

## Introduction

Factor VIII plays an important role in the regulation of coagulation and a severe depletion (<5%) causes a moderate haemophilia A. However, mildly reduced FVIII activity does not cause bleeding problems and is associated with reduced instances of thrombosis. TB-402 is a partially inhibiting antibody to Factor VIII that was derived from LE2E9, a virtually complete inhibitor which arose during the treatment of a haemophiliac patient with FVIII.

The partially inhibitory nature of TB-402 limits its pharmacological effect. This series of studies was designed to characterise the pharmacology of TB-402 and to determine if addition of agents such as Novo 7, Kogenate or FEIBA could restore functional measures of coagulation *in vitro*.

## Methods

A pool of citrated platelet poor human plasma was prepared from healthy volunteers. Following incubations with inhibitory antibodies and/or rhFVIII (Kogenate), residual factor VIII activity was measured using a modified Coatest assay. aPTT was measured on the BCS-XP coagulation analyzer (Siemens). Thrombin generation was assessed with the Calibrated Automated Thrombogram (CAT, Thrombinoscope) using low concentrations of tissue factor and phospholipids.

## Results

### Factor VIII activity

The inhibitory effect of TB-402 and its parent antibody on FVIII activity are shown in figure 1. The almost complete inhibitory effect of LE2E9 is apparent. For TB-402, inhibition of FVIII activity occurred over a similar concentration range as for LE2E9 but the extent of inhibition is limited to <40% even at concentrations up to 1mg/mL. The co-incubation of TB-402 with Kogenate is shown in figure 2 and demonstrates that factor VIII activity is restored by Kogenate in a concentration-dependent manner. Interestingly, the presence of TB-402 causes Kogenate to increase FVIII activity to approximately 60% of that expected, suggesting that TB-402 also inhibits the activity of the added factor VIII. At higher concentrations of Kogenate (upon exhaustion of available TB-402) factor VIII activity increases at a higher rate.

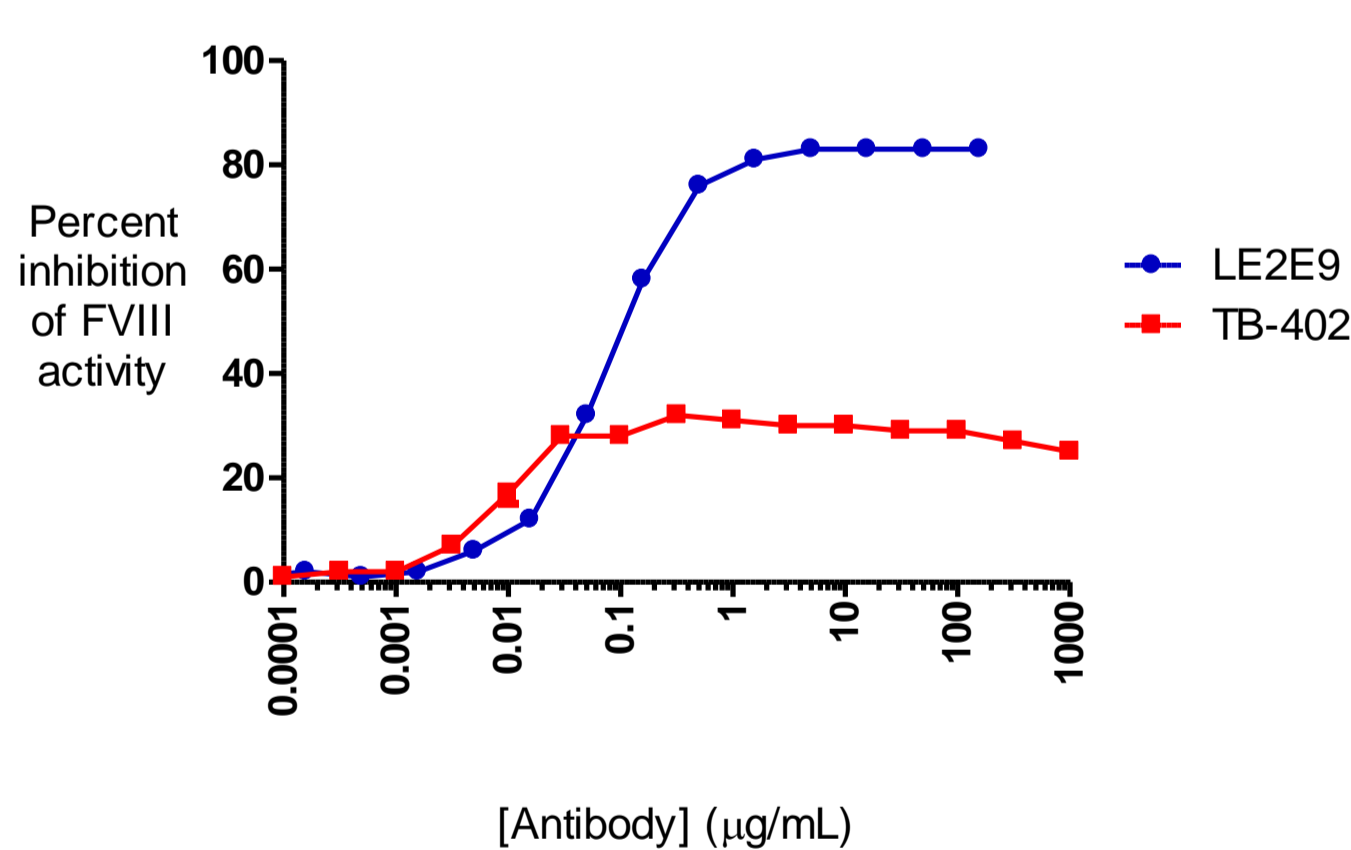


Figure 1: Inhibition of factor VIII activity in human plasma due to TB-402 or LE2E9.

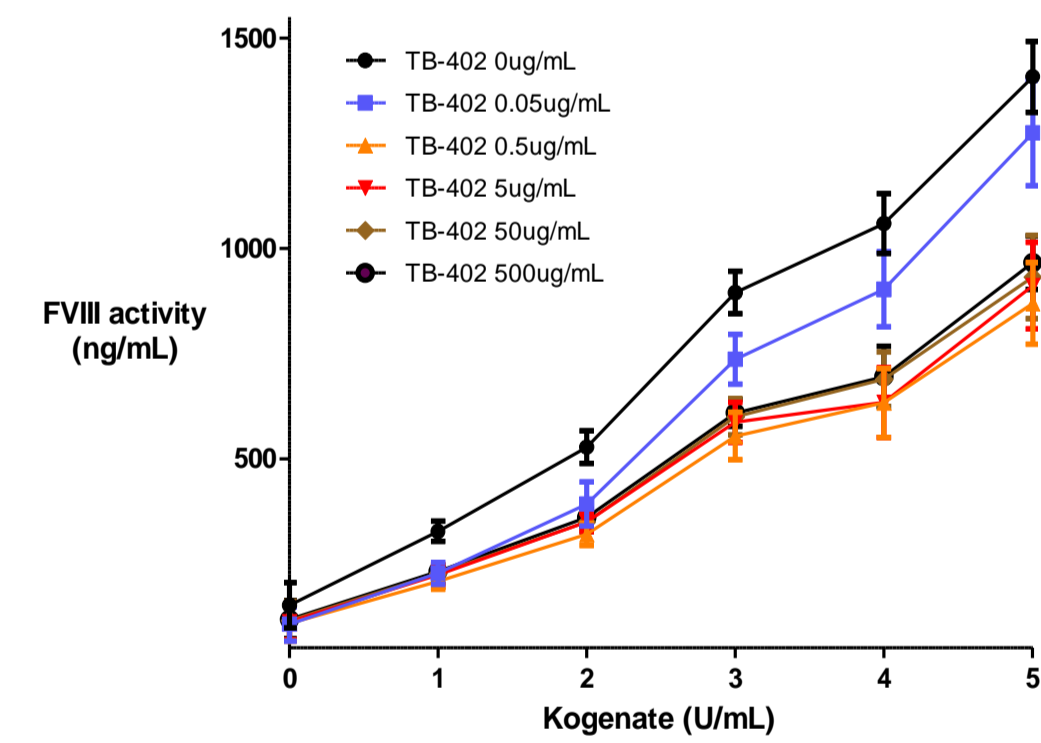


Figure 2: Restoration of factor VIII activity due to the addition of Kogenate together with TB-402 in human plasma.

### Effects on aPTT

The mildly prolonging effect of TB-402 on aPTT is shown in figure 3. As was noted with the inhibition of FVIII activity, the effect of TB-402 reaches its maximum at approximately 1ug/mL, with an increase in aPTT of approx 4 sec. By contrast, the more complete inhibitor LE2E9 induced a more substantial prolongation of aPTT (approximately 14 sec). As expected, Kogenate reduced aPTT, and when co-administered with TB-402 reversed the prolongation induced by TB-402 (figure 4).

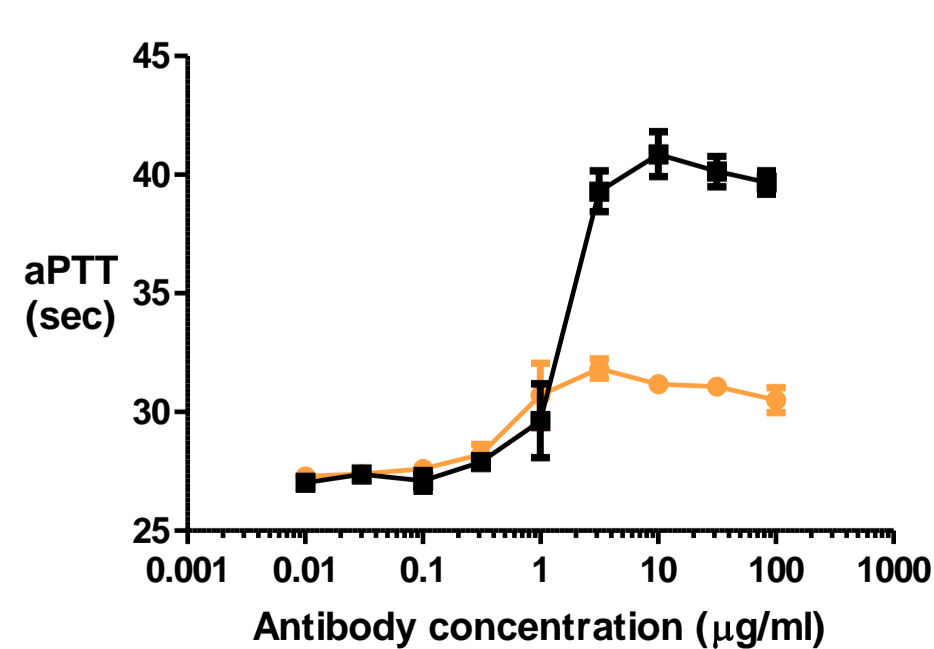


Figure 3: Prolongation of aPTT by TB-402 or LE2E9 in human plasma.

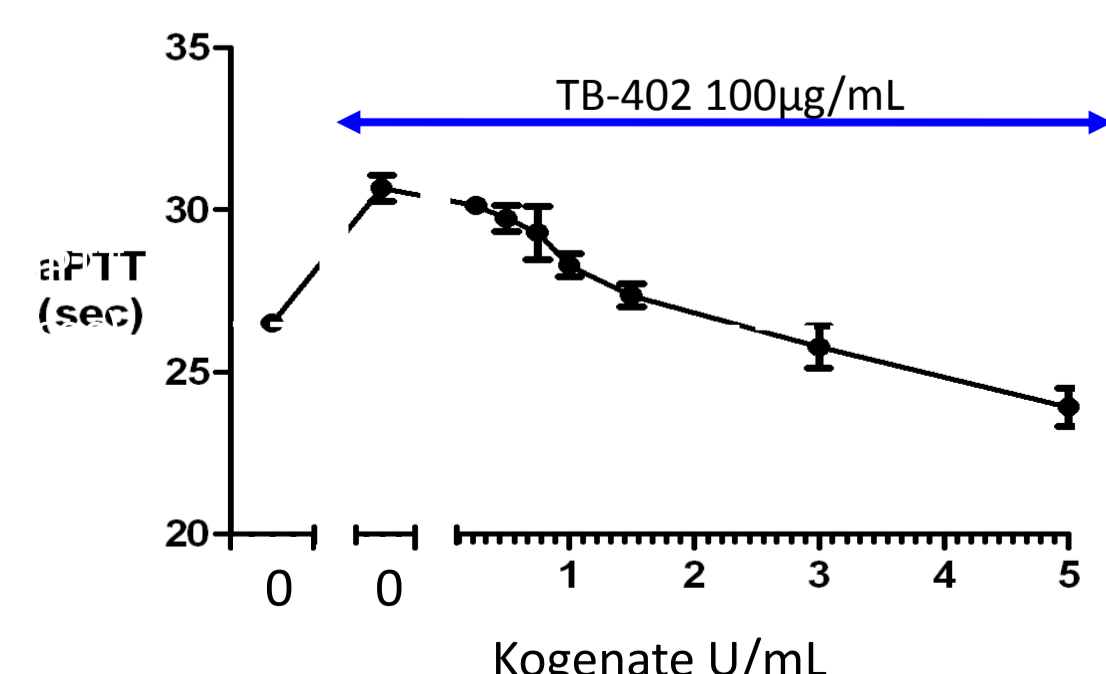


Figure 4: Prolongation of aPTT by TB-402 in human plasma and its reversal by Kogenate.

### Effects on thrombin generation

Thrombin generation was measured using various concentrations of tissue factor (1-5pM) and phospholipid (0.2-4µM). The responsiveness of blood for peak height of thrombin increased with both tissue factor and phospholipid (figure 5 left).

Under thrombin generation conditions of 1pM tissue factor and 0.8 µM phospholipid, TB-402 induced a concentration-dependent mild inhibition of thrombin generation in platelet poor plasma from normal healthy humans. At its maximum, achieved at 10ug/mL TB-402, thrombin generation was inhibited by approx 50% (figure 5 right), whereas the more complete inhibitor LE2E9 induced an approx 80% inhibition

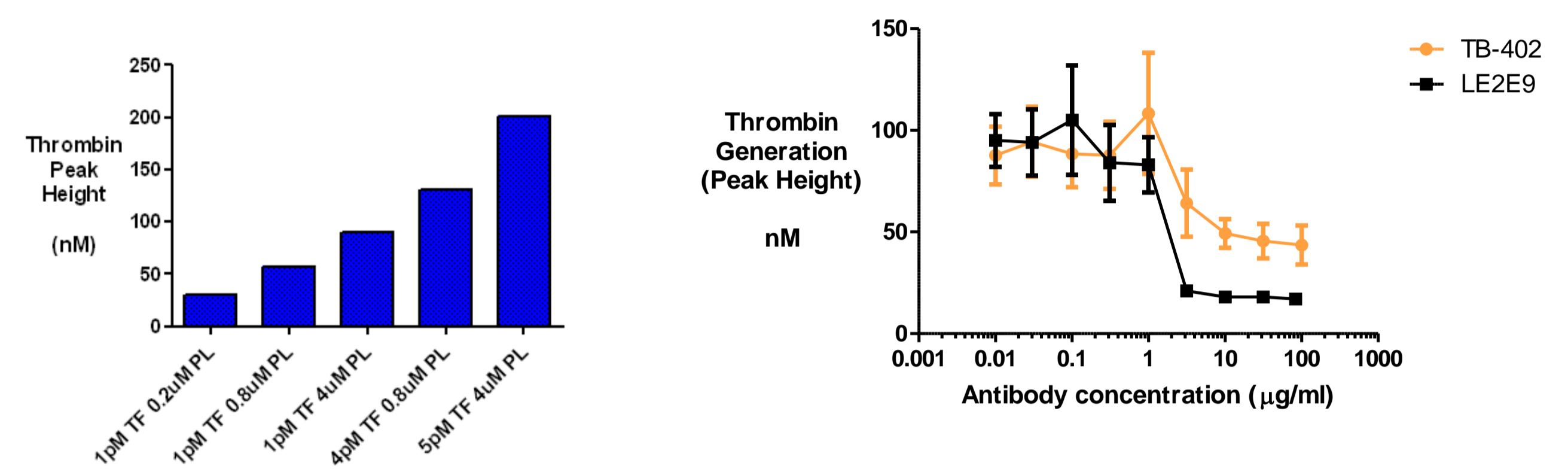


Figure 5: Effect of TF and PL on thrombin peak height (left) and inhibition of thrombin generation by TB-402 and LE2E9 (right).

Kogenate reversed the inhibition on the generation of thrombin, in a concentration-dependent manner, such that approximately 1U/mL Kogenate was sufficient to restore the thrombin generating capacity of TB-402-treated plasma (figure 7).

Agents such as Novo7, Aafact, PPSB and FEIBA were also able to restore thrombin generation, also in concentration-dependent manners (figure 7).

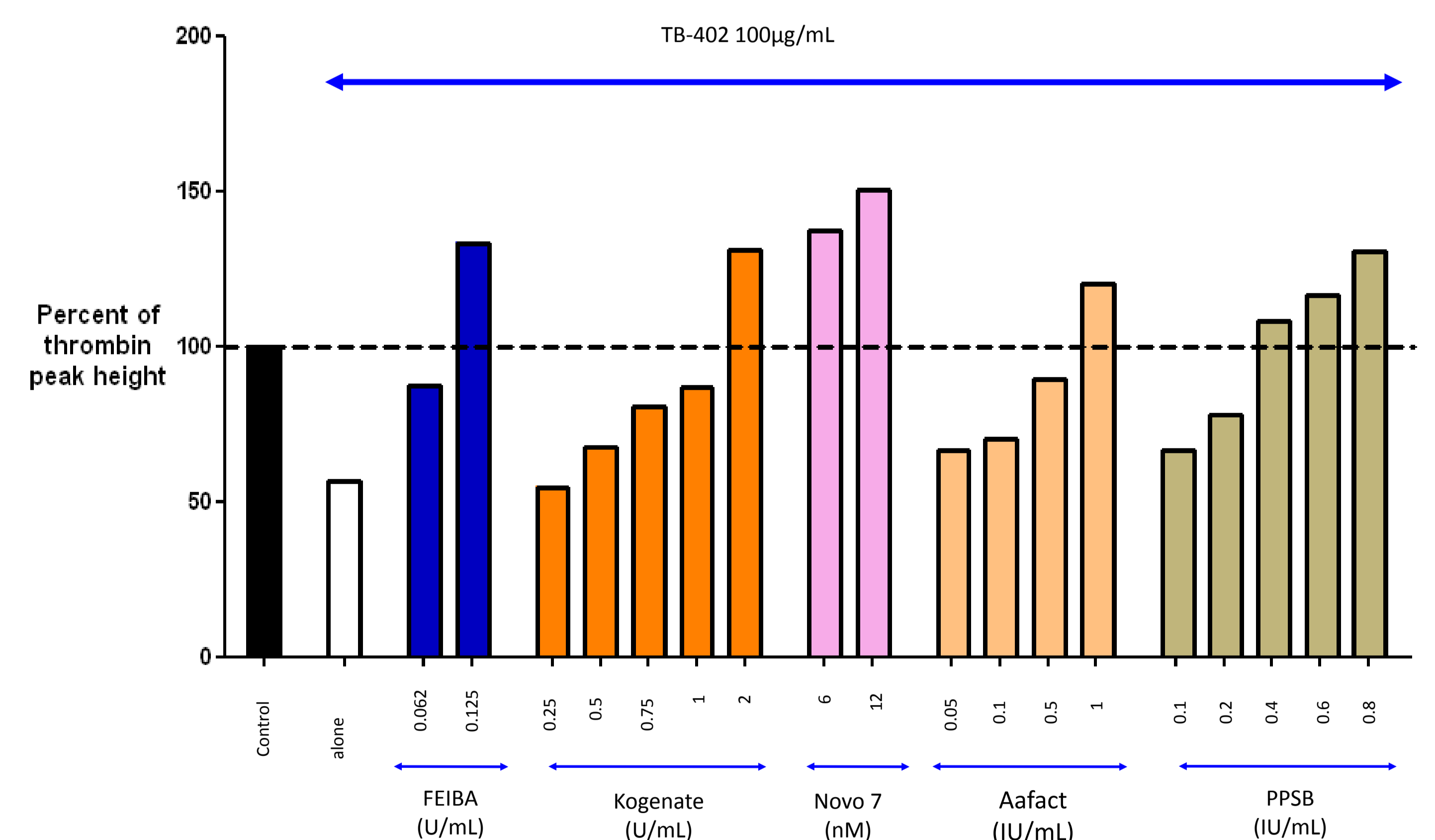


Figure 7: Restoration of TB-402 inhibited thrombin generation with Kogenate, Novo7, PPSB, Aafact and FEIBA.

## Conclusions

- TB-402 is a partial inhibitor of factor VIII in human plasma.
- TB-402 induces a mild prolongation of aPTT, maximally 4 sec.
- All the effects on FVIII:C and aPTT are reversed by Kogenate.
- TB-402 induces a partial inhibition of thrombin generation which is reversed by Kogenate, Novo7 or FEIBA.