

Oral FXIIa inhibitor KV998086 suppresses FXII zymogen mediated kallikrein kinin system activation

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Background

- Prophylactic use of an anti-activated Factor XII monoclonal antibody, garadacimab, has been shown to reduce HAE attacks by >90% in a phase 2 trial¹
- We have discovered potent, selective, and orally available FXIIa inhibitors that may provide a novel therapeutic opportunity to treat HAE and other Kallikrein Kinin System (KKS)-mediated diseases
- Previous studies have shown that FXII zymogen has proteolytic activity that can initiate contact system activation², and thereby may contribute to disease
- The ability of small molecule FXIIa inhibitors to also inhibit the active conformation of the FXII zymogen is unknown

Purpose

This study examines the effects of the novel oral small molecule FXIIa inhibitor KV998086 on both FXIIa- and FXII-mediated kallikrein kinin system activation

Methods

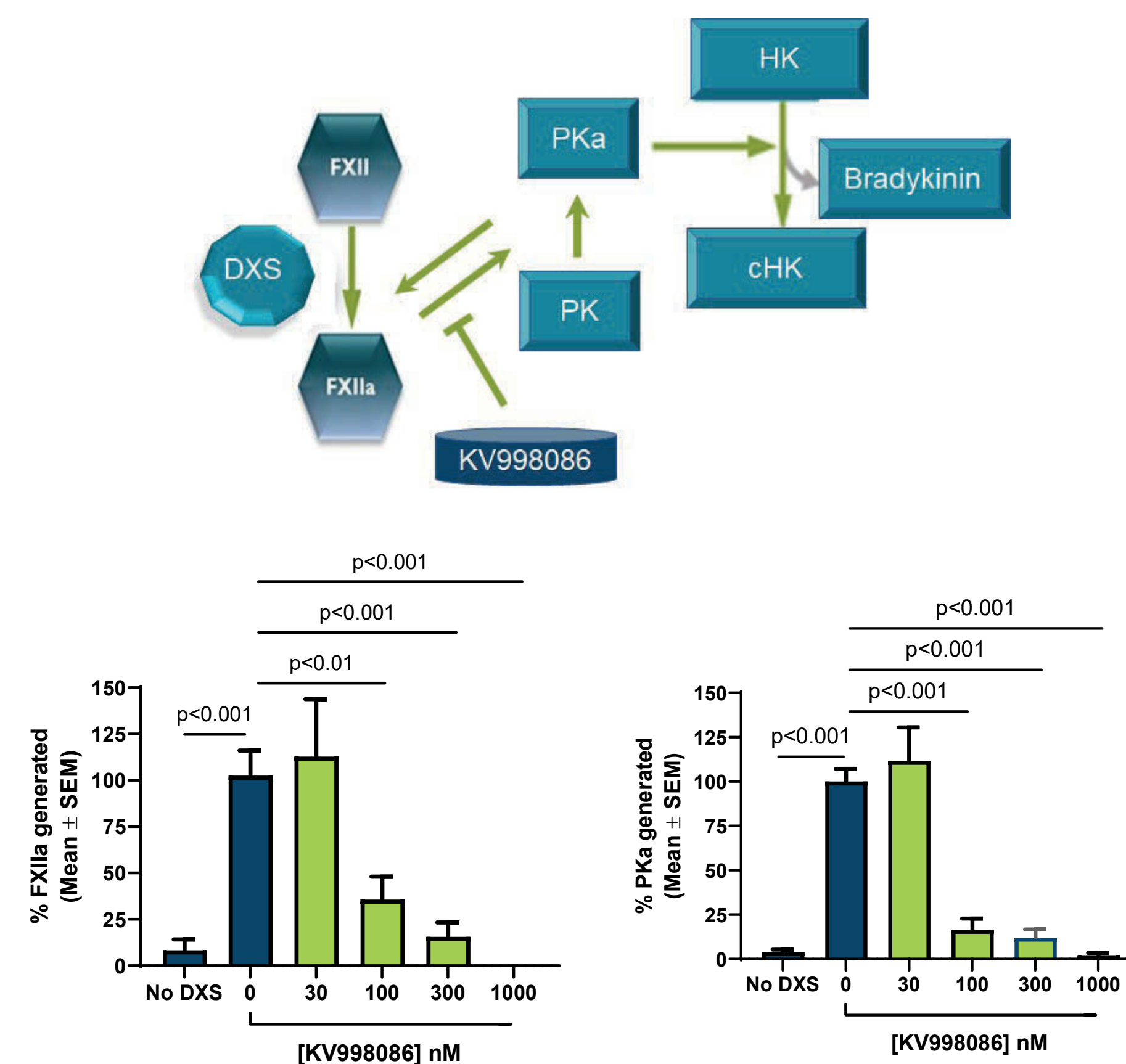
- Dextran Sulfate (DXS) plasma HK cleavage assay:** Whole human plasma was pre-treated with KV998086 for 15 minutes. DXS (6.25µg/mL) stimulation of human plasma (+/- KV998086) was conducted for 17 minutes on ice. Capillary based immunoassay was used to measure plasma kallikrein (PKa) and FXIIa (WES, Protein Simple).
- FXIIa and FXII-T enzyme activity assay:** FXII-T (a triple mutant of FXII with R334A, R343A and R353A substitutions that does not undergo conversion to FXIIa) and wild-type (WT)FXII were generated in HEK293-6E cells. FXIIa (1nM) and FXII-T (200nM) amidolytic activity was measured as the rate of fluorogenic substrate H-D-Pro-Phe-Arg-AFC cleavage at 37°C across 5 minute and 60 minutes assays, respectively. A range of concentrations of KV998086/KV998083 were added prior to substrate addition. The IC₅₀ was calculated as the concentration of inhibitor that exerts half maximal inhibitory effect.
- PolyP stimulated PKa generation:** FXII-T was incubated with plasma prekallikrein (PK) and stimulated with long-chain polyphosphates (PolyP, p700) at 37°C. Time course and KV998086 dose response experiments were conducted. For mouse whole plasma studies, wild type recombinant human FXII, WT-FXII and FXII-T were incubated with FXII-KO plasma and PolyP at 37°C. High molecular weight kininogen (HK) was measured.

Table 1. Structurally diverse small molecule FXIIa inhibitors

| | KV998083 | KV998086 |
|-------------------|---------------------------|---------------------------|
| Enzyme | IC₅₀ nM | IC₅₀ nM |
| Factor XIIa | 36.9 | 10.0 |
| Factor Xa | >40000 | >40000 |
| Plasma Kallikrein | >40000 | >40000 |
| Thrombin | >40000 | >40000 |
| Plasmin | >40000 | >40000 |
| Trypsin | >40000 | >40000 |

Results

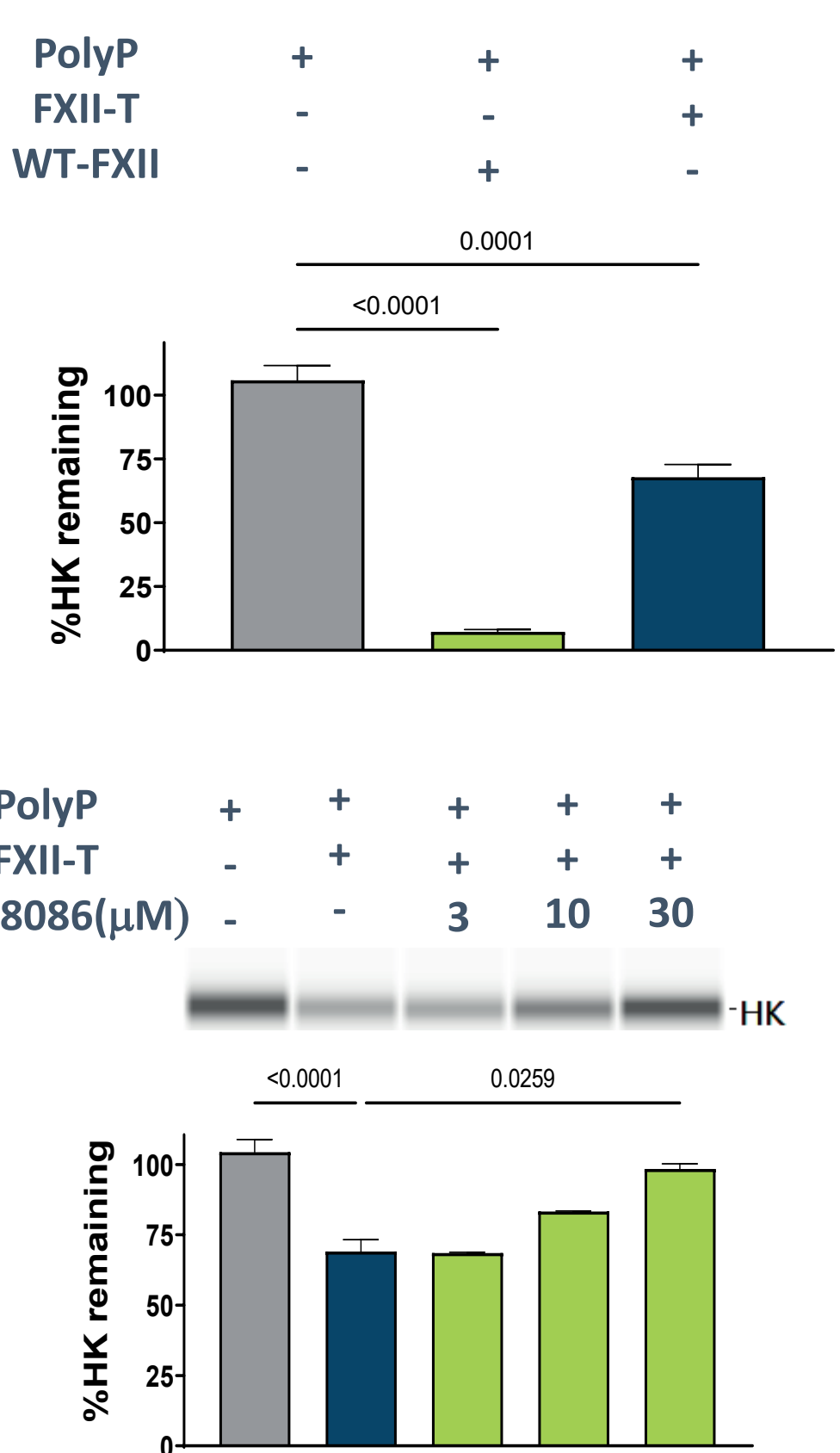
Figure 1: Effects of KV998086 on dextran sulfate (DXS) stimulated KKS activation in whole human plasma



KV998086 blocks the generation of FXIIa and PKa in human plasma

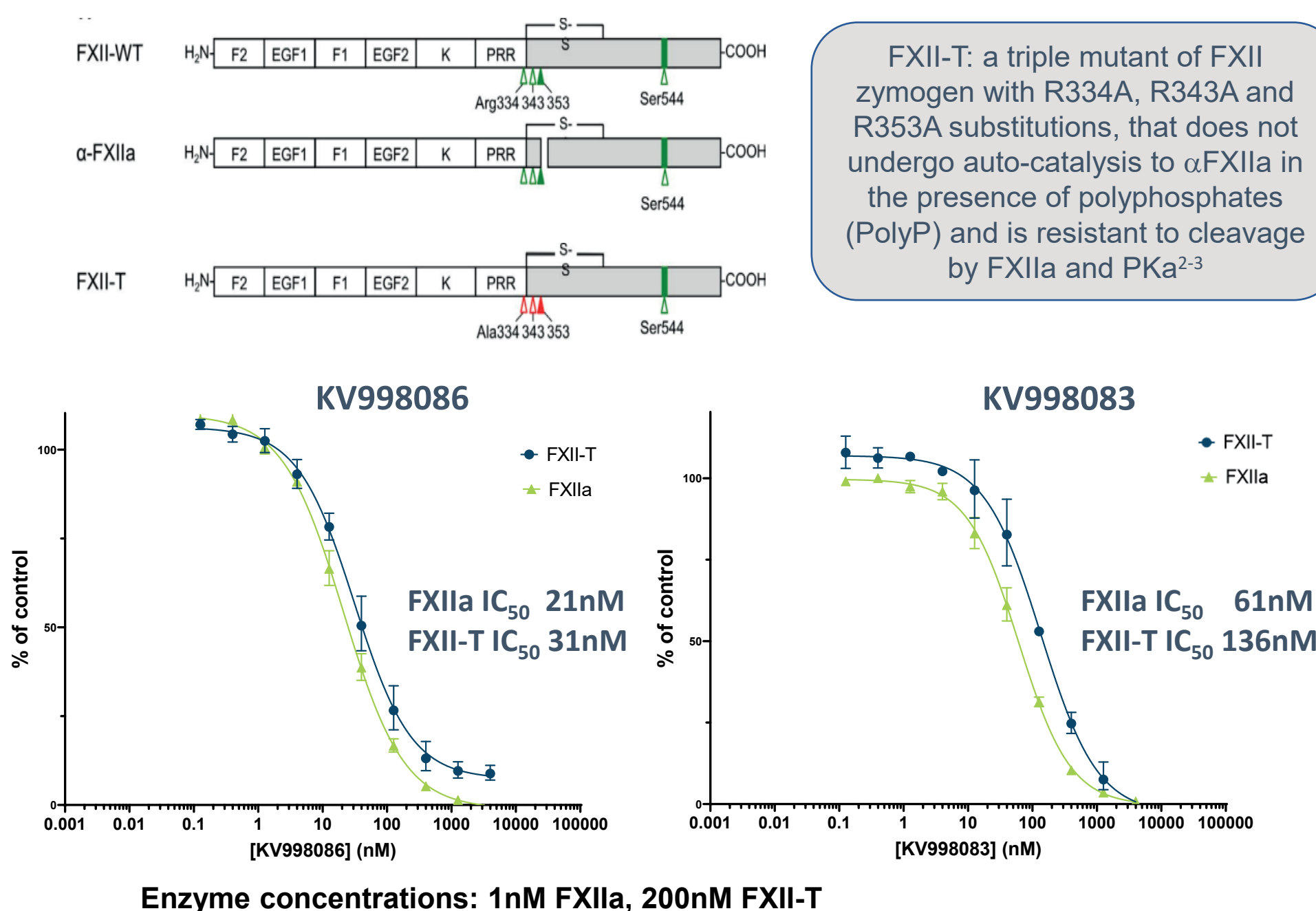
Results

Figure 4: Effects of WT-FXII, FXII-T and KV998086 on HK cleavage in murine FXII KO plasma



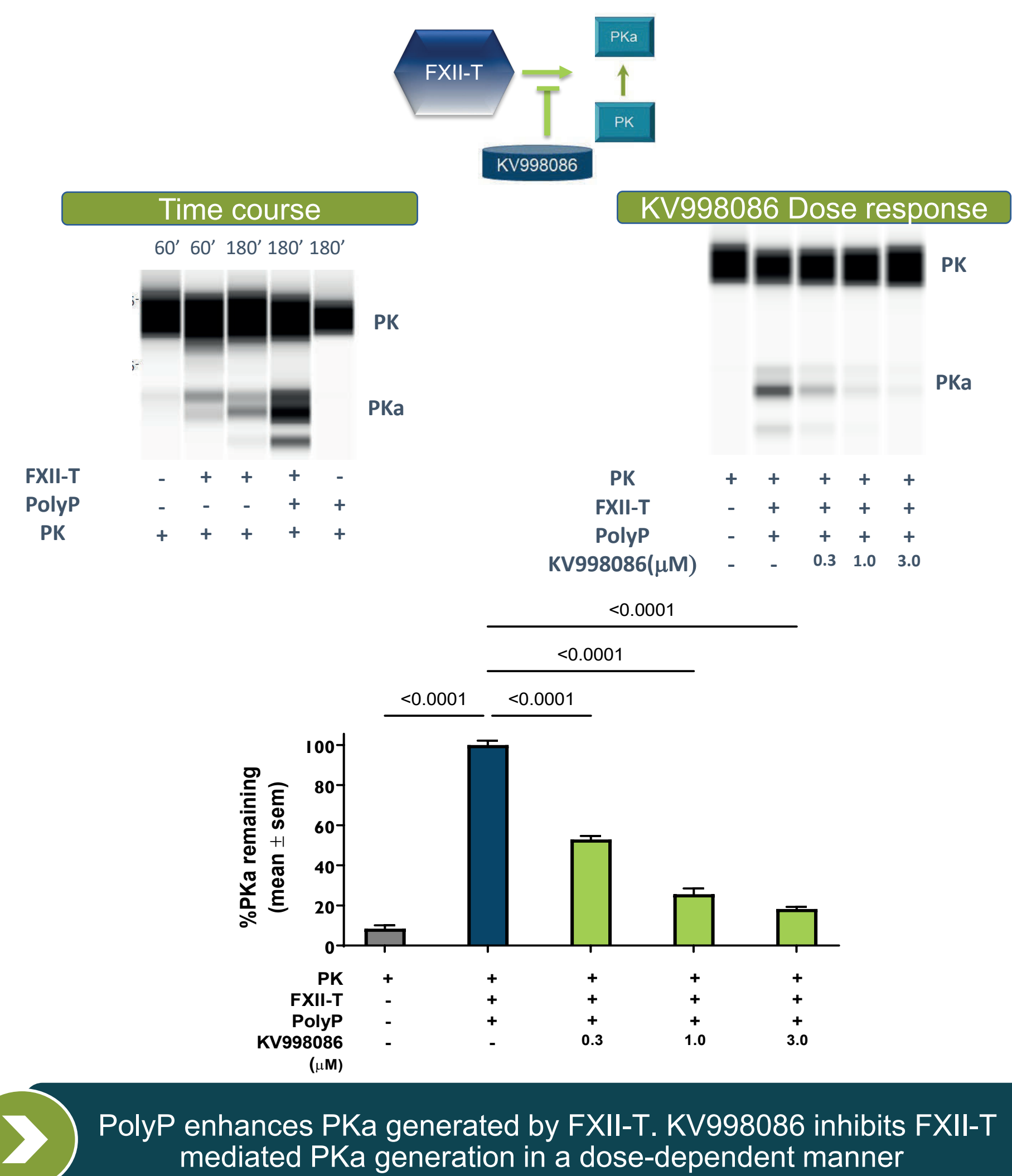
KV998086 inhibits FXII-T stimulated HK cleavage in FXII KO mouse plasma

Figure 2: Effect of small molecule FXIIa inhibitors on FXIIa and FXII zymogen protease activity



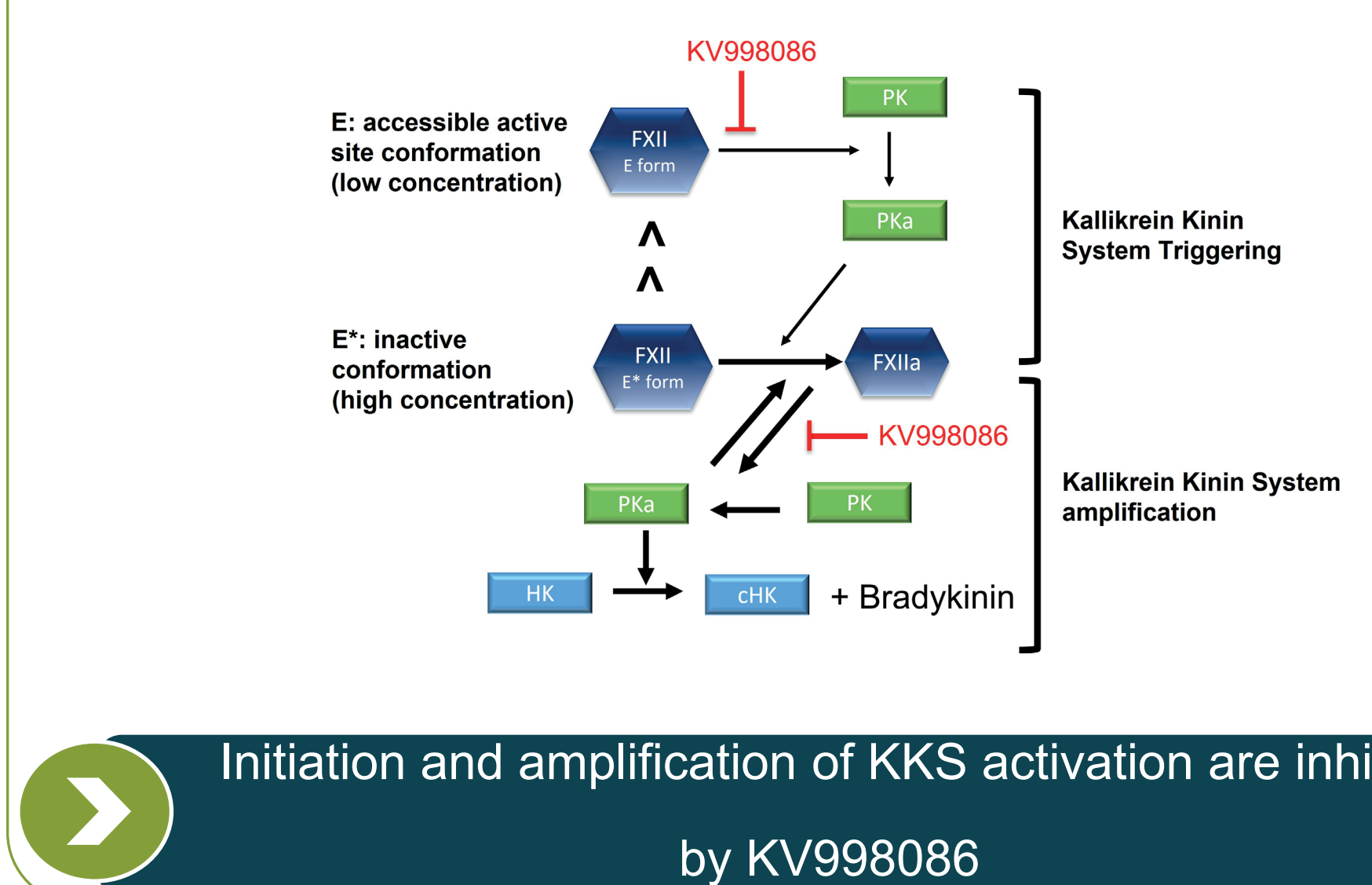
Structurally diverse FXIIa inhibitors inhibit FXIIa and FXII-T enzyme activity with comparable IC₅₀s

Figure 3: Effect of KV998086 on PolyP stimulated PKa generation by FXII-T



PolyP enhances PKa generated by FXII-T. KV998086 inhibits FXII-T mediated PKa generation in a dose-dependent manner

Figure 5: Effects of KV998086 on FXII zymogen and FXIIa mediated KKS activation



Initiation and amplification of KKS activation are inhibited by KV998086

Conclusions

- KV998086 is a potent FXIIa inhibitor that also suppresses FXII zymogen mediated kallikrein kinin system activation
- IC₅₀ results suggest:
 - Only a small fraction of FXII-T is in the conformation with an accessible active site
 - Interactions of KV998086 with the active site that mediate proteolytic inhibition are preserved in FXII and FXIIa
- KV998086 inhibits zymogen activity that can contribute to the initiation of KKS activation

Acknowledgements

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References

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Conflict of interest disclosure

All authors are employees of KalVista Pharmaceuticals Inc.