Preclinical safety and toxicokinetics of VMX-C001 – an intravenous bypassing agent for factor Xa inhibitors

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INTRODUCTION

VMX-C001, a modified form of human zymogen factor X, is being developed to stop or prevent bleeding in patients taking factor Xa inhibitors and is currently in human phase I studies.

AIM

To examine the safety pharmacology of VMX-C001 in rats and cynomolgus monkeys in a formal GLP toxicology format.

METHODS

All animal studies were performed at Charles River Laboratories using GMP grade VMX-C001 drug product. After dose range finding studies, 2-week toxicity studies were performed at doses of 20, 50 and 100 IU/kg/day VMX-C001, followed by a two-week recovery period. Safety parameters included: clinical signs, body weight, food consumption, ECGs, respiratory function, hematology, coagulation (including TAT, D-dimer and thrombin generation), clinical chemistry, urinalysis, histology and anti-drug antibodies (ADAs).

RESULTS

Analysis of VMX-C001 levels in plasma revealed linear and dose proportional toxicokinetic without an effect of sex or period of administration. Accumulation of VMX-C001 was not observed after repeated dosing. The distribution volume of VMX-C001 was 55-80 ml/kg in monkeys and 100 – 150 ml/kg in rats, indicating a single compartment model limited to the blood circulation. The half-life of VMX-C001 was 4-5 hours in rats and 6-8 hours in monkeys (**Table 1**).

ADAs for anti VMX-C001 were observed on Day 29 in two female rats at 100 IU.kg.day. One of these females also tested positive for anti (human) Factor X antibodies. In monkeys, two female monkeys were positive for anti VMX-C001 on Day 15, of which one was also positive for anti factor X. On Day 29, one male tested positive for anti VMX-C001 and one female tested positive for both ADA type (**Figure 1**). None of the animals developed a coagulation abnormality indicating the antibodies had no functional significance.

There were no unscheduled deaths, no clinical signs, no effects on body weight or food consumption nor on ECGs. There were also no coagulation changes (**Figure 2 and Figure 3**), as well as no hematology, clinical chemistry and urinalysis changes during the dosing and recovery periods.

CONCLUSIONS

Administration of VMX-C001 for 2 weeks was clinically well tolerated in rats and cynomolgus monkeys at levels of 20, 50 and 100 IU/kg/. The No-Observed-Adverse-Effect Level (NOAEL) of VMX-C001 was considered to be the highest dose administered in both species.

Table 1: Pharmacokinetic parameters of VMX-C001 in monkeys after the first dose. Legend: Concentration at time of dosing (C_0), Area Under the Concentration-time curve from the time of dosing to the last observed concentration, Dose Normalized ($AUC_{last\ ,\ DN}$), Mean Residence Time (MRT), apparent elimination Half-life ($t_{1/2}$), total systemic drug Clearance (CL) Volume of distribution (V_d). Note: a dose of 20 IU/kg equals 2 mg/kg.

First dose (Day 1) – Male, cyno

Dose	N	C_0 (µg/mL)	$AUC_{last, DN}$ (µg.h/mL)	MRT (h)	$t_{1/2}$ (h)	CL (mL/h/kg)	V_d (mL/kg)
2 mg/kg	3	44.56	138.3	6.949	6.197	7.033	62.88
5 mg/kg	3	94.51	140.4	7.302	6.893	6.498	64.34
10 mg/kg	3	190.0	165.6	7.501	7.024	5.521	55.88

First dose (Day 1) – Female, cyno

2 mg/kg	3	36.22	127.6	7.355	7.845	7.399	83.05
5 mg/kg	3	88.69	134.8	7.329	7.413	6.687	71.54
10 mg/kg	3	200.5	166.8	7.427	7.000	5.616	56.23

First dose (Day 1) – Male, rat

Dose	N	C_0 (µg/mL)	$AUC_{last, DN}$ (µg.h/mL)	MRT (h)	$t_{1/2}$ (h)	CL (mL/h/kg)	V_d (mL/kg)
2 mg/kg	10	34.36	64.04	4.413	4.746	15.31	104.8
5 mg/kg	10	85.70	58.56	4.315	4.438	16.81	107.6
10 mg/kg	10	175.0	58.86	4.313	4.985	16.65	119.7

First dose (Day 1) – Female, rat

2 mg/kg	10	33.60	49.89	4.051	5.325	19.59	150.5
5 mg/kg	10	94.63	49.98	4.049	4.969	19.65	140.9
10 mg/kg	10	192.5	53.86	4.179	5.053	18.21	132.8

Figure 1: Anti-drug antibodies in cynomolgus monkeys. ADAs specific for human Factor X (circles) and for VMX-C001 (black triangles) are indicated. Legend: Day 15 (D15), recovery animals (D29).

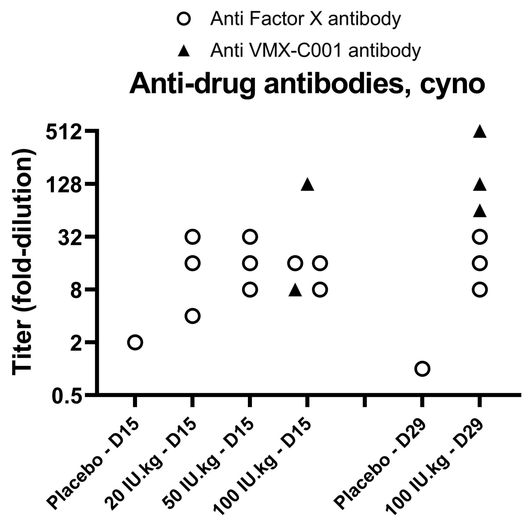

Figure 2: Safety coagulation. D-dimer and TAT complex ELISA were measured in samples from monkeys before VMX-C001 administration (Pretreatment), and 30 minutes (H0.5) plus 4 hours (H4) after administration on the first day of dosing (Day 1). Additionally, sampling was done before (H0) and after the last dose (H0.5 + H4) on Day 13, and at the end of the study (Day 15) and from recovery animals (Day 29). D-dimer and TAT complexes were measured using commercially available reagents. Data from female and male monkeys are shown together for all timepoints except for Pretreatment.

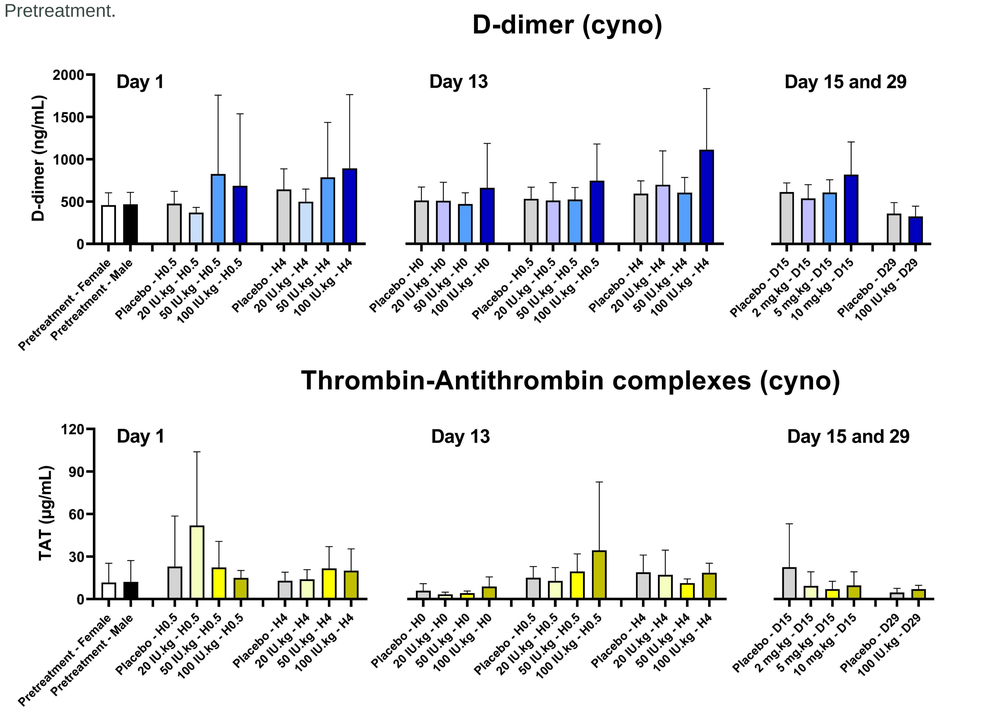

Figure 3: Thrombin generation. Samples were collected before and 5 minutes after VMX-C001 dosing on the first day (D1) and last day (D13) of dosing and calibrated automated thrombography was carried out using platelet poor plasma reagent (PPP; 5 pM TF, 4 µM PCPS, Stago Diagnostica). Data from female and male animals are shown together for all timepoints except for Pretreatment.