uniQure

Case Study:

Measuring transgene product activity as a demonstration of therapeutic efficacy

Paula Miranda, PhD
Team Lead Bioanalytics

EBF – Training day: BA strategies for Cell & Gene therapies - 17 September 2020

uniQure

AAV-mediated transgene augmentation mechanism-of-action

- AAV-mediated therapies:
  - Transgene augmentation (Gain-of function protein)
  - Protein or Substrate reduction (RNAi, miRNAs)
- Preclinical studies in multiple species:
  - Dose-ranging and biodistribution studies
  - TOX studies
- Enzymatic activity assays are critical component to the bioanalytical package GT
  - Potency assays
  - Transgene therapeutic efficacy

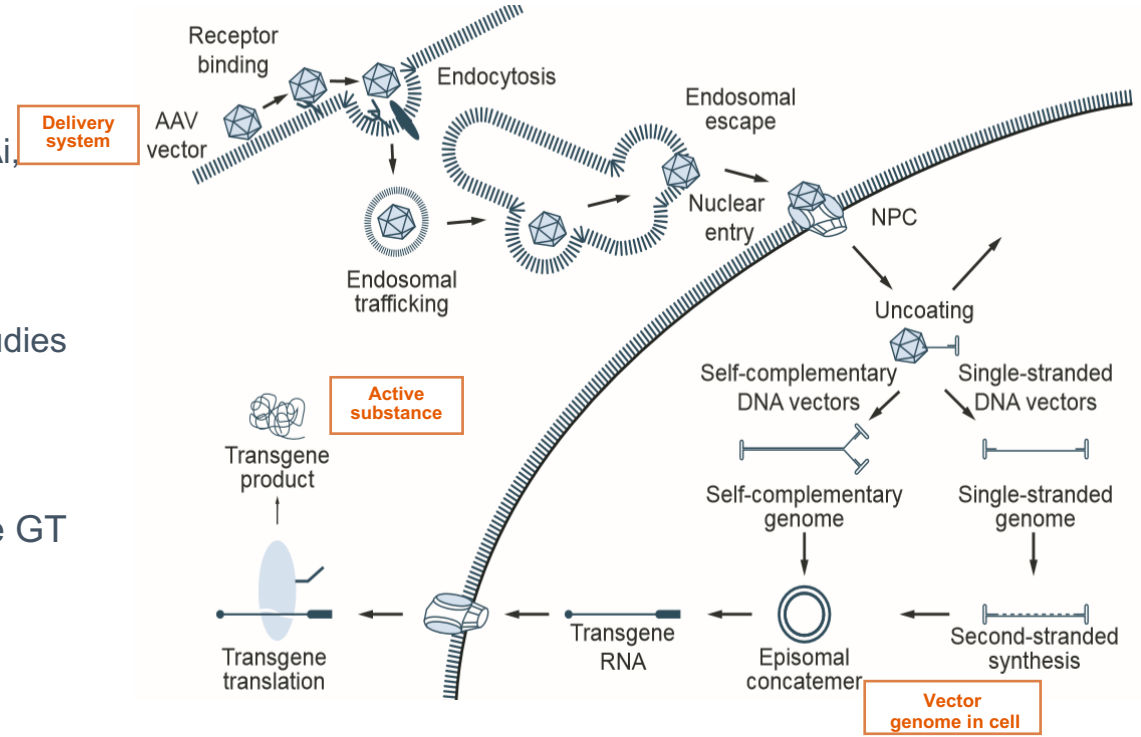


Image reproduced from: Salganik, *et al.* Microbiol Spectr, 2015.

uniQure

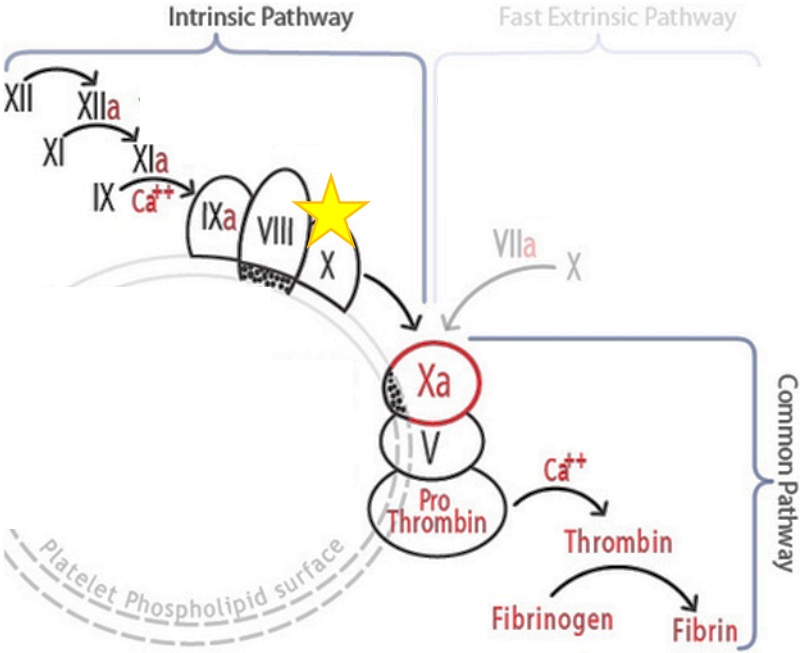
Enzymatic Activity Assays

- Complex assay system
- Enzyme activity assays are:
  - Dynamic nature
  - Sensitivity to lab conditions (temperature, reaction time…)
- GTs approaches with gain-of-function proteins:
  - Lack of true reference material (recombinant proteins)
  - Enzyme stability
  - Dynamic range of assay
  - Synergetic activity within host species
- If possible, IVD kits (assays) are preferable

uniQure

Case study: AAV-mediated Hemophilia B therapy

- Hemophilia B – Coagulation FIX deficiency
- Coagulation cascade: series of reactions in which a coagulation factor and its glycoprotein co-factor (FVIII & FV) are activated that then catalyze the next reaction in the cascade, resulting in cross linked fibrin.
- AAV-mediated therapy:
  - **hFIX**
  - **Gain-of-function FIX padua variant (naturally occurring)**
- Gain of function FIX Padua variant
  - Mutation identified in a trombophilic patient in Padua (arginine to leucine (R338L)[1]
  - Normal FIX protein levels
  - ~5- fold increase of FIX activity

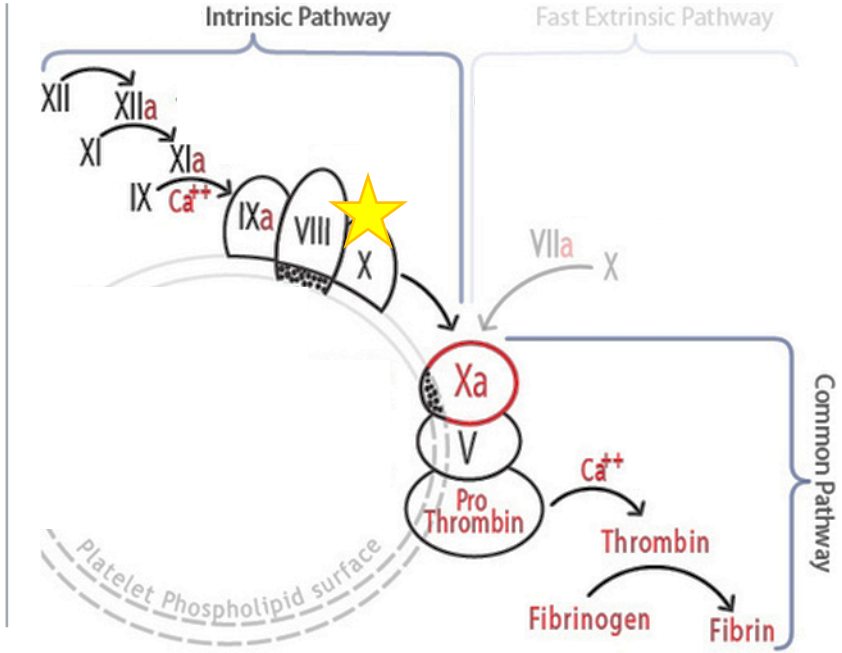


[1]Simioni, et al, N Eng J Med, 2009

uniQure

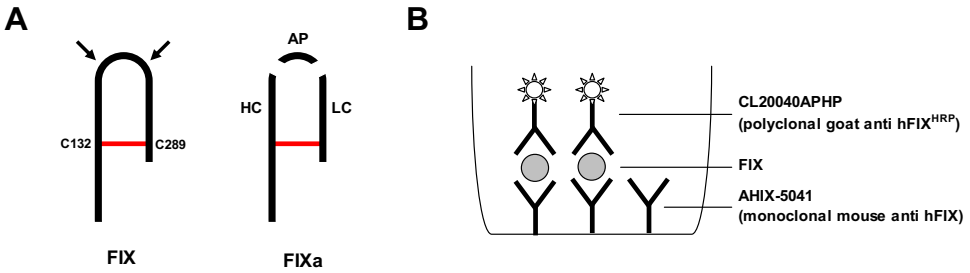
Case study: AAV-mediated Hemophilia B therapy

- Pre-clinical strategy:
  - Mice
  - Pigs
  - NHP
- Part of our BA package:
  - **Normal hFIX protein levels - ELISA**
  - **~5- fold increase of FIX activity – Chromogenic assay**



[1]Simioni, et al, N Eng J Med, 2009

ELISA - hFIX protein in plasma

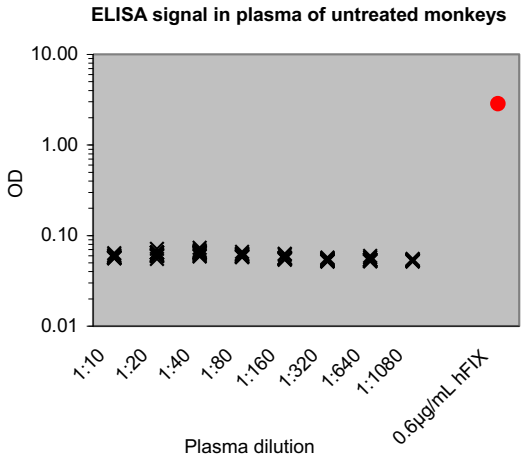


Capture antibody AHIX-5041) recognizes an epitope in the heavy chain region of human FIX, which is allegedly absent in other species

FIX: Factor IX. Arrows: (FVIIa/FXIa) cleavage sites. FIXa: activated FIX. HC: Heavy chain. AP: activation peptide. LC: light chain.
Red: disulfide bond.

Jiang H et al, Blood, 2006.

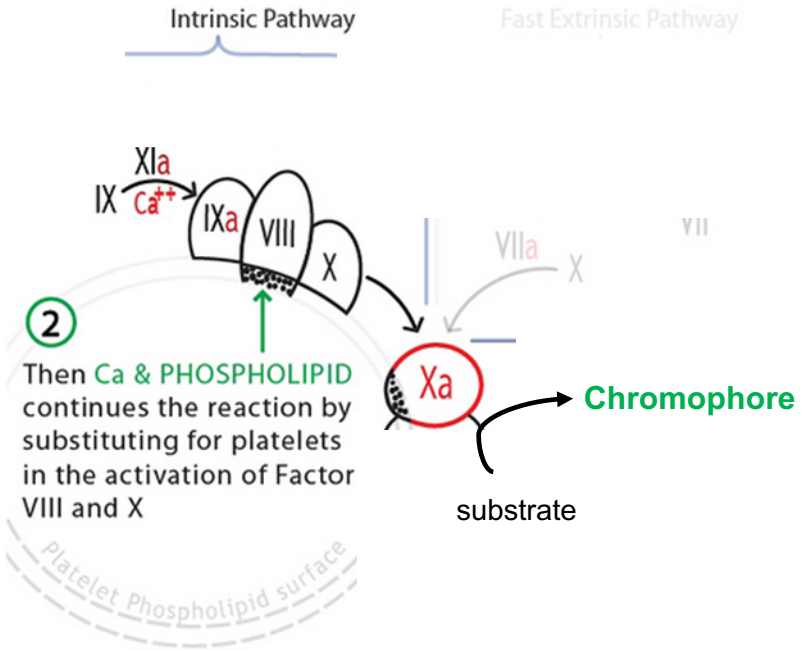
- Validated method
- Selectivity: Proven specificity for human FIX in mouse, pig and NHP plasma



uniQure

Chromogenic- FIX activity levels (kit)

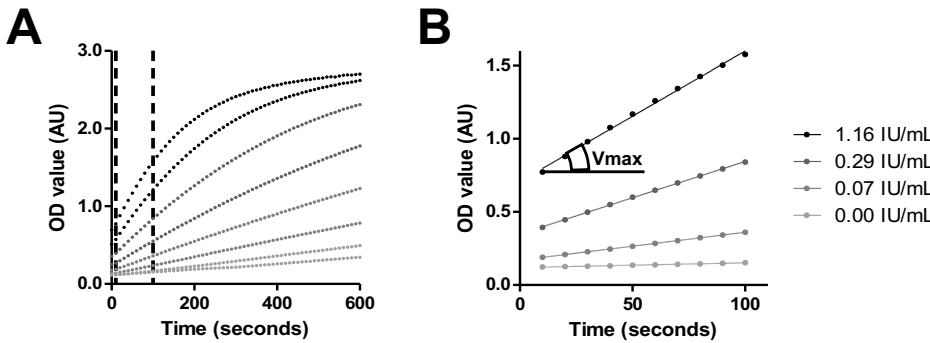
- FXIa (in excess) activates FIX from the test sample, which forms a complex, that activate FX.
- Then a chromogenic substrate specific to FXa is added, resulting in the formation of a chromophore (color development).
- The speed at which chromophore is converted is directly proportional to the amount of FIX activity present in the test sample.



uniQure

Chromogenic- FIX activity levels (kit)

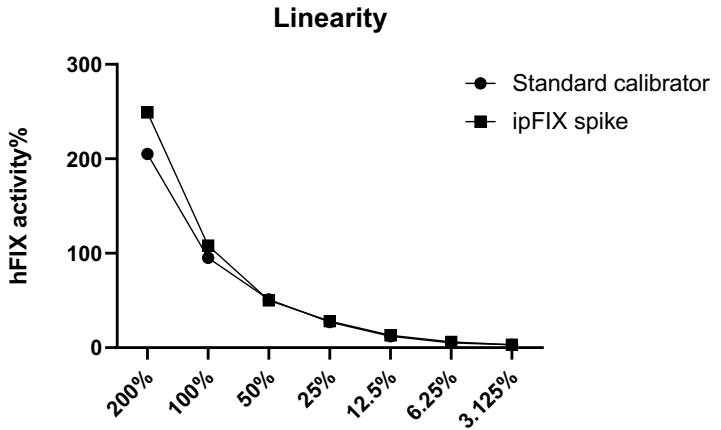
- Kinetic mode measurement substrate conversion rate, rather than an amount of converted substrate at a single moment
- In each reaction, substrate conversion was observed at speeds that seemed to be proportional to the input (Figure A).
- The slope of each line represents the initial, or maximum, conversion speed *Vmax (B)*.
- The detected FIX activity is back-fitted from the returned Vmax values



uniQure

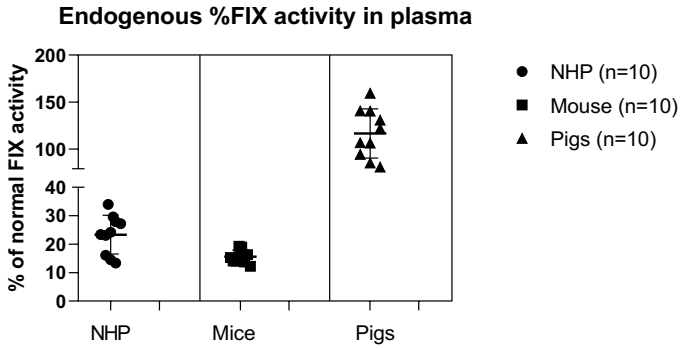
Chromogenic- FIX activity levels (kit)

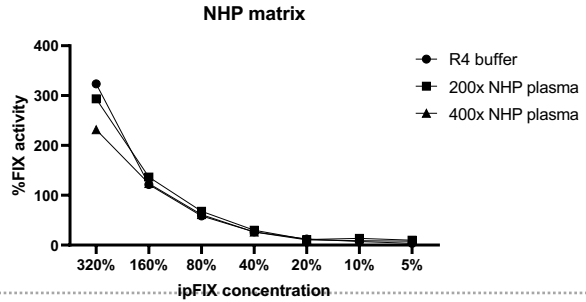
- The dynamic range of the assay sufficiently covers achievable FIX activity levels (C)
- Immunopurified hFIX was used for spiking experiments
- For assay setup, however, immunopurified hFIX presented stability issues
- Assay setup:
  - Calibrator human plasma (%hFIX normal levels)
  - QCs: Human Abnormal and Normal Calibrator plasmas (commercially available)

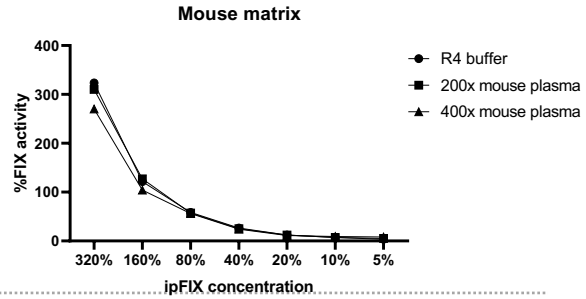


Pre-clinical studies and FIX activity levels

- This assay enables specific, sensitive, and accurate detection of FIX. However not specific to human FIX, since it measures host FIX endogenous activity.
- In pigs: FIX activity levels are very high (chromogenic assay).
- Immunopurified human FIX spike in different matrixes, indicate linearity in NHP and mouse plasma samples
- Good hFIX recovery rates within the dynamic range



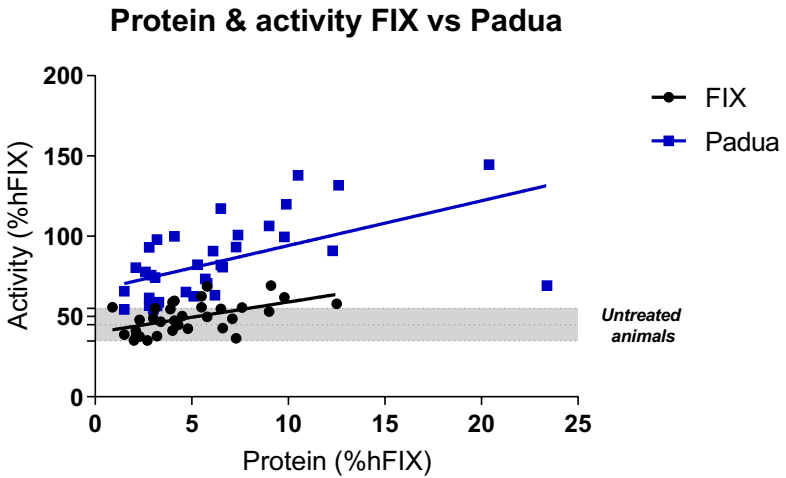




uniQure

FIX enzymatic activity of both variants correlate well with FIX protein levels

- The FIX (transgene) activity correlates well hFIX transgene protein across different pre-clinical studies
- FIX Padua gain-of-function higher % normal hFIX activity
- Most importantly, pre-clinical data was translatable to the obtained in the clinical setting (in NHPs).



uniQure

Enzymatic Activity Assays for transgene proteins

- Complex assay system
- IVD kits are preferable
- Guarantee a reliable transgene protein quantification (ELISA)
- When developing/qualifying an enzymatic activity assays for a transgene protein:
  - Dynamic nature
  - Sensitivity to lab conditions (temperature, reaction time…)
  - Gain-of-function proteins
  - Dynamic range of the assay
  - Lack of true reference material (recombinant proteins)
  - Enzyme stability (QCs)
- Host endogenous activity, can blunt/enhance the transgene product activity

Thank you!