# PHARMACOKINETICS OF SUBCUTANEOUSLY ADMINISTERED CB2679D/ISU304 IN WILD-TYPE AND HEMOPHILIA B MICE

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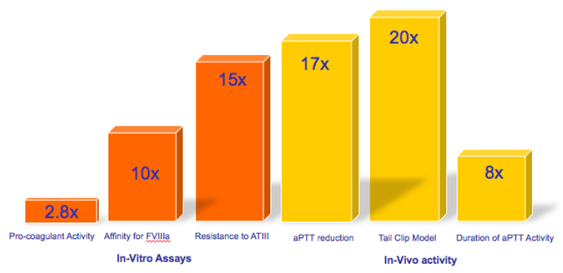
## STUDY OBJECTIVES

Determine the pharmacokinetic and pharmacodynamic parameters for subcutaneous administration of a highly potent factor IX (FIX) variant in normal and hemophilia B mice

### Introduction

- The rapid clearance of FIX necessitates frequent intravenous (IV) administrations to achieve effective prophylaxis for patients with hemophilia B (HB)
- Subcutaneous (SC) administration would be a preferred route of administration but has been limited by low bioavailability and potency of the marketed FIX products
- CB2679d/ISU304 has enhanced biological properties including resistance to inhibition by ATIII, increased affinity for FVIIIa, and increased catalytic activity compared with wild-type FIX
- The variant has three mutations: R318Y/R338E/T343R that were introduced using rational design



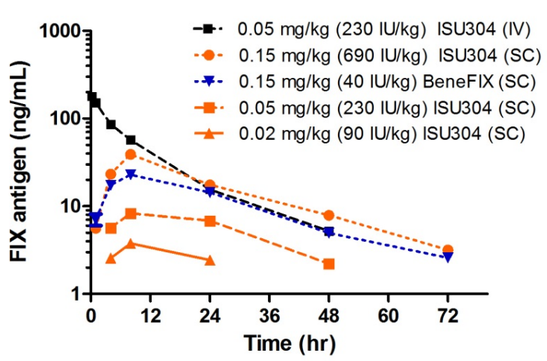

## METHODS

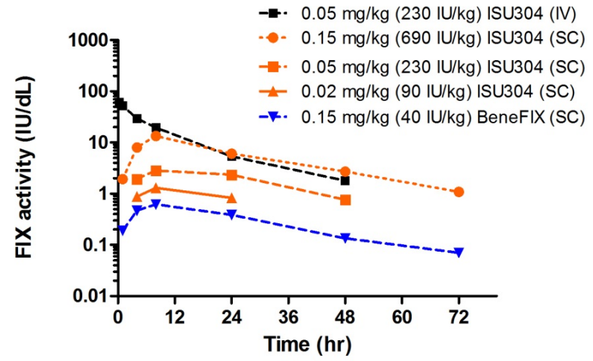
| Number of mice | Test article | Dose IU/kg | Dose mg/kg | Dosing time (hr) | Sampling time (hr) |
|---|---|---|---|---|---|
| Single dose subcutaneous injection Hemophilia B mice | | | | | |
| 1 | ISU304 | 90 | 0.02 | 0 | 4, 6, 8, 24 |
| 3 | ISU304 | 230 | 0.05 | 0 | 4, 6, 8, 24 |
| 1 | ISU304 | 690 | 0.15 | 0 | 4, 6, 8, 24 |
| Daily dose subcutaneous injection Hemophilia B mice | | | | | |
| 3 | ISU304 | 230 | 0.05 | 0, 24. 48, 72 | 24, 48, 72, 96 |
| Single dose subcutaneous injection Normal mice (except IV as indicated) | | | | | |
| 10 | ISU304 | 90 | 0.02 | 0 | 0.25, 1, 4, 8, 24, 48 |
| 10 | ISU304 | 230 | 0.05 | 0 | 0.25, 1, 4, 8, 24, 48 |
| 10 | ISU304 | 690 | 0.15 | 0 | 0.25, 1, 4, 8, 24, 48, 72, 96 |
| 10 | BeneFIX | 40 | 0.15 | 0 | 0.25, 1, 4, 8, 24, 48, 72, 96 |
| 6 | IV - ISU304 | 230 | 0.05 | 0 | 0.25, 1, 4, 8, 24, 48 |

- All mice were injected with a volume of 0.3 mL of test article
- FIX antigen was measured using a sandwich ELISA
- FIX activity was measured using a one-stage clotting assay on Stago Compact
- Pharmacokinetics of FIX was analyzed using PKSolver

## BLOOD LEVELS OF FIX ANTIGEN AND PREDICTED ACTIVITY AFTER IV OR SUBCUTANEOUS FIX ADMINISTRATION IN NORMAL MICE

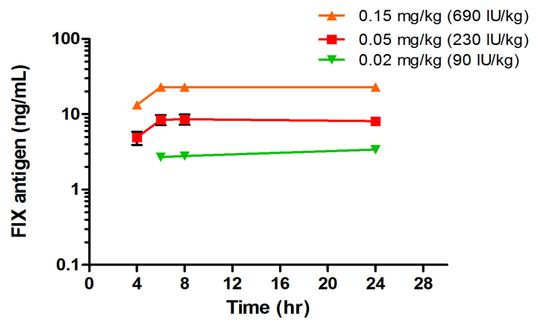
[CALCULATED FROM ANTIGEN/ACTIVITY RATIO IN HEMOPHILIA B MICE]

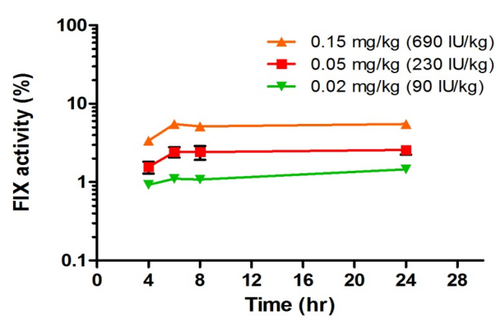



- Dose dependent increase in antigen and activity levels
- Subcutaneous activity can equate to IV-administered activity

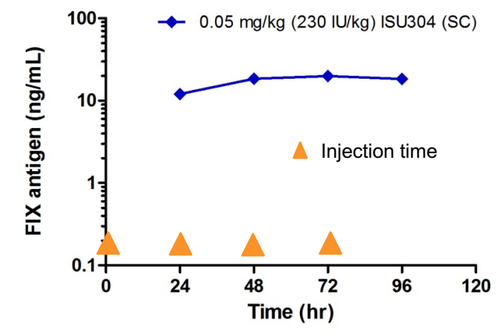
## BLOOD LEVELS OF FIX ANTIGEN AND ACTIVITY AFTER SINGLE DOSE SUBCUTANEOUS ADMINISTRATION OF CB2679D/ISU304 IN HEMOPHILIA B MICE

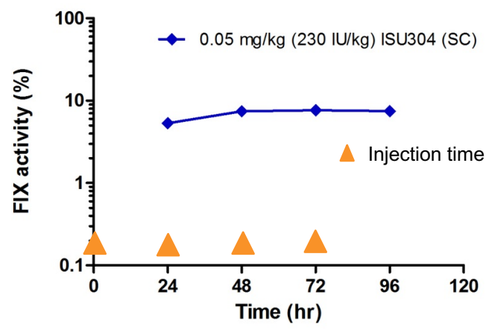



- Dose dependent increase in antigen and measured activity levels

## BLOOD LEVELS OF FIX ANTIGEN AND ACTIVITY AFTER DAILY SUBCUTANEOUS ADMINISTRATION OF CB2679D/ISU304 IN HEMOPHILIA B MICE




- **Daily subcutaneous dosing achieved steady-state levels after 3 doses moved severe hemophilia to mild hemophilia range**

## PHARMACOKINETICS IN NORMAL MICE

| Route | | Intravenous | Subcutaneous | | | |
|---|---|---|---|---|---|---|
| Test Article | | ISU304 | ISU304 | BeneFIX | ISU304 | ISU304 |
| Dose mg/kg | | 0.05 | 0.15 | 0.15 | 0.05 | 0.02 |
| Dose IU/kg | | 230 | 690 | 40 | 230 | 90 |
| Lambda_z | 1/h | 0.069 | 0.038 | 0.035 | 0.034 | - |
| $t_{1/2}$ | hour | 10 | 18 | 20 | 20 | - |
| Tmax | hour | 0.25 | 8 | 8 | 8 | 8 |
| Cmax | ng/ml | 179 | 39 | 23 | 8.3 | 3.8 |
| AUC 0-t | ng/ml*h | 1638 | 1,061 | 738 | 265 | 67 |
| AUC 0 inf_obs | ng/ml*h | 1713 | 1,144 | 811 | 330 | - |
| Bioavailability | % | 100 | 22 | 16 | 19 | - |

- Due to the high specific activity of CB 2679d/ISU304, subcutaneous dosing yields much higher FIX activities in mouse plasma compared with the same mass dose of BeneFIX

## SUMMARY

- CB2679d/ISU304 with enhanced biological properties was developed using a rational protein design approach and has increased resistance to inhibition by ATIII, increased affinity for FVIIIa, and increased catalytic activity compared to wild-type FIX
- There was a dose-dependent increase in plasma Factor IX antigen with subcutaneous injection of CB 2679d/ISU304
- The pharmacokinetic profile of CB 2679d/ISU304 was similar to BeneFIX when dosed using the same mass. However, CB 2679d/ISU304 has approximately 17-times greater potency and therefore can achieve higher activity at an equal mass dosing level
- Bioavailability of subcutaneous injection of CB2679D/ISU304 was 19-22% in normal mice
- Daily subcutaneous dosing of CB 2679d/ISU304 demonstrated the effects of the bioavailability, potency, time to maximal concentration, and half-life by reaching a steady-state activity sufficient to correct severe hemophilia to mild hemophilia, in hemophilia B mice, after three days

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**Disclosures:**
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