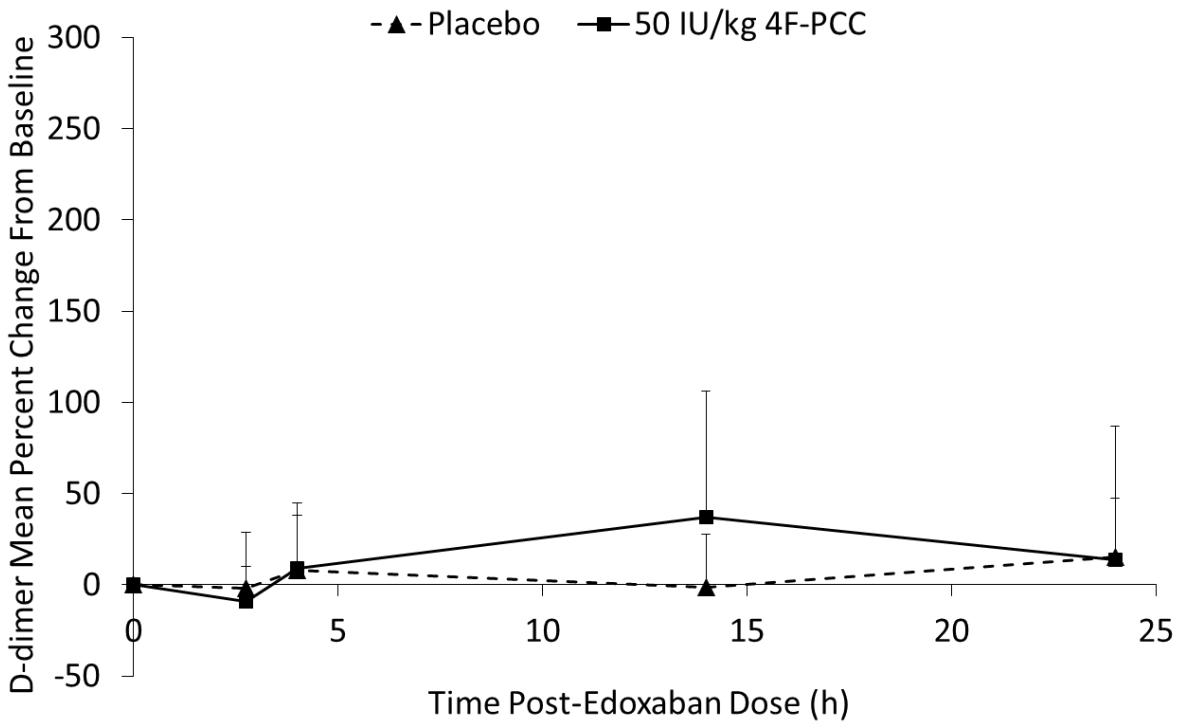

Placebo
25 IU/kg 4F-PCC
F1+2 Mean Percent Change From Baseline
2000
1500
1000
500
0
-500
0
5
10
15
20
25
Time Post-Edoxaban Dose (h)

**C**

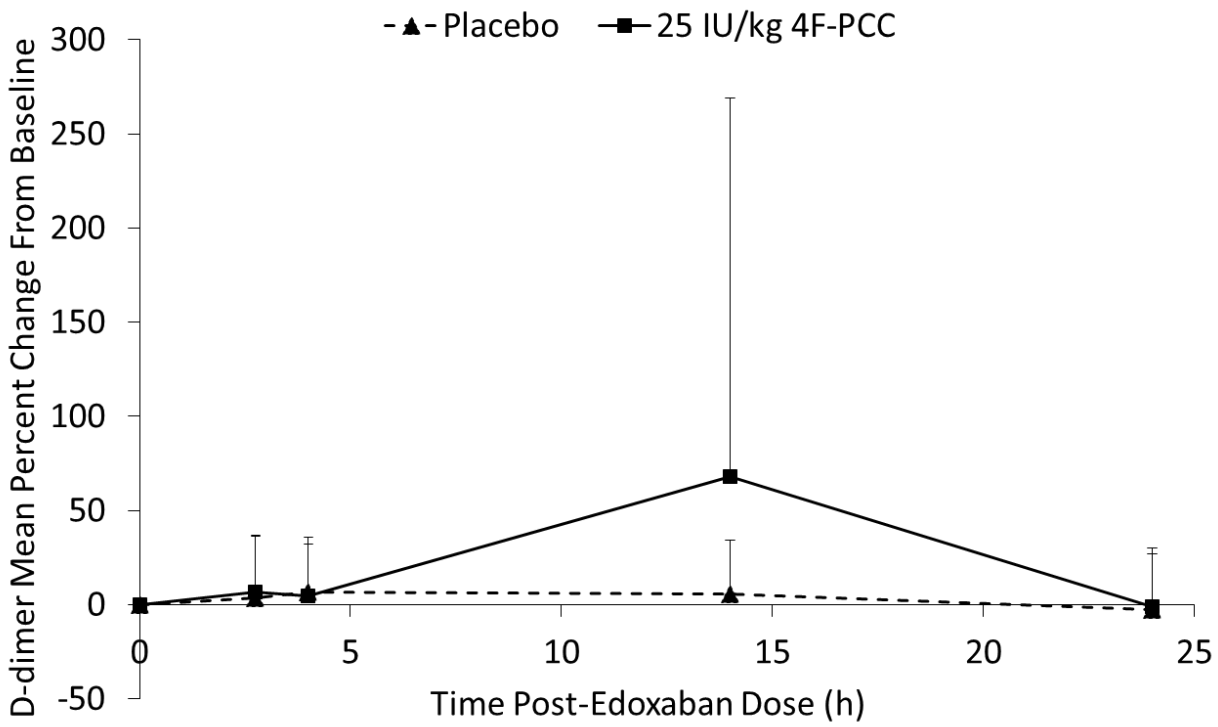

Placebo
10 IU/kg 4F-PCC
F1+2 Mean Percent Change From Baseline
2000
1500
1000
500
0
-500
0
5
10
15
20
25
Time Post-Edoxaban Dose (h)

**Data Supplement Figure IV:** Time course of D-dimer mean percent change from baseline for treatment with **(A)** 50 IU/kg, **(B)** 25 IU/kg, or **(C)** 10 IU/kg 4F-PCC or placebo. Error bars represent standard deviation. 4F-PCC, 4-factor prothrombin complex concentrate.

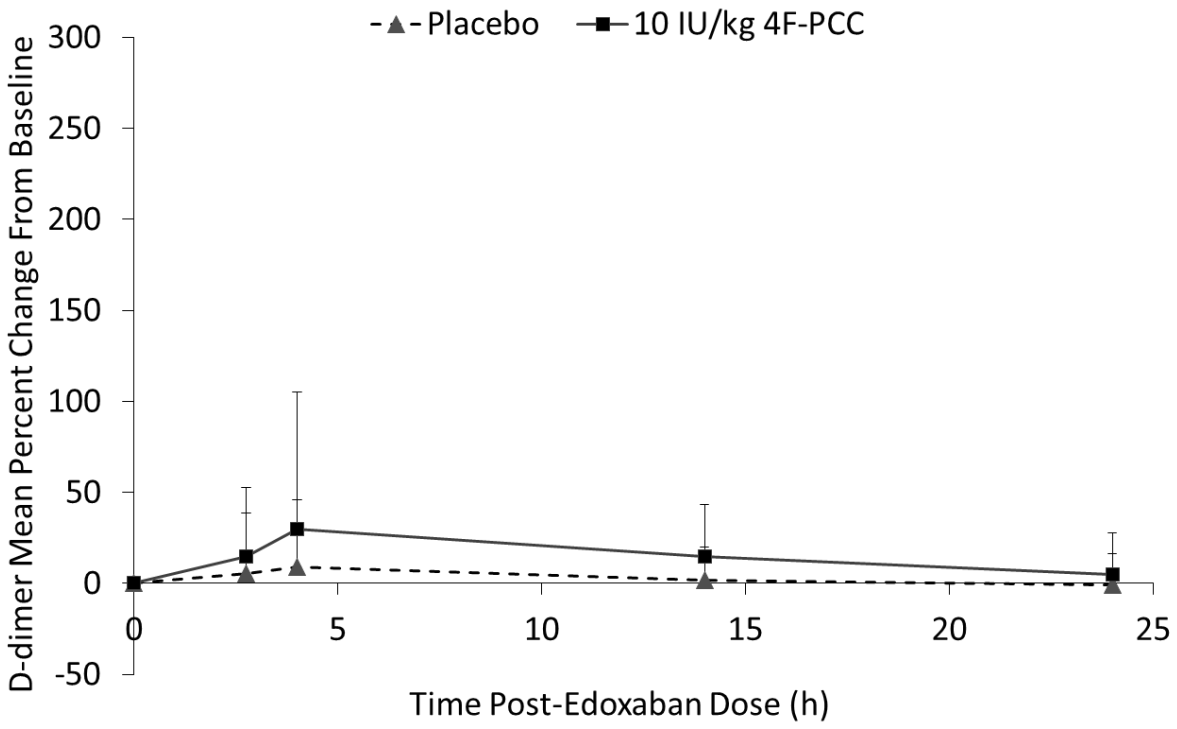
**A**



**B**


Placebo
25 IU/kg 4F-PCC
D-dimer Mean Percent Change From Baseline
300
250
200
150
100
50
0
-50
0
5
10
15
20
25
Time Post-Edoxaban Dose (h)

**C**


Placebo
10 IU/kg 4F-PCC
D-dimer Mean Percent Change From Baseline
300
250
200
150
100
50
0
-50
0
5
10
15
20
25
Time Post-Edoxaban Dose (h)