

## ORIGINAL ARTICLE

# Andexanet versus prothrombin complex concentrates: Differences in reversal of factor Xa inhibitors in in vitro thrombin generation

Genmin Lu PhD | Joyce Lin BS | Khanh Bui BS | John T. Curnutte MD, PhD |  
Pamela B. Conley PhD

Research Biology, Portola Pharmaceuticals, Inc., South San Francisco, CA, USA; a wholly owned subsidiary of Alexion Pharmaceuticals, Inc.

**Correspondence**

Genmin Lu, Portola Pharmaceuticals, Inc., 270 E Grand Ave, South San Francisco, CA 94080.

Email: [glu@portola.com](mailto:glu@portola.com)

**Funding information**

This study was funded by Portola Pharmaceuticals, Inc.; a wholly owned subsidiary of Alexion Pharmaceuticals, Inc.

**Handling Editor:** Dr Pantep Angchaisuksiri.

**Abstract**

**Background:** Andexanet alfa (andexanet) is a modified human factor Xa (FXa) approved for anticoagulation reversal in patients with life-threatening bleeding treated with rivaroxaban or apixaban. Four-factor prothrombin complex concentrates (4F-PCCs) are approved for reversal of vitamin K antagonist-induced anticoagulation but not FXa inhibitors. The mechanism and effectiveness of 4F-PCCs for FXa inhibitor reversal are unclear.

**Objective:** To investigate the mechanism and impact of 4F-PCCs on reversal of rivaroxaban and apixaban in vitro compared to andexanet.

**Methods:** The effect of 4F-PCCs (or individual factors) on tissue factor-initiated thrombin generation (TF-TG) was evaluated in human plasma, with or without rivaroxaban or apixaban, and compared with andexanet under the same conditions.

**Results:** In the TF-TG assay, 4F-PCC completely reversed warfarin anticoagulation. Andexanet normalized TF-TG over a wide range of apixaban and rivaroxaban concentrations tested (19-2000 ng/mL). However, 4F-PCC (or individual factors) was unable to normalize endogenous thrombin potential (ETP) or peak thrombin (Peak) in the presence of apixaban or rivaroxaban (75-500 ng/mL). TF-TG was only normalized by 4F-PCC at inhibitor concentrations <75 ng/mL (ETP) or <37.5 ng/mL (Peak). These data can be explained by the estimated thresholds of FXa activity required to support normal TF-TG based on the inhibitor:FXa ratios and levels of uninhibited FXa. The data are consistent with healthy volunteer studies where TF-TG is not normalized until inhibitor levels are substantially decreased.

**Conclusions:** Both the theoretical calculations and experimental data demonstrated that 4F-PCCs are only able to normalize TG over a low and narrow range of FXa inhibitor concentrations (<75 ng/mL).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Research and Practice in Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis.

**KEY WORDS**

andexanet, blood coagulation factors, factor Xa inhibitors, prothrombin complex concentrates, reversal, thrombin generation

**Essentials**

- Andexanet alfa is the only approved specific reversal agent for factor Xa (FXa) inhibitors.
- Prothrombin complex concentrates (PCCs) are not approved for FXa inhibitor reversal.
- Four-factor PCCs did not normalize thrombin generation in vitro at inhibitor levels  $\geq 75$  ng/mL.
- Andexanet normalized thrombin generation at inhibitor concentrations of 19-2000 ng/mL.

**1 | INTRODUCTION**

Direct oral factor Xa (FXa) inhibitors, such as apixaban and rivaroxaban, are used for the prevention and treatment of various thromboembolic disorders.<sup>1,2</sup> These agents exert their anticoagulant activity by specifically targeting FXa with subnanomolar affinities.<sup>3,4</sup> Although reported rates of major bleeding complications are low (~1%–3%/year),<sup>5,6</sup> these complications can have substantial morbidity and mortality. Therefore, reversal of the effects of FXa inhibitors may be necessary in the event of spontaneous or traumatic bleeding, or if a patient requires urgent surgery.<sup>7</sup>

Andexanet alfa (andexanet) is a modified human FXa approved in the United States and European Union as a specific reversal agent for patients treated with apixaban or rivaroxaban, when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding.<sup>8,9</sup> Andexanet rapidly binds and sequesters FXa inhibitors, reduces the anticoagulant activity, and restores endogenous FXa activity.<sup>10</sup> Andexanet is currently the only approved agent available to reverse the anticoagulant effects of apixaban or rivaroxaban. Factor replacement therapies, such as four-factor prothrombin complex concentrates (4F-PCCs), are approved for reversal of anticoagulation with vitamin K antagonists (VKA; eg, warfarin) and serve to replace factors VII (FVII), IX (FIX), X (FX), and II (FII) rendered functionally deficient by the anticoagulant.<sup>11</sup> Although 4F-PCCs have been studied for FXa inhibitor reversal in nonclinical in vitro/ex vivo assays,<sup>12-14</sup> animal models,<sup>15</sup> healthy volunteers,<sup>16-23</sup> and observational clinical studies in patients taking FXa inhibitors,<sup>24-27</sup> the effectiveness and clinical benefit of 4F-PCCs on hemostatic efficacy and reversal of key biomarkers are mixed,<sup>7,28-31</sup> and their potential mechanism of action has not been critically elucidated. There have been no controlled studies evaluating the effectiveness of 4F-PCCs for reversal of FXa inhibitor-mediated bleeding (or anticoagulation), and they are not approved for this indication.

Several studies in healthy subjects anticoagulated with rivaroxaban or apixaban have assessed the effect of 4F-PCCs on anti-FXa activity and/or tissue factor-initiated thrombin generation (TF-TG) as pharmacodynamic markers of reversal activity.<sup>16-22</sup> None of the studies that measured anti-FXa activity showed any effect of 4F-PCCs versus saline in reversing anti-FXa levels.<sup>17-22</sup> Of the studies that used TF-TG as a biomarker (Table S1), most (5/7) showed

only a small correction at 15-30 minutes following PCC administration, with normalization of endogenous thrombin potential (ETP) to baseline levels occurring much later at 4-12 hours,<sup>17-21</sup> which coincided with substantial clearance and reduction of FXa inhibitor levels. Two of the 7 studies showed a more rapid normalization of ETP to baseline (15-30 minutes after PCC administration),<sup>16,22</sup> which may be explained by differences in TF concentration and plasma dilution used in the TF-TG assay,<sup>22</sup> or the pretreatment level of inhibition by the inhibitor in the 4F-PCC arm versus placebo.<sup>16</sup> In contrast to ETP, PCCs did not normalize peak thrombin (Peak) to baseline until >20 hours in any of the studies where data were provided.<sup>17-21</sup> These data raise questions regarding the limitations of 4F-PCCs on normalizing thrombin generation in that it is dependent on substantial clearance of the anticoagulants, a process that can take several hours and could delay therapeutic benefit to bleeding patients.

Since published data demonstrated no effect of 4F-PCCs on anti-FXa levels in subjects anticoagulated with FXa inhibitors,<sup>17,21,28</sup> we evaluated the hypothesis that PCCs could have an alternative mechanism, such as providing additional enzyme (FX that can be converted to FXa) and substrate (FII) sufficient to overcome FXa inhibition and thereby normalize thrombin generation.<sup>30</sup> A central question is to what degree FXa inhibition needs to be relaxed (via clearance of the anticoagulant) for PCCs to normalize thrombin generation. The aims of the current study were to (i) establish the threshold of FXa inhibitor levels where PCCs may show effects on thrombin generation; and (ii) compare directly the effectiveness of andexanet versus 4F-PCCs, assessed by TF-TG in vitro.

**2 | METHODS****2.1 | Calibrated automated thrombography**

TF-TG was measured using a Calibrated Automated Thrombogram (Diagnostics Stago, Inc, Parsippany, NJ, USA) per the manufacturer's recommendations using the PPP-reagent (5 pM TF/4  $\mu$ M phospholipids). TF-TG profiles were analyzed using Thrombinoscope software (Diagnostics Stago) with five parameters: ETP, Peak, lag time, time-to-peak, and velocity index. The results were reported as mean  $\pm$  standard deviation.

## 2.2 | Contribution of individual coagulation factors and 4F-PCC to TF-TG in the absence of anticoagulant

Purified plasma proteins (FVII, FIX, FX, FII; Haematologic Technologies, Inc, Essex Junction, VT, USA) were added to pooled human platelet-poor plasma (PPP; CRYOCheck, Precision BioLogic, Dartmouth, NS, Canada) between 0-1.0 IU/mL (with 1 IU/mL equivalent to the corresponding factor level in normal plasma).

A commercially available 4F-PCC (Kcentra/Beriplex, CSL Behring LLC, Kankakee, IL, USA) was added to PPP (0-1.0 IU/mL). Dosing for Kcentra was obtained from the package insert.<sup>32</sup> The recommended Kcentra dose of 50 IU/kg is equivalent to ~1.0 IU/mL 4F-PCC plasma level. To eliminate the potential effect of heparin in Kcentra, 4F-PCC was used with or without pretreatment with the heparinase cup (Haemonetics Corporation, Braintree, MA, USA) at room temperature for 30 minutes before adding to plasma.

To estimate the FXa levels required for normal thrombin generation, pooled human PPP was mixed with FX-depleted plasma (Haematologic Technologies, Inc) by varying PPP% in the mixture from 0% to 100%. TF-TG at different FX levels was compared to normal PPP (100% PPP).

## 2.3 | Reversal of FXa inhibitor-induced inhibition of thrombin generation

TF-TG was measured in plasma with different rivaroxaban or apixaban concentrations. Rivaroxaban was purchased from a commercial source and prepared as 1 mg/mL dimethyl sulfoxide (DMSO) stock. Apixaban (Bristol-Myers Squibb, Princeton, NJ, USA) was provided by the manufacturer and prepared as 1 mg/mL DMSO stock.

For evaluation of the potential effect of coagulation factors or 4F-PCC, pooled plasma was supplemented with different rivaroxaban or apixaban concentrations (0-500 ng/mL), plus individual coagulation factors (FVII, FIX, FX, FII) or Kcentra (0-1.0 IU/mL). TF-TG was measured as described in the Methods above.

For evaluation of dose-dependent reversal by andexanet (Portola Pharmaceuticals, Inc), pooled plasma was supplemented with rivaroxaban or apixaban (0-2000 ng/mL), plus andexanet (0-4.0  $\mu$ M), a range corresponding to approximately the maximum plasma concentrations ( $C_{max}$ ) with andexanet low dose (400-mg bolus plus 4 mg/min  $\times$  2-hour infusion,  $C_{max}$  ~2.0  $\mu$ M) and high dose (800-mg bolus plus 8 mg/min  $\times$  2-hour infusion,  $C_{max}$  ~4.0  $\mu$ M). Andexanet was provided as lyophilized powder and reconstituted with H<sub>2</sub>O as 10 mg/mL stock.

## 2.4 | Reversal of warfarin anticoagulation by 4F-PCC

Reversal of warfarin anticoagulation by 4F-PCC was performed under similar conditions using individual plasma from warfarin-treated patients, with international normalized ratio (INR) = 1.5-6.9

(George King Bio-Medical, Inc, Overland Park, KS, USA). Individual patient plasma was supplemented with 4F-PCC (0-1.0 IU/mL) followed by assessment with TF-TG as described above.

## 3 | RESULTS

### 3.1 | Contribution of coagulation factors to thrombin generation in the absence or presence of an anticoagulant

To assess the contribution of major coagulation factors in 4F-PCCs (FVII, FIX, FX, FII) to TF-TG, we first evaluated PPP supplemented with each factor with or without an anticoagulant. In the absence of anticoagulant, addition of FII alone (1.0 IU/mL) increased both ETP (~2-fold) and Peak (~50%) while the others had moderate (FIX) to minimal (FVII, FX) effects (Figure 1A). These observations are consistent with the relative plasma concentration and affinity of each coagulation factor as the substrate for the respective enzyme complex and demonstrate the predominant role of prothrombinase (FXa/FVa/phospholipid/Ca<sup>2+</sup>) activity in thrombin generation. The addition of 4F-PCC (1.0 IU/mL, equivalent to a 50 IU/kg therapeutic dose) to PPP caused increases in ETP (~2.4-fold) and Peak (~40%) similar to that seen with the addition of FII alone.

We next assessed the effect of individual coagulation factors in 4F-PCCs on TF-TG in the presence of rivaroxaban. Addition of each factor (FVII, FIX, FX, FII) had minimal impact on reversal of rivaroxaban (250 ng/mL) inhibition assessed by the TF-TG profiles compared to PPP control (Figure 1B). At lower rivaroxaban concentrations, addition of prothrombin had some effect on ETP (but not Peak; Figure S1), suggesting that supplement of individual factors (up to levels similar to the supplement of 4F-PCCs), including prothrombin, is unable to overcome FXa inhibition by rivaroxaban. Similar results and conclusions were obtained with apixaban (Figure S2).

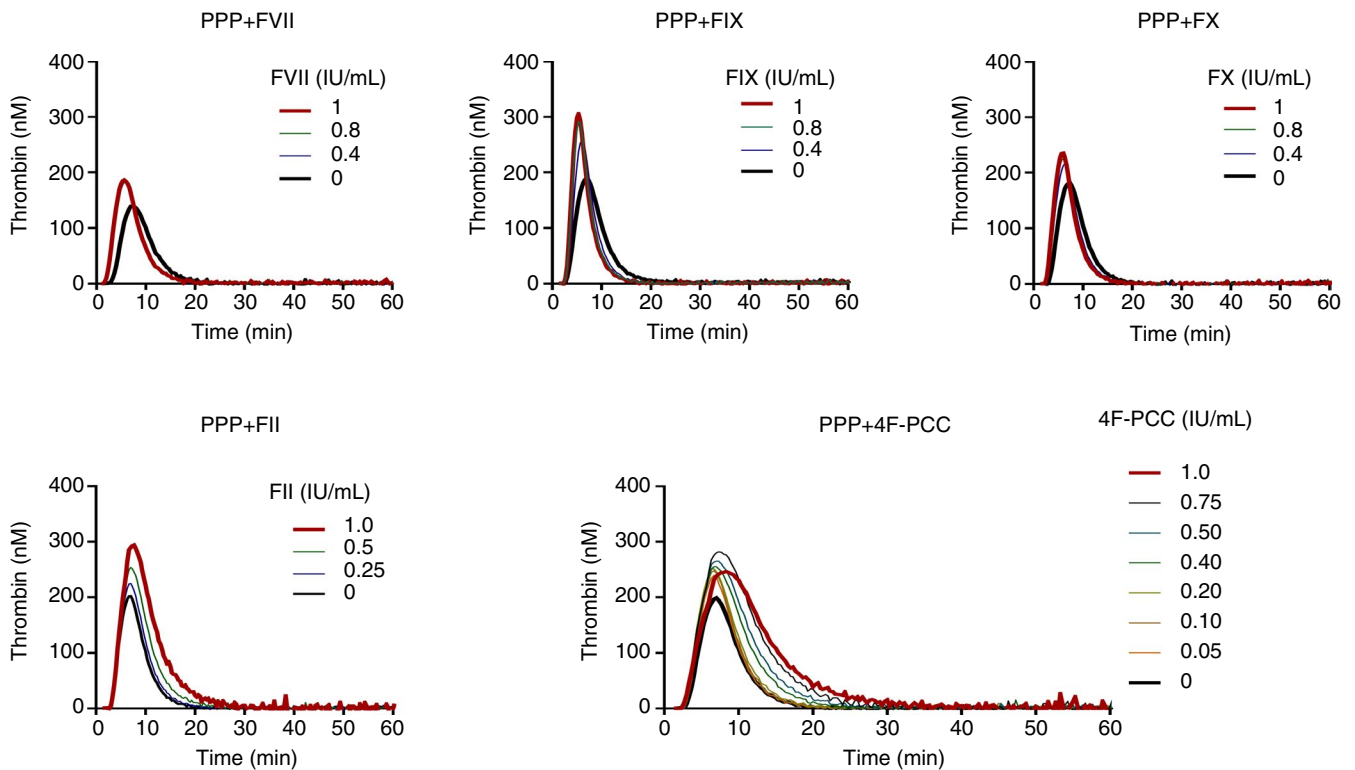
### 3.2 | Effect of 4F-PCC on thrombin generation in warfarin-treated patient plasma

Because 4F-PCCs are approved for VKA reversal, we assessed the effect of 4F-PCC on TF-TG in warfarin-treated patient plasma using the same assay. As expected, 4F-PCC dose dependently and completely normalized TF-TG profiles in warfarin-treated patient's plasma with an INR of 4.8 (Figure S3A) or TF-TG parameters (ETP; Peak) over a wide INR range (Figure S3B), consistent with the recommended dosing of PCCs based on INR.

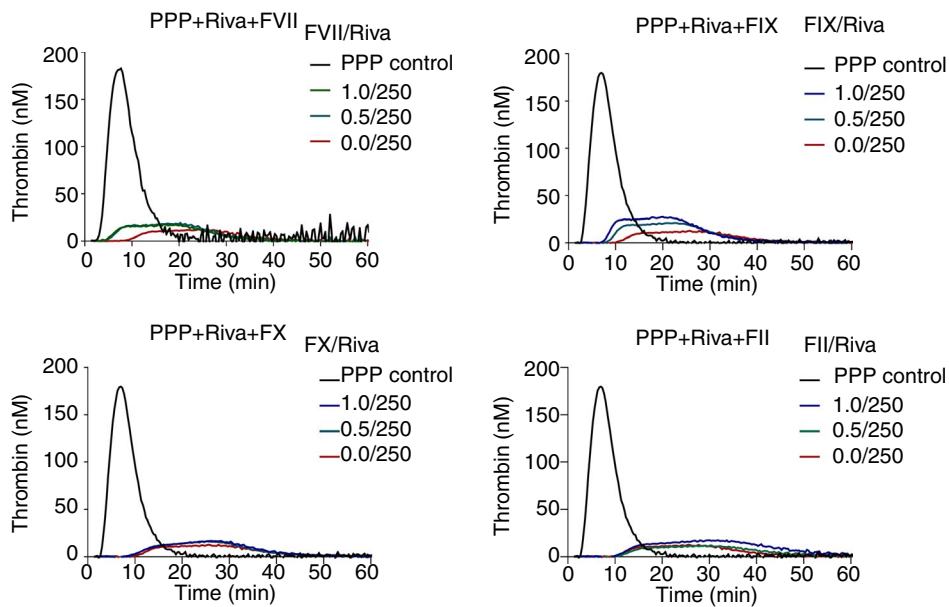
### 3.3 | Effect of 4F-PCC on thrombin generation in the presence of rivaroxaban or apixaban

The effect of 4F-PCC on TF-TG in the presence of rivaroxaban or apixaban was assessed by detailed titration of the anticoagulant

**(a) Thrombin generation profiles in the absence of a FXa inhibitor compared to 4F-PCC**

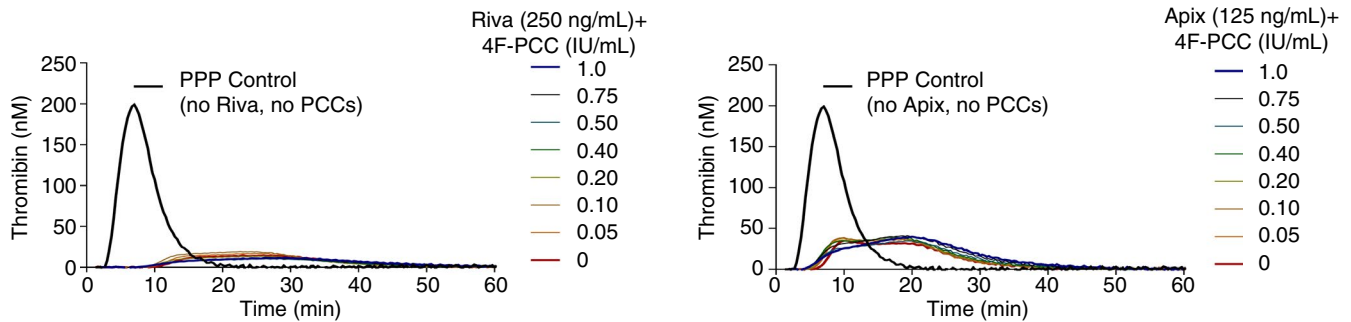


**(b) Thrombin generation profiles in the presence of rivaroxaban (250 ng/mL)**

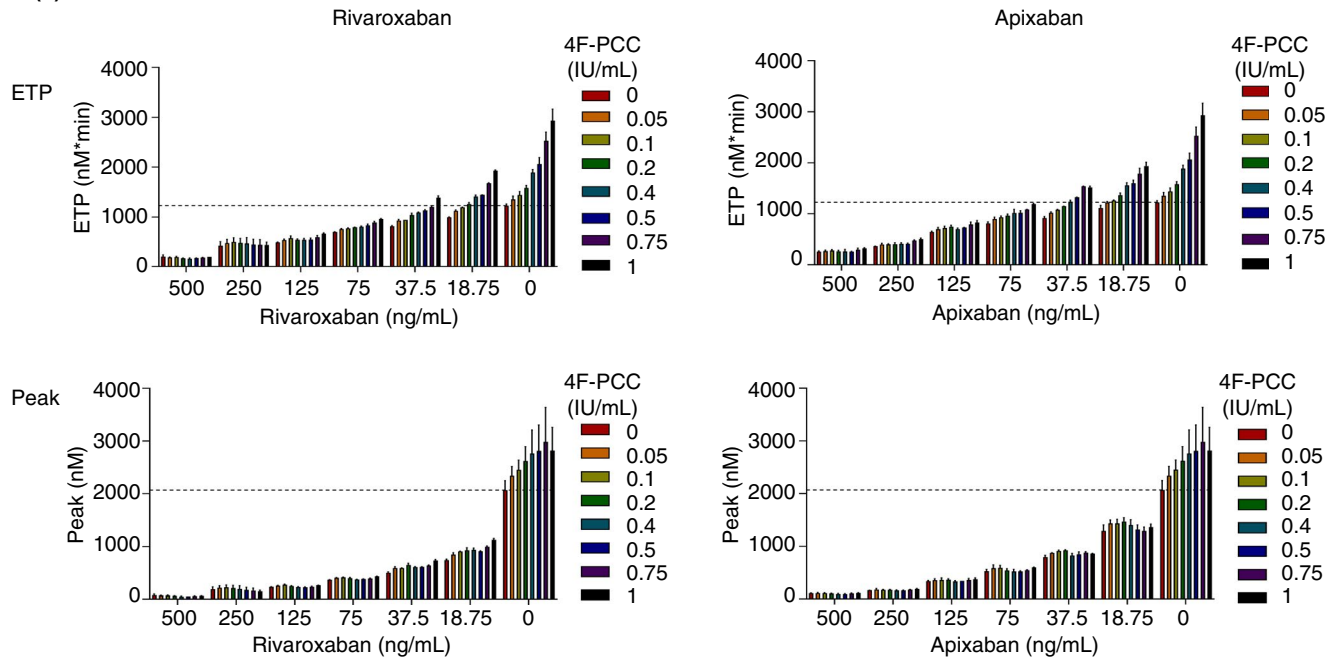


**FIGURE 1** Contribution of individual coagulation factors (FVII, FIX, FX, FII) in 4F-PCCs to TF-TG in normal plasma with or without rivaroxaban. A, Thrombin generation profiles in normal plasma supplemented with different coagulation factors compared to 4F-PCCs in the absence of a FXa inhibitor. Purified plasma coagulation factor was added to PPP (0-1.0 IU/mL); 1.0 IU/mL is equivalent to the normal plasma level of each factor in healthy subjects (the addition of 1.0 IU/mL of an individual factor therefore doubles the plasma concentration of that factor). Addition of FVII or FX had minimal effect whereas FIX (1.0 IU/mL) increased Peak by approximately 60% as would be expected since FIXa can activate additional FX to FXa and accelerate thrombin generation. Addition of FII (1.0 IU/mL) alone increased the ETP similar to that seen with the addition of 4F-PCC (1.0 IU/mL). Shown are representative thrombin generation profiles with each coagulation factor or 4F-PCC. B, Contribution of individual coagulation factors (FVII, FIX, FX, FII) to TF-TG in plasma with rivaroxaban. Representative thrombin generation profiles in the presence of rivaroxaban (250 ng/mL) and different levels of coagulation factor (0-1.0 IU/mL). 4F-PCC, four-factor prothrombin complex concentrate; ETP, endogenous thrombin potential; FII, factor II; FVII, factor VII; FIX, factor IX; FX, factor X; FXa, factor Xa; Peak, peak thrombin; PPP, platelet-poor plasma; Riva, rivaroxaban; TF-TG, tissue factor-initiated thrombin generation

## (a) Thrombin generation profiles



## (b) ETP and Peak



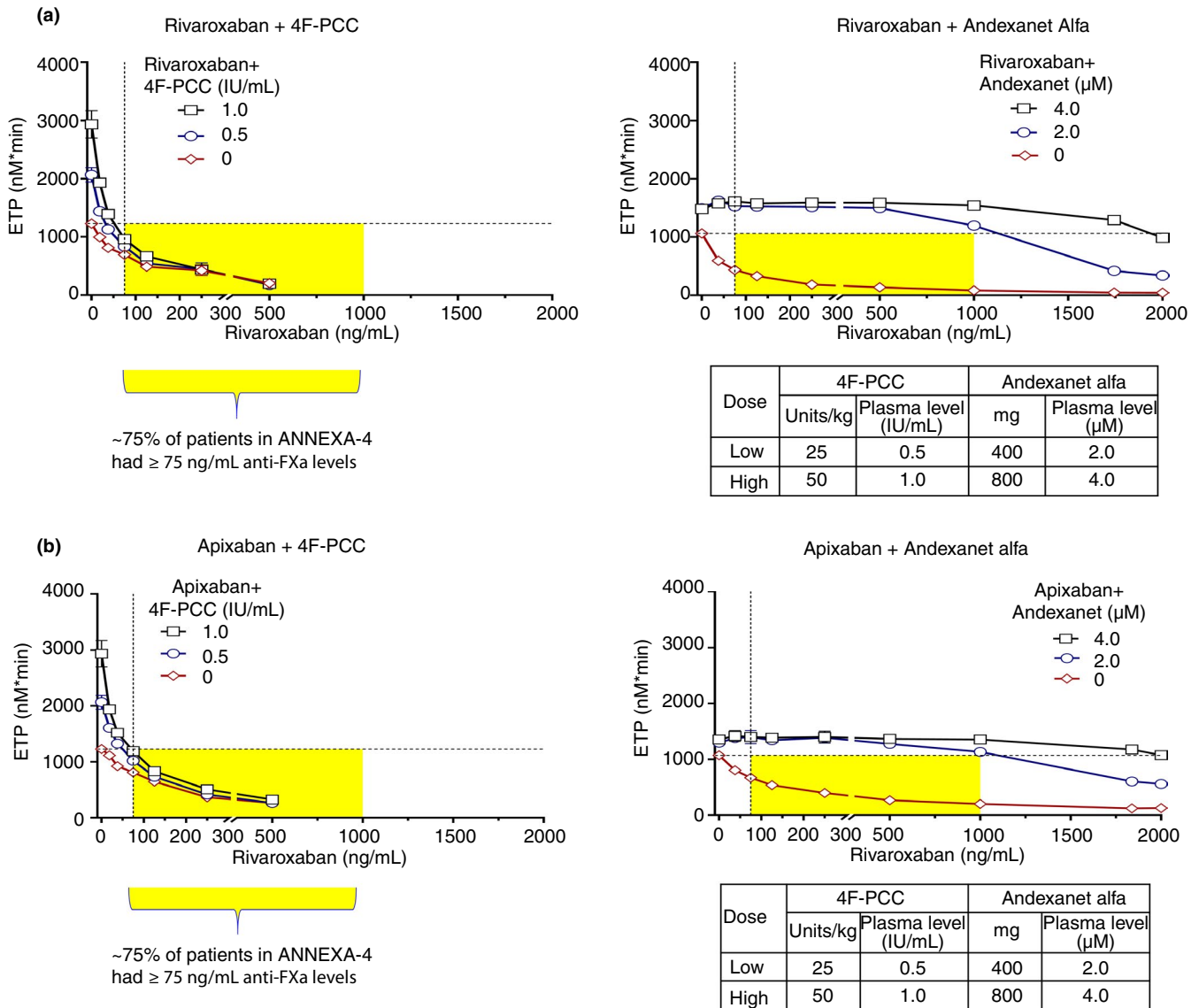
**FIGURE 2** Effect of 4F-PCC on TF-TG in the presence of rivaroxaban or apixaban. A, TF-TG profiles in PCC-spiked plasma with a FXa inhibitor at  $\sim C_{max}$ . Representative thrombin generation profiles are shown in the presence of rivaroxaban (250 ng/mL) or apixaban (125 ng/mL) with 4F-PCC (0–1.0 IU/mL). B, Effect of 4F-PCC on FXa inhibitor-induced anticoagulation as assessed by ETP and Peak. Data are shown as mean  $\pm$  SD (N = 2). The dashed lines represent normal ETP and Peak in PPP. 4F-PCC, four-factor prothrombin complex concentrate; Apix, apixaban;  $C_{max}$ , maximum plasma concentration; ETP, endogenous thrombin potential; FXa, factor Xa; Peak, peak thrombin; PPP, platelet-poor plasma; Riva, rivaroxaban; SD, standard deviation; TF-TG, tissue factor-initiated thrombin generation

(0–500 ng/mL) and 4F-PCC (0–1.0 IU/mL). As demonstrated in Figure 2A with the time-course profiles generated in the TF-TG assay with rivaroxaban (250 ng/mL,  $\sim C_{max}$  for 20-mg once-daily dose) or apixaban (125 ng/mL,  $\sim C_{max}$  for 5-mg twice-daily dose), 4F-PCC ( $\leq 1.0$  IU/mL) had no effect on TF-TG profiles. Assessment of the effect of 4F-PCC on a range of rivaroxaban and apixaban concentrations (0–500 ng/mL; Figure 2B) showed that 4F-PCC did not restore ETP to normal baseline levels unless inhibitor concentrations were  $<75$  ng/mL (apixaban) or  $<37.5$  ng/mL (rivaroxaban). The threshold concentration was even lower for restoration of Peak to normal levels; the apixaban or rivaroxaban concentration was  $<18.75$  ng/mL before 4F-PCC restored Peak, a level below the estimated 30 ng/mL no-effect level for rivaroxaban and apixaban.<sup>33</sup> Similar conclusions can be drawn based on other CAT parameters (Figure S4). Note that in the absence of rivaroxaban or apixaban, 4F-PCC (0–1.0 IU/mL) caused a dose-dependent increase in both

ETP ( $\sim 2.4$ -fold) and Peak ( $\sim 40\%$ ), reflecting the procoagulant nature of the added factors. These results were similar if 4F-PCC was pre-treated with heparinase before adding to plasma to eliminate potential interference of heparin in 4F-PCC (Figure S5).

### 3.4 | Effect of andexanet on thrombin generation in the presence of rivaroxaban or apixaban

Using the same assay under similar conditions, we further assessed the effects of andexanet on TF-TG in the presence of rivaroxaban or apixaban compared with 4F-PCC. As illustrated in Figure 3A, 4F-PCC at approved low and high doses was ineffective in reversing rivaroxaban- and apixaban-induced inhibition of TF-TG when inhibitor concentrations were  $\geq 75$  ng/mL. In contrast, andexanet at



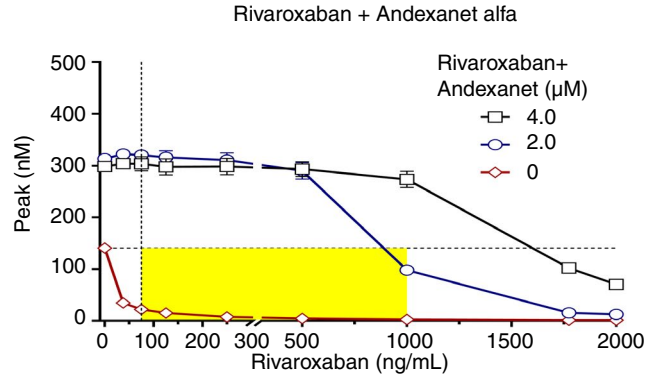
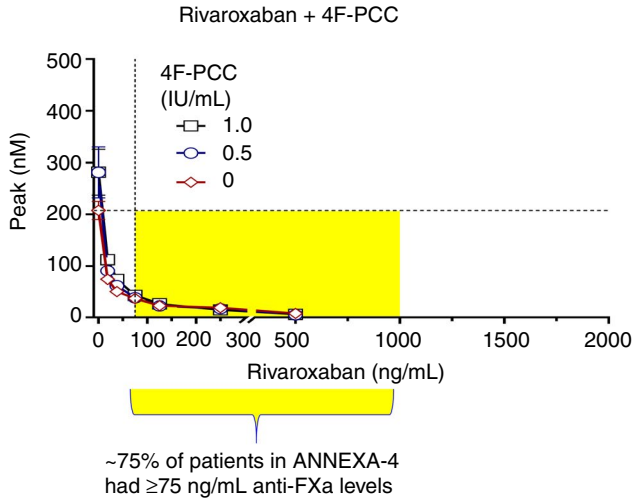
**FIGURE 3** Effect of andexanet on TF-TG (ETP) in the presence of rivaroxaban or apixaban compared with 4F-PCCs. A, Rivaroxaban; and B, apixaban. Data are shown as mean ± SD (N = 2). In each panel, the horizontal dashed line represents the ETP level in normal plasma. The vertical dashed line indicates the 75-ng/mL inhibitor level. The shaded area represents the range of inhibitor levels (75-1000 ng/mL) found in the ANNEXA-4 efficacy population (≥75 ng/mL). The inset table shows the plasma level of 4F-PCC or andexanet at their approved low and high doses, respectively. 4F-PCC, four-factor prothrombin complex concentrate; ETP, endogenous thrombin potential; FXa, factor Xa; SD, standard deviation; TF-TG, tissue factor-initiated thrombingeneration. <sup>a</sup>At the approved andexanet low and high doses, the andexanet C<sub>max</sub> is ~2.0 μM at low dose (400-mg bolus plus 4 mg/min 2-hour infusion) and ~4.0 μM at high dose (800-mg bolus plus 8 mg/min × 2-hour infusion). The 2 and 4 μM levels are maintained by the infusion.

therapeutic plasma concentrations achieved in humans at the approved low and high doses (~2.0 and 4.0 μM, respectively) dose dependently and completely reversed rivaroxaban- and apixaban-induced inhibition of TF-TG up to 1000 ng/mL (with 2.0 μM andexanet) and 2000 ng/mL inhibitors (with 4.0 μM andexanet; Figure 3B). In the absence of FXa inhibitor, ETP level was 1.4× over the normal plasma with andexanet at either 2.0 μM or 4.0 μM. Similar conclusions can be drawn by comparing Peak, although the effect of 4F-PCC on Peak was much less compared to ETP. Peak was not normalized at the lowest inhibitor levels (18.75 ng/mL) tested (Figure 4A-B).

#### 4 | DISCUSSION

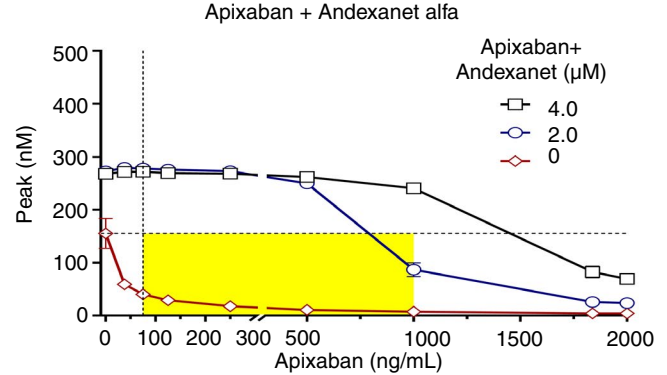
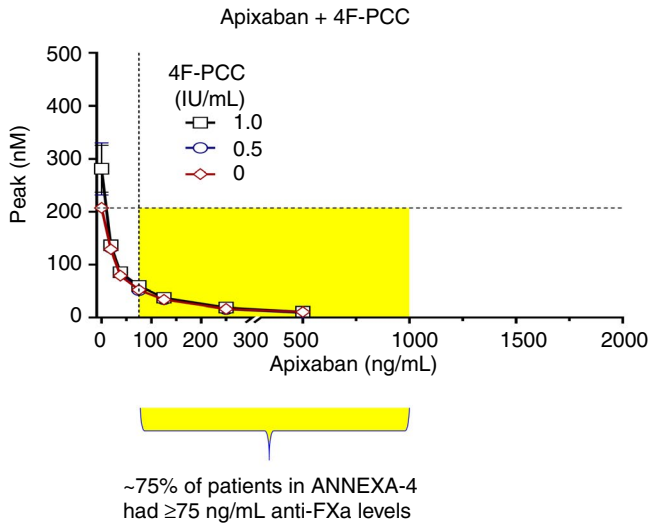
Since thrombin generation is the last step in the coagulation cascade leading to fibrin/clot formation, the TF-TG assay has been used as a pharmacodynamic marker for restoration of hemostasis for hemophilia treatment and anticoagulation reversal, although plasma thrombin generation assays do not include cellular components of whole blood,<sup>34,35</sup> and a correlation between restoration of thrombin generation and hemostasis has not been established in bleeding patients taking FXa inhibitors. In the present study, we used pooled human plasma and the same version of TF-TG assay as

**(a) Rivaroxaban**



Dose	4F-PCC		Andexanet alfa	
	Units/kg	Plasma level (IU/mL)	mg (bolus)	Plasma level (μM) <sup>a</sup>
Low	25	0.5	400	2.0
High	50	1.0	800	4.0

**(b) Apixaban**



Dose	4F-PCC		Andexanet alfa	
	Units/kg	Plasma level (IU/mL)	mg (bolus)	Plasma level (μM) <sup>a</sup>
Low	25	0.5	400	2.0
High	50	1.0	800	4.0

**FIGURE 4** Effect of andexanet on TF-TG (Peak) in the presence of rivaroxaban or apixaban compared with 4F-PCCs. A, Rivaroxaban; and B, apixaban. Data are shown as mean ± SD (N = 2). In each panel, the horizontal dashed line represents the Peak level in normal plasma. The vertical dashed line indicates the 75-ng/mL inhibitor level. The shaded area represents the range of inhibitors (75-1000 ng/mL) found in the ANNEXA-4 efficacy population (≥75 ng/mL). The inset table shows the plasma level of 4F-PCC or andexanet at their approved low and high doses, respectively. 4F-PCC, four-factor prothrombin complex concentrate; FXa, factor Xa; Peak, peak thrombin; SD, standard deviation; TF-TG, tissue factor-initiated thrombin generation. <sup>a</sup>At the approved andexanet low and high doses, the andexanet C<sub>max</sub> is ~2.0 μM at low dose (400-mg bolus plus 4 mg/min 2-hour infusion) and ~4.0 μM at high dose (800-mg bolus plus 8 mg/min × 2-hour infusion). The 2 and 4 μM levels are maintained by the infusion.

our previous clinical studies and showed here that reversal of FXa inhibitor-mediated anticoagulation by andexanet is effective over a wide range of inhibitor concentrations (19-2000 ng/mL), covering the range observed in bleeding patients in clinical studies. In contrast, 4F-PCCs only affect TF-TG over a low and narrow portion of this range (<75 ng/mL) and demonstrate an inverse relationship between FXa inhibitor concentrations and 4F-PCC-mediated increase in thrombin generation. The different thresholds of FXa inhibitor concentration where 4F-PCCs or andexanet may be effective is discussed based on distinct mechanisms of action for each

strategy as well as the theoretical threshold calculations and observed experimental results.

Andexanet binds FXa inhibitors with high affinity and 1:1 stoichiometry, neutralizes their anticoagulant activity, and rapidly leads to restoration of FXa activity and normal thrombin formation. 4F-PCCs are effective for warfarin reversal over a range of INRs by supplementation of normal coagulation factors inactivated by warfarin treatment, including prothrombin as one of the major components due to its high plasma concentration and longer half-life compared to other factors.<sup>36,37</sup>

The fundamental difference between warfarin and FXa inhibitors is that the former depletes functional coagulation factors (which can be replaced by 4F-PCCs), while the latter is a potent and specific enzyme inhibitor that causes a substantial blockage at a critical step in the coagulation cascade (ie, FXa). Replacement or supplementation strategies are less likely to work for FXa inhibitors due to two mechanistic reasons discussed below.

First, 4F-PCCs are unable to overcome FXa inhibition by direct binding and neutralization of FXa inhibitors. Since 4F-PCCs provide

FX (not FXa), and FXa inhibitors do not bind FX, treatment with 4F-PCCs has no direct effect on the anti-FXa activity of rivaroxaban or apixaban.<sup>17,21,28</sup> 4F-PCCs could theoretically cause the production of sufficient FXa through FX activation to overwhelm the FXa inhibitor. This does not appear to be the case, and, in fact, even the highest 4F-PCC dose falls substantially short of achieving this effect, as demonstrated by the calculations summarized in Table 1. In the best-case scenario, assuming 10% of FX could be converted to FXa, the estimated molar ratios (inhibitor:FXa) would be 48:1 for

**TABLE 1** Estimation of FXa inhibitor:FXa molar ratios in the absence and presence of 1.0 IU/mL 4F-PCC compared with andexanet

FX and FXa inhibitor concentrations			
Component	FX	Apixaban	Rivaroxaban
Molecular weight, Da	59,000 <sup>49</sup>	459.5	435.9
Plasma concentration, ng/mL	10,000	150	300
Plasma concentration, nM	170 <sup>50</sup>	326	688
Extravascular, ng/mL <sup>a</sup>	NA	225	900
Total, ng/mL	10,000	375	1200
<b>Total, nM</b>	<b>170</b>	<b>816</b>	<b>2753</b>
Molar ratios in the absence of 4F-PCC			
Potential FXa formation, %FX <sup>b</sup>	FXa, nM	Apixaban:FXa	Rivaroxaban:FXa
1% FX	1.7	480	1619
10% FX	17	48	162
Molar ratios in the presence of 1.0 IU/mL 4F-PCC			
Potential FXa formation, %FX <sup>b</sup>	FXa, nM	Apixaban:FXa	Rivaroxaban:FXa
2× 1% FX	3.4	240	810
2× 10% FX	34	24	81
Molar ratios in the presence of andexanet			
AnXa dose <sup>c</sup>	AnXa, nM	Apixaban:AnXa	Rivaroxaban:AnXa
Low dose	2000	0.43	NA
High dose	4000	NA	0.69

Abbreviations: 4F-PCC, four-factor prothrombin complex concentrate; AnXa, andexanet;  $C_{max}$ , maximum plasma concentration; FX, factor X; FXa, factor Xa; NA, not applicable; TF, tissue factor. Bold numbers signify the molar concentrations used for the molar ratio calculations in the rest of the table.

<sup>a</sup> For apixaban (5 mg twice daily), the  $C_{max}$  is approximately 150 ng/mL, with  $\sim 1.5\times$  (225 ng/mL) distributed in tissues.<sup>51</sup> For rivaroxaban (20 mg once daily), the  $C_{max}$  is approximately 300 ng/mL, with  $\sim 3\times$  (900 ng/mL) distributed in tissues.<sup>52</sup>

<sup>b</sup> Assuming 1% or 10% FX would be converted to FXa. Addition of 1.0 IU/mL 4F-PCC would provide  $\sim 2\times$  FX levels compared to normal plasma. Although it is difficult to measure active FXa in circulation due to its low abundance and very short half-life, previous computer modeling and measurement in a TF-initiated whole blood clotting reaction indicated that about 1%-10% of FX would be converted to FXa (1.7-17 nM),<sup>53</sup> of which only a small fraction (0.004%-0.09% FX) would be functional as the active prothrombinase (0.007-0.155 nM)<sup>54</sup> due to inhibition of FXa by endogenous inhibitors such as TF pathway inhibitor and antithrombin III. Addition of high-dose 4F-PCC (50 IU/kg,  $\sim 1.0$  IU/mL) would increase FX and FXa levels by  $\sim 2$ -fold. However, this increase in FX and FXa concentrations would have negligible effect in the presence of FXa inhibitors because of the vast molar excess of the inhibitors relative to any additional active FXa that could be potentially generated in the presence of 4F-PCCs.

<sup>c</sup> At the approved andexanet low and high doses, the andexanet  $C_{max}$  is  $\sim 2.0$   $\mu$ M at low dose (400-mg bolus plus 4 mg/min 2-hour infusion) and  $\sim 4.0$   $\mu$ M at high dose (800-mg bolus plus 8 mg/min  $\times$  2-hour infusion).



apixaban and 162:1 for rivaroxaban. The addition of 1.0 IU/mL from 4F-PCC would provide ~2-fold increase in FX levels. While this approximately doubles the FXa level, the enhanced FXa concentration would still only be able to negate <5% of the anticoagulant concentration (Table 1). Looked at another way, the 4F-PCC dose required to overcome the FXa inhibitor via this mechanism would be 20× higher than the highest approved dose (ie, ~1000 IU/kg or 150 vials each containing 500 units). The actual effect of 4F-PCCs on FXa inhibitors is far less if only 1% FX is converted to FXa (Table 1). These stoichiometric limitations were not considered in previously published PCCs studies,<sup>16</sup> and although based on several assumptions, they clearly indicate that inhibitor concentrations are in vast molar excesses over FXa levels that could possibly be formed in plasma, even with high-dose 4F-PCCs. These theoretical considerations are supported by clinical studies in healthy subjects where no anti-FXa activity reversal was observed with PCCs.<sup>18,19,22,30</sup> In contrast, low and high andexanet doses can achieve a molar excess (inhibitor:andexanet) over apixaban (0.43:1) and rivaroxaban (0.69:1) (Table 1).

The second mechanistic limitation is that 4F-PCCs are unable to bypass FXa inhibition by overloading substrate (prothrombin) in the presence of excess FXa inhibitors. Supplementation with high-dose 4F-PCCs (50 IU/kg, ~1.0 IU/mL) would double the prothrombin level. However, this increase in substrate concentration would

have limited effect on restoration of TF-TG because the FXa activity required for activation of prothrombin to thrombin is blocked by the FXa inhibitor. An important question, therefore, is to what level does the FXa inhibitor need to fall in order to achieve normal thrombin generation, either in the absence or presence of the highest PCC dose.

The degree of FXa blockade can be calculated for various FXa inhibitor levels (Table 2). A 150-ng/mL plasma apixaban level will result in residual FXa activity of ~3.0% (compared to normal plasma)—a 97% inhibition. Similarly, 300 ng/mL rivaroxaban will result in residual FXa activity of ~1.4%, or a 98.6% inhibition. Thus, therapeutic levels of apixaban and rivaroxaban result in strong FXa inhibition that substantially impairs conversion of prothrombin to thrombin, even if the prothrombin concentration is doubled with the highest 4F-PCC dose.

To estimate the FXa levels required to support normal thrombin generation, TF-TG was measured in plasma containing different FX levels by titrating normal PPP into FX-depleted plasma (Table S2; Figure S6). This information was used to estimate the %FXa levels required to support the targeted range of TF-TG (assuming that FXa levels change proportionally, as FX levels do). As shown in Table S2, 30% FXa is required to achieve near-normal ETP (90%), and 50% FXa would normalize all TF-TG parameters.

**TABLE 2** Estimation of residual FXa activity in the presence of a FXa inhibitor and the potential contribution of 4F-PCCs to FXa and thrombin generation

Total inhibitor, ng/mL	Free inhibitor, ng/mL <sup>a</sup>	Free inhibitor, nM <sup>b</sup>		Residual FXa, %, in PPP <sup>c</sup>		Residual FXa, %, in PPP + 4F-PCCs <sup>d</sup>	
		Apixaban	Rivaroxaban	Apixaban	Rivaroxaban	Apixaban	Rivaroxaban
500	50	108.8	114.7	0.91	0.86	1.8	1.7
300	30	65.3	68.8	1.5	1.4	3.0	2.9
250	25	54.4	57.4	1.8	1.7	3.6	3.4
150	15	32.6	34.4	3.0	2.8	5.9	5.6
75	7.5	16.3	17.2	5.8	5.5	11.5	11.0
37.5	3.75	8.2	8.6	10.9	10.4	21.8	20.8
18.75	1.88	4.1	4.3	19.7	18.9	39.4	37.7
9.38	0.94	2.0	2.2	32.9	31.7	65.8	63.5
4.69	0.47	1.0	1.1	49.5	48.2	99.0	96.4
2.34	0.23	0.5	0.5	66.2	65.0	132.4	130.1
0	0	0.0	0.0	100.0	100.0	200.0	200.0

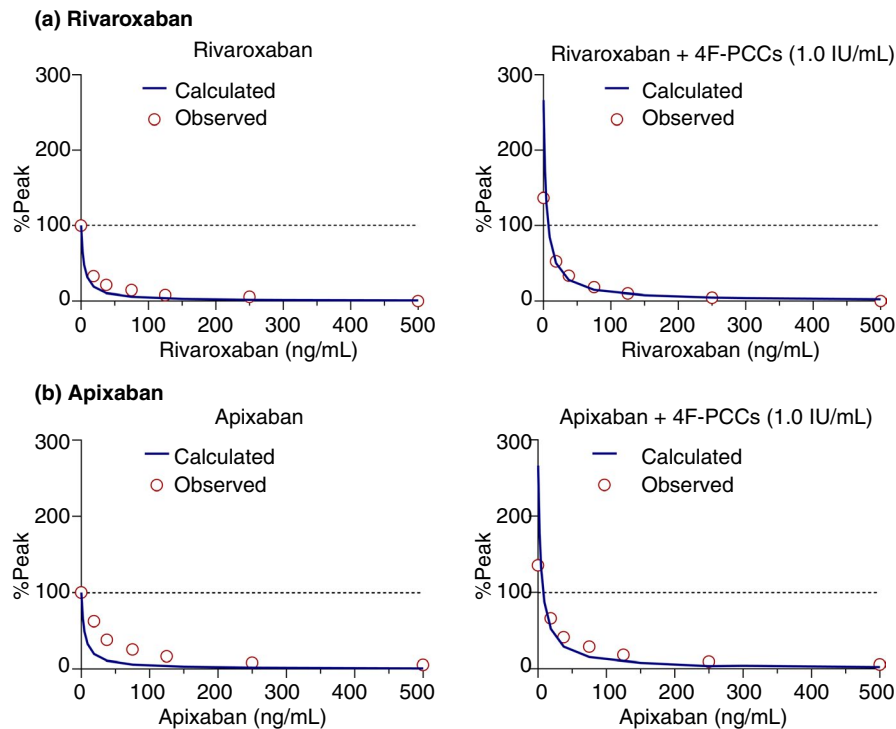
Abbreviations: 4F-PCC, four-factor prothrombin complex concentrate; FXa, factor Xa; PPP, platelet-poor plasma.

<sup>a</sup>Free inhibitor concentrations were based on 10% of the total inhibitor concentrations.

<sup>b</sup>The free inhibitor molar concentrations were calculated using molecular weight 459.6 Da for apixaban and 435.9 Da for rivaroxaban.

<sup>c</sup>Residual FXa activity (%) in the presence of a FXa inhibitor was calculated by  $(K_i / (K_i + [I])) \times 100$ , where [I] is the free inhibitor molar concentration (nM), and  $K_i$  is the equilibrium dissociation constant of the inhibitor to FXa. The residual FXa activity was calculated assuming  $K_i = 1.0$  nM, which might represent an upper limit of FXa activity because apixaban and rivaroxaban are potent FXa inhibitors with subnanomolar affinities ( $K_i < 1.0$  nM).<sup>3,4</sup>

<sup>d</sup>Assuming high-dose 4F-PCCs (50 IU/kg, ~1.0 IU/mL) would increase FX level by 2-fold and increase the FXa level proportionally by 2-fold. In addition, supplementation of 1.0 IU/mL 4F-PCCs would also increase the prothrombin level by 2-fold. The substrate effect on thrombin generation could be estimated by  $[S] / (K_m + [S])$ , where [S] is the prothrombin concentration in plasma and  $K_m$  is the equilibrium dissociation constant. Assuming  $K_m = [S]$ , a 2-fold increase in prothrombin concentration would increase thrombin generation by 1.33-fold. Thus, the overall effect of 1.0 IU/mL 4F-PCCs would increase thrombin generation by  $2 \times 1.33 = 2.66$ -fold. This combined effect of 1.0 IU/mL 4F-PCCs on thrombin generation is comparable to the in vitro experimental results shown in Figure 5 over a range of FXa inhibitor concentrations.



**FIGURE 5** Calculated versus observed effect of 4F-PCCs on TF-TG (Peak) as a function of FXa inhibitor concentrations. A, Rivaroxaban; and B, apixaban. Data were normalized to normal PPP (100%, horizontal dashed line). For clarity, the results are presented in two separate panels for each inhibitor with or without 4F-PCCs. The solid line represents the calculated values from Table 2 after taking into account the prothrombin component effect (1.33 $\times$ ). The open circle represents the observed effect of 4F-PCCs (0, 1.0 IU/mL) on Peak (data from Figure 4). Note that at the same level of inhibitor concentrations, apixaban appears to have less inhibitory effect on thrombin generation compared with rivaroxaban (Figures 2-4), which could be explained by the different rate of inhibition ( $k_{on}$ ) in addition to the affinity ( $K_i$ ) between the two inhibitors. The  $k_{on}$  value for apixaban is several-fold slower than rivaroxaban.<sup>55</sup> The calculations shown in Table 2 and Figure 5 were solely based on  $K_i$  and may represent an over-estimation of the apixaban inhibitory effect compared to rivaroxaban. 4F-PCC, four-factor prothrombin complex concentrate; FXa, factor Xa; Peak, peak thrombin; PPP, platelet-poor plasma; TF-TG, tissue factor-initiated thrombin generation

Without a specific reversal agent to sequester FXa inhibitors, apixaban and rivaroxaban would have to be extensively cleared (to  $\leq 10$  ng/mL) before 30% FXa activity can be achieved (Table 2). Table 2 also shows the impact of high-dose 4F-PCCs on FXa activity. The combined effects of FX and FII in 4F-PCCs are calculated to achieve the equivalent of 30% FXa activity (and thereby normalize TF-TG) at an apixaban or rivaroxaban concentration of  $\sim 35$  ng/mL (Table 2, calculations in legend). The experimental data in Figures 2-4 are in close accord with these calculated values: high-dose 4F-PCCs (1 IU/mL) normalized ETP for 37.5 ng/mL rivaroxaban and 75 ng/mL apixaban. However, as seen in Figure 4, even at the lowest inhibitor concentrations tested (18.75 ng/mL), high-dose 4F-PCC is unable to normalize the Peak, which is a direct measure of FXa enzymatic activity and therefore most closely congruent with the theoretical calculations (Table 2; Figure 5). Of note is the close agreement between the experimental data (Peak) and theoretical calculations for both apixaban and rivaroxaban in the absence or presence of 4F-PCCs (Figure 5). Similar conclusions can be drawn from other TF-TG parameters (Figure S4). Taken together, the 75 ng/mL threshold for 4F-PCCs effectiveness is the upper limit and is supported by both experimental data (ETP and Peak) and theoretical calculations (Peak). As discussed below, this threshold has clinical implications. In

the Andexanet Alfa, a Novel Antidote to the Anticoagulation Effects of Factor Xa Inhibitors (ANNEXA-4) study, 75% of the bleeding patients had anti-FXa levels  $\geq 75$  ng/mL at the time of andexanet administration<sup>38</sup> and would be predicted, based on these data, to have little or no benefit from 4F-PCCs on restoring thrombin generation to normal baseline levels.

These results provide a new lens through which to interpret published reports on the use of 4F-PCCs for FXa inhibitor reversal in both healthy volunteers and bleeding patients. Several studies have evaluated PCCs in healthy volunteers treated with rivaroxaban and apixaban<sup>16-23,30,31</sup> (Table S1). As discussed above, anti-FXa levels were not impacted by PCCs in these studies.<sup>17,21,28</sup> Moreover, thrombin generation was not normalized in most cases with 4F-PCCs until the inhibitors had been substantially cleared,<sup>21</sup> a finding consistent with the present study showing that 4F-PCCs did not normalize TF-TG unless FXa inhibitor levels were  $< 35$ -75 ng/mL (Table 2; Figure 5). Regarding bleeding patients on FXa inhibitors, several observational studies have reported on the use of 4F-PCCs but have shown inconsistent efficacy results.<sup>24-27</sup> In the light of the current study, the data in these four major reports are difficult to interpret because anti-FXa levels were measured in only 122 of 361 (34%) patients taking apixaban and rivaroxaban

spanning these four PCC studies. Furthermore, anti-FXa levels in the PCC responders and nonresponders were not reported, a critical variable that should correlate with whether the patient had good hemostasis with 4F-PCC. Despite this, some important new insights can be drawn from these studies. First, the retrospective cohort study by Gerner et al<sup>26</sup> in 131 patients with intracerebral hemorrhage (ICH) taking rivaroxaban or apixaban reported no benefit of 4F-PCCs on either mortality or the arrest of hematoma expansion as determined by serial brain imaging (computed tomography [CT] or magnetic resonance imaging). Of note, this is the only study of the four that had consistent serial imaging of ICH, similar to the mandatory CTs required in ANNEXA-4. The other three studies reported a benefit of PCCs in patients with major bleeding on rivaroxaban or apixaban with overall hemostatic efficacies of 69% (84 patients, Majeed et al<sup>24</sup>), 65% (66 patients, Schulman et al<sup>25</sup>), and 74% (80 patients, Arachchillage et al<sup>27</sup>). In these three studies, one can estimate that ~25% or more of the patients were in the lower ranges of anticoagulation, similar to that seen in ANNEXA-4 for patients with anti-FXa levels <75 ng/mL. These patients would be expected to have a good response to 4F-PCCs and thus may have contributed disproportionately to the overall efficacy. Another factor contributing to the observed efficacy is the percentage of patients for whom hemostasis had already been achieved spontaneously (ie, without treatment) by the time they arrived at the hospital—a number that may be as high as 50%, as seen in the ICH patients in the Gerner et al<sup>26</sup> study. Recently, two other observational cohort studies on 4F-PCCs showed hemostatic efficacies of 70%<sup>39</sup> and 82%,<sup>40</sup> but again with anti-FXa levels available in only 30% and 15% of patients, respectively.

In ANNEXA-4, anti-FXa levels measured at the time of andexanet administration were not clustered around the 75 ng/mL threshold. The apixaban anti-FXa median level (N = 134) was 149.7 ng/mL (including up to 950 ng/mL), and the rivaroxaban median level (N = 100) was 211.8 ng/mL (including up to 850 ng/mL) in the efficacy population.<sup>38</sup> Hemostatic efficacy was 82% across the broad range of anti-FXa levels, even in outliers with very high inhibitor levels at the time of andexanet administration (discussed in the supplement of Connolly et al<sup>38</sup>). Importantly, all patients in the efficacy population (defined in the protocol as those with anti-FXa levels ≥75 ng/mL) would be expected to have minimal improvement in thrombin generation regardless of use of either low- or high-dose 4F-PCCs based on the *in vitro* data presented herein.

One limitation of the current study is the use of a plasma-based *in vitro* TF-TG assay in the absence of cellular components (eg, platelets<sup>41,42</sup>). Additionally, the difference in thrombin generation potential between healthy subjects and bleeding patients remains unknown, and a correlation between restoration of thrombin generation and hemostasis has not been established in bleeding patients taking FXa inhibitors. Ultimately, the hemostatic efficacy and thrombotic potential of reversal agents (beyond the intrinsic risk simply due to reversal itself) need to be determined in a randomized controlled trial and compared head to head. The ongoing ANNEXA-I study (ClinicalTrials.gov: NCT03661528) is such a study that is comparing

the hemostatic efficacy of andexanet with usual care (4F-PCCs and other agents) in ICH patients.

In summary, this study used TF-TG to compare the effectiveness of 4F-PCC and andexanet to reverse the anticoagulant effects of apixaban and rivaroxaban. As expected, 4F-PCCs completely corrected the inhibition of TF-TG by warfarin in a dose-responsive manner. However, they were only effective in reversing the inhibition of TF-TG by FXa inhibitors over a narrow range of inhibitor concentrations (<75 ng/mL). The results are consistent with previous studies in healthy subjects that showed thrombin generation was not normalized until 4–6 hours after PCC treatment, when FXa inhibitor concentrations have declined significantly from maximum levels. In contrast, andexanet, at the approved doses, was able to fully restore TF-TG over a wide range (19–2000 ng/mL) of inhibitor concentrations. Specific reversal agents such as andexanet are recommended as the preferred treatment over 4F-PCCs by at least 17 guidelines as of this writing<sup>43–46</sup> and by the Joint Commission.<sup>47</sup> These recommendations are based on the distinct mechanism of action of andexanet versus 4F-PCCs, as delineated in the current and previous studies, in that sequestration of the FXa inhibitor by andexanet allows for immediate restoration of thrombin generation to normal baseline levels<sup>38,48</sup> over a broad range of inhibitor concentrations. In contrast, these *in vitro* data suggest that 4F-PCCs can only be effective at restoring TF-TG when inhibitor concentrations are <75 ng/mL, which corresponds to a small proportion (<25%) of pretreatment baseline anti-FXa levels documented in bleeding patients.<sup>38</sup>

## ACKNOWLEDGMENTS

Medical writing and editorial support were provided by Kim Fuller, PhD, of SciFluent Communications, and were financially supported by Portola Pharmaceuticals, Inc.

## RELATIONSHIP DISCLOSURE

All authors are employees and stockholders of Portola Pharmaceuticals, Inc.

## AUTHOR CONTRIBUTIONS

GL contributed to the concept and design of the study and the acquisition, analysis, and interpretation of data; and drafted, revised, and reviewed the final manuscript. JL and KB contributed to the acquisition and analysis of data and reviewed the manuscript. JTC and PBC contributed to the concept and design of the study and interpretation of data; and drafted, revised, and reviewed the final manuscript.

## REFERENCES

1. XARELTO® (rivaroxaban) tablets, for oral use [package insert]. Titusville, NJ: Janssen Pharmaceuticals, Inc.; 2018.
2. ELIQUIS® (apixaban) tablets for oral use [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; 2012.
3. Perzborn E, Strassburger J, Wilmen A, Pohlmann J, Roehrig S, Schlemmer KH, et al. *In vitro* and *in vivo* studies of the novel anti-thrombotic agent BAY 59-7939—an oral, direct factor Xa inhibitor. *J Thromb Haemost*. 2005;3(3):514–21.

4. Luetggen JM, Knabb RM, He K, Pinto DJ, Rendina AR. Apixaban inhibition of factor Xa: microscopic rate constants and inhibition mechanism in purified protein systems and in human plasma. *J Enzyme Inhib Med Chem*. 2011;26(4):514–26.
5. Riva N, Dentali F, Permian ET, Ageno W. Major bleeding and case fatality rate with the direct oral anticoagulants in orthopedic surgery: a systematic review and meta-analysis. *Semin Thromb Hemost*. 2016;42(1):42–54.
6. Gomez-Outes A, Lecumberri R, Suarez-Gea ML, Terleira-Fernandez AI, Monreal M, Vargas-Castrillon E. Case fatality rates of recurrent thromboembolism and bleeding in patients receiving direct oral anticoagulants for the initial and extended treatment of venous thromboembolism: a systematic review. *J Cardiovasc Pharmacol Ther*. 2015;20(5):490–500.
7. Gulseth MP. Overview of direct oral anticoagulant therapy reversal. *Am J Health Syst Pharm*. 2016;73(Suppl 2):S5–S13.
8. ANDEXXA<sup>®</sup> (coagulation factor Xa (recombinant), inactivated-zhzo). Lyophilized powder for solution for intravenous injection [package insert]. South San Francisco, CA: Portola Pharmaceuticals, Inc.; 2018.
9. European Medicines Agency. Summary of Product Characteristics. Ondexxya (andexanet alfa); 2019.
10. Lu G, DeGuzman FR, Hollenbach SJ, Karbarz MJ, Abe K, Lee G, et al. A specific antidote for reversal of anticoagulation by direct and indirect inhibitors of coagulation factor Xa. *Nat Med*. 2013;19(4):446–51.
11. Franchini M, Lippi G. Prothrombin complex concentrates: an update. *Blood Transfus*. 2010;8(3):149–54.
12. Perzborn E, Heitmeier S, Laux V, Buchmuller A. Reversal of rivaroxaban-induced anticoagulation with prothrombin complex concentrate, activated prothrombin complex concentrate and recombinant activated factor VII in vitro. *Thromb Res*. 2014;133(4):671–81.
13. Escolar G, Arellano-Rodrigo E, Lopez-Vilchez I, Molina P, Sanchis J, Reverter JC, et al. Reversal of rivaroxaban-induced alterations on hemostasis by different coagulation factor concentrates – in vitro studies with steady and circulating human blood. *Circ J*. 2015;79(2):331–8.
14. Schultz NH, Tran HTT, Bjornsen S, Henriksson CE, Sandset PM, Holme PA. The reversal effect of prothrombin complex concentrate (PCC), activated PCC and recombinant activated factor VII in apixaban-treated patients in vitro. *Res Pract Thromb Haemost*. 2017;1(1):49–56.
15. Godier A, Miclot A, Le Bonniec B, Durand M, Fischer AM, Emmerich J, et al. Evaluation of prothrombin complex concentrate and recombinant activated factor VII to reverse rivaroxaban in a rabbit model. *Anesthesiology*. 2012;116(1):94–102.
16. Eerenberg ES, Kamphuisen PW, Sijkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation*. 2011;124(14):1573–9.
17. Levi M, Moore KT, Castillejos CF, Kubitzka D, Berkowitz SD, Goldhaber SZ, et al. Comparison of three-factor and four-factor prothrombin complex concentrates regarding reversal of the anticoagulant effects of rivaroxaban in healthy volunteers. *J Thromb Haemost*. 2014;12(9):1428–36.
18. Cheung YW, Barco S, Hutten BA, Meijers JC, Middeldorp S, Coppens M. In vivo increase in thrombin generation by four-factor prothrombin complex concentrate in apixaban-treated healthy volunteers. *J Thromb Haemost*. 2015;13(10):1799–805.
19. Barco S, Whitney Cheung Y, Coppens M, Hutten BA, Meijers JC, Middeldorp S. In vivo reversal of the anticoagulant effect of rivaroxaban with four-factor prothrombin complex concentrate. *Br J Haematol*. 2016;172(2):255–61.
20. Nagalla S, Thomson L, Oppong Y, Bachman B, Chervoneva I, Kraft WK. Reversibility of apixaban anticoagulation with a four-factor prothrombin complex concentrate in healthy volunteers. *Clin Transl Sci*. 2016;9(3):176–80.
21. Song Y, Wang Z, Perlstein I, Wang J, LaCreta F, Frost RJA, et al. Reversal of apixaban anticoagulation by four-factor prothrombin complex concentrates in healthy subjects: a randomized three-period crossover study. *J Thromb Haemost*. 2017;15(11):2125–37.
22. Levy JH, Moore KT, Neal MD, Schneider D, Marcscisin VS, Ariyawansa J, et al. Rivaroxaban reversal with prothrombin complex concentrate or tranexamic acid in healthy volunteers. *J Thromb Haemost*. 2018;16(1):54–64.
23. Zahir H, Brown KS, Vandell AG, Desai M, Maa JF, Dishy V, et al. Edoxaban effects on bleeding following punch biopsy and reversal by a 4-factor prothrombin complex concentrate. *Circulation*. 2015;131(1):82–90.
24. Majeed A, Agren A, Holmstrom M, Bruzelius M, Chairati R, Odeberg J, et al. Management of rivaroxaban- or apixaban-associated major bleeding with prothrombin complex concentrates: a cohort study. *Blood*. 2017;130(15):1706–12.
25. Schulman S, Gross PL, Ritchie B, Nahriani S, Lin Y, Lieberman L, et al. Prothrombin complex concentrate for major bleeding on factor Xa inhibitors: a prospective cohort study. *Thromb Haemost*. 2018;118(5):842–51.
26. Gerner ST, Kuramatsu JB, Sembill JA, Sprugel MI, Endres M, Haeusler KG, et al. Association of prothrombin complex concentrate administration and hematoma enlargement in non-vitamin K antagonist oral anticoagulant-related intracerebral hemorrhage. *Ann Neurol*. 2018;83(1):186–96.
27. Arachchilage DRJ, Alavian S, Griffin J, Gurung K, Szydlo R, Karawitige N, et al. Efficacy and safety of prothrombin complex concentrate in patients treated with rivaroxaban or apixaban compared to warfarin presenting with major bleeding. *Br J Haematol*. 2019;184(5):808–16.
28. Hoffman M, Goldstein JN, Levy JH. The impact of prothrombin complex concentrates when treating DOAC-associated bleeding: a review. *Int J Emerg Med*. 2018;11(1):55.
29. Piran S, Khatib R, Schulman S, Majeed A, Holbrook A, Witt DM, et al. Management of direct factor Xa inhibitor-related major bleeding with prothrombin complex concentrate: a meta-analysis. *Blood Adv*. 2019;3(2):158–67.
30. Grottke O, Schulman S. Four-factor prothrombin complex concentrate for the management of patients receiving direct oral activated factor X inhibitors. *Anesthesiology*. 2019;131(5):1153–65.
31. Dzik WH. Reversal of oral factor Xa inhibitors by prothrombin complex concentrates: a re-appraisal. *J Thromb Haemost*. 2015;13(suppl 1):S187–S194.
32. KCENTRA<sup>®</sup> (prothrombin complex concentrate (human)) for intravenous use [package insert]. Kankakee, IL: CSL Behring; 2018.
33. Pernod G, Albaladejo P, Godier A, Samama CM, Susen S, Gruel Y, et al. Management of major bleeding complications and emergency surgery in patients on long-term treatment with direct oral anticoagulants, thrombin or factor-Xa inhibitors: proposals of the Working Group on Perioperative Haemostasis (GIHP) - March 2013. *Arch Cardiovasc Dis*. 2013;106(6–7):382–93.
34. Prior SM, Mann KG, Freeman K, Butenas S. Continuous thrombin generation in whole blood: new applications for assessing activators and inhibitors of coagulation. *Anal Biochem*. 2018;551:19–25.
35. Danforth CM, Orfeo T, Everse SJ, Mann KG, Brummel-Ziedins KE. Defining the boundaries of normal thrombin generation: investigations into hemostasis. *PLoS One*. 2012;7(2):e30385.
36. Xi M, Beguin S, Hemker HC. The relative importance of the factors II, VII, IX and X for the prothrombinase activity in plasma of orally anticoagulated patients. *Thromb Haemost*. 1989;62(2):788–91.
37. Dusel CH, Grundmann C, Eich S, Seitz R, Konig H. Identification of prothrombin as a major thrombogenic agent in prothrombin complex concentrates. *Blood Coagul Fibrinolysis*. 2004;15(5):405–11.

38. Connolly SJ, Crowther M, Eikelboom JW, Gibson CM, Curnutte JT, Lawrence JH, et al. Full study report of andexanet alfa for bleeding associated with factor Xa inhibitors. *N Engl J Med*. 2019;380(14):1326–35.
39. Bavalia R, Abdoellakhan R, Brinkman HJM, Brekelmans MPA, Hamulyák EN, Zuurveld M, et al. Emergencies on direct oral anticoagulants: management, outcomes, and laboratory effects of prothrombin complex concentrate. *Res Pract Thromb Haemost*. 2020;4(4):569–81.
40. Panos NG, Cook AM, John S, Jones GM; Neurocritical Care Society (NCS) Pharmacy Study Group. Factor Xa inhibitor-related intracranial hemorrhage: results from a multicenter, observational cohort receiving prothrombin complex concentrates. *Circulation*. 2020;141(21):1681–9.
41. Allen GA, Wolberg AS, Oliver JA, Hoffman M, Roberts HR, Monroe DM. Impact of procoagulant concentration on rate, peak and total thrombin generation in a model system. *J Thromb Haemost*. 2004;2:402–13.
42. Hoffman M, Volovyk Z, Monroe DM. Reversal of dabigatran effects in models of thrombin generation and hemostasis by factor VIIa and prothrombin complex concentrate. *Anesthesiology*. 2015;122:353–62.
43. Cuker A, Burnett A, Triller D, Crowther M, Ansell J, Van Cott EM, et al. Reversal of direct oral anticoagulants: guidance from the Anticoagulation Forum. *Am J Hematol*. 2019;94(6):697–709.
44. January CT, Wann LS, Calkins H, Chen LY, Cigarroa JE, Cleveland Jr JC, et al. 2019 AHA/ACC/HRS focused update of the 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation. *Circulation*. 2019;140:e125–e151.
45. Tomaselli GF, Mahaffey KW, Cuker A, Dobesh PP, Doherty JU, Eikelboom JW, et al. 2017 ACC expert consensus decision pathway on management of bleeding in patients on oral anticoagulants: a report of the American College of Cardiology task force on expert consensus decision pathways. *J Am Coll Cardiol*. 2017;70(24):3042–67.
46. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology (NCCN guidelines): cancer-associated venous thromboembolic disease. Version 1.2019-February 28, 2019.
47. The Joint Commission. Managing the risks of direct oral anticoagulants. Sentinel Event Alert 61. 2019. Available at: <https://www.jointcommission.org/-/media/tjc/documents/resources/patient-safety-topics/sentinel-event/sea-61-doacs-final.pdf>. Accessed June 16, 2020
48. Siegal DM, Curnutte JT, Connolly SJ, Lu G, Conley PB, Wiens BL, et al. Andexanet alfa for the reversal of factor Xa inhibitor activity. *N Engl J Med*. 2015;373(25):2413–24.
49. Leytus SP, Foster DC, Kurachi K, Davie EW. Gene for human factor X: a blood coagulation factor whose gene organization is essentially identical with that of factor IX and protein C. *Biochemistry*. 1986;25(18):5098–102.
50. Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood*. 1990;76(1):1–16.
51. Siegal D, Lu G, Leeds JM, Karbarz M, Castillo J, Mathur V, et al. Safety, pharmacokinetics, and reversal of apixaban anticoagulation with andexanet alfa. *Blood Adv*. 2017;1(22):1827–38.
52. Lu G, Conley PB, Leeds JM, Karbarz MJ, Levy GG, Mathur VS, et al. A phase 2 PK/PD study of andexanet alfa for reversal of rivaroxaban and edoxaban anticoagulation in healthy volunteers. *Blood Adv*. 2020;4(4):728–39.
53. Brummel-Ziedins KE, Orfeo T, Gissel M, Mann KG, Rosendaal FR. Factor Xa generation by computational modeling: an additional discriminator to thrombin generation evaluation. *PLoS One*. 2012;7(1):e29178.
54. Rand MD, Lock JB, van't Veer C, Gaffney DP, Mann KG. Blood clotting in minimally altered whole blood. *Blood*. 1996;88(9):3432–45.
55. Jourdi G, Siguret V, Martin AC, Golmard JL, Godier A, Samama CM, et al. Association rate constants rationalise the pharmacodynamics of apixaban and rivaroxaban. *Thromb Haemost*. 2015;114(1):78–86.

#### SUPPORTING INFORMATION

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

**How to cite this article:** Lu G, Lin J, Bui K, Curnutte JT, Conley PB. Andexanet versus prothrombin complex concentrates: Differences in reversal of factor Xa inhibitors in in vitro thrombin generation. *Res Pract Thromb Haemost*. 2020;4:1282–1294. <https://doi.org/10.1002/rth2.12418>