

Towards AAV5-mediated Gene Therapy for Hemophilia A with a Factor IX Variant that functions independently of FVIII

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uniQure's approach: FVIII-independent FIX variant

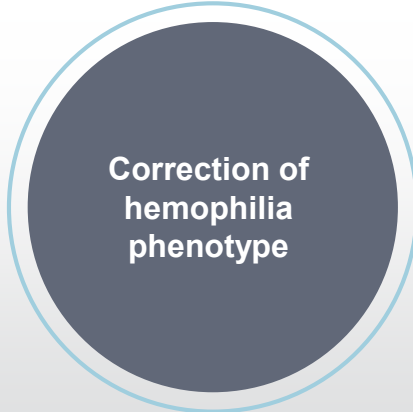
- **Novel Approach / Aspirational Goals**

- Expression of a FIX variant with FVIII-independent FX activity using AAV5 vector



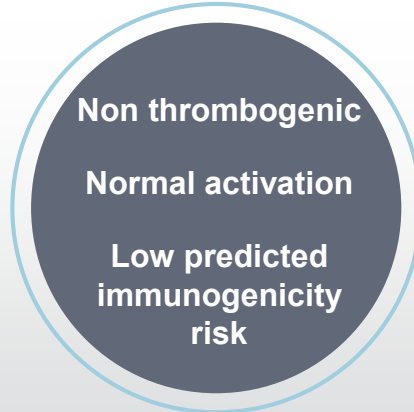
Hepatocyte friendly
Non immunogenic

Long-term expression



Correction of
hemophilia
phenotype

Intended for patients with
and without inhibitors



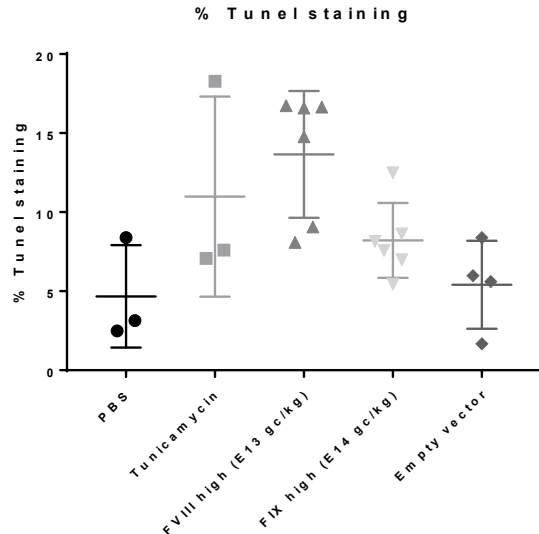
Non thrombogenic
Normal activation
Low predicted
immunogenicity
risk

Safety

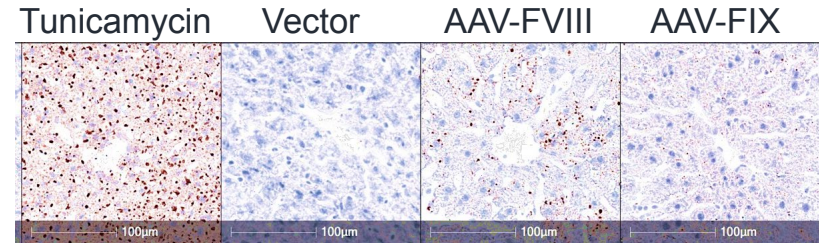
Why not express FVIII in the liver?

- Endogenous FVIII synthesis in endothelial cells and not hepatocytes
- Production site and protein load may activate the unfolded protein response *in vitro* and *in vivo*¹⁻⁵

Liver hepatocyte apoptosis



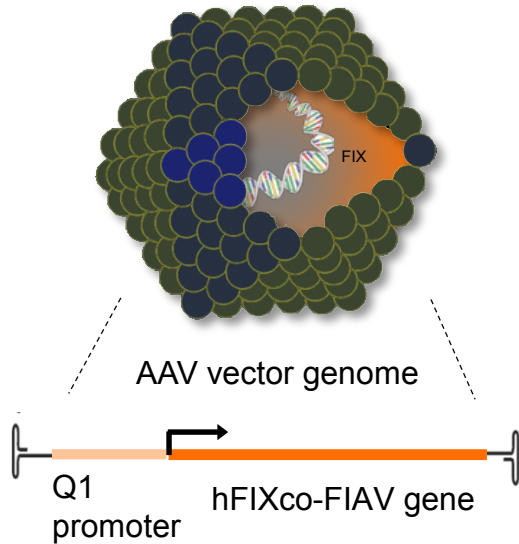
Hepatic lipid accumulation



Oil red O staining, representative staining of 1 animal

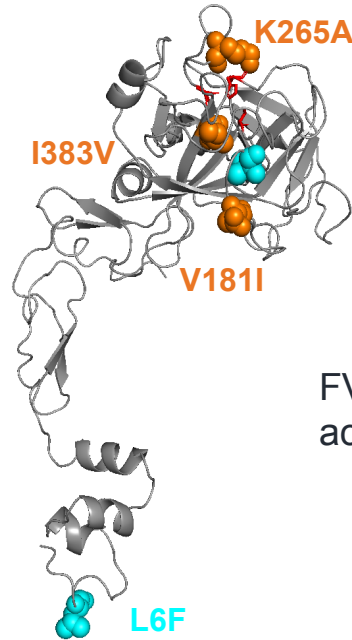
AMT-180 encodes FIX-FIAV that activates FX in the absence of FVIII

AAV5-FIX-FIAV

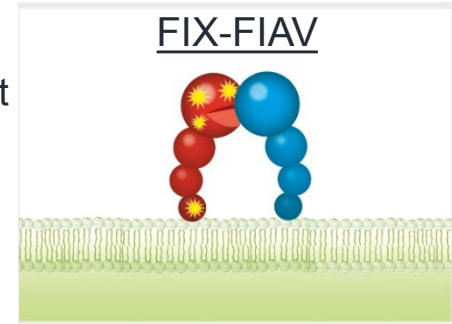
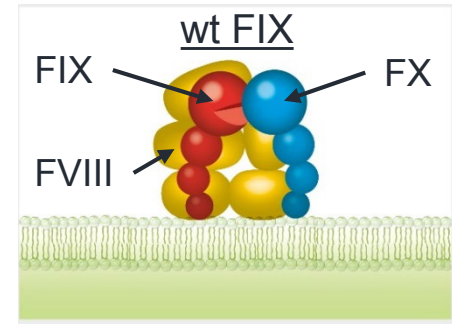


L6F, V181I, K265A, I383V

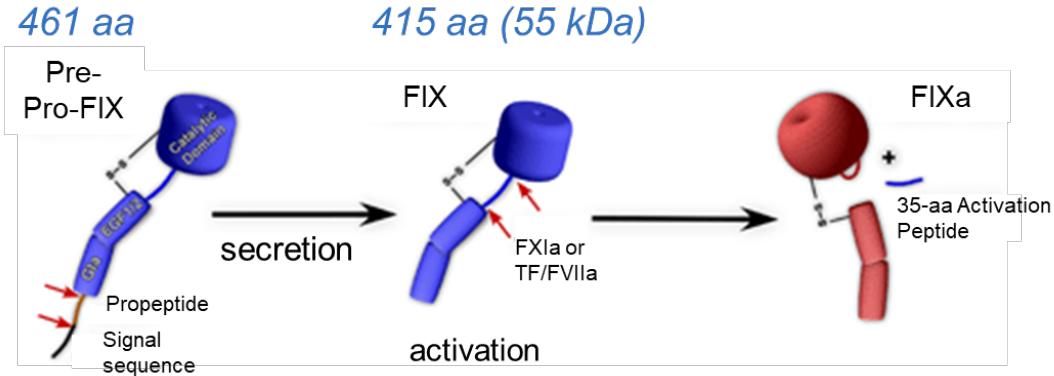
FIX-FIAV



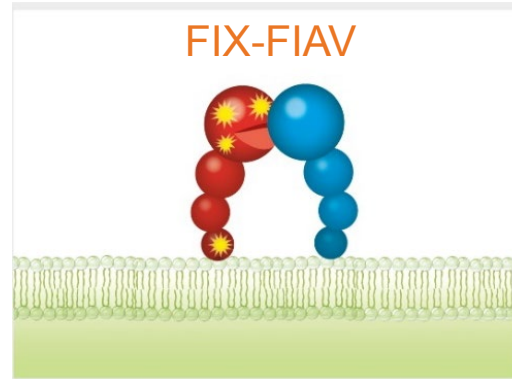
FVIII-independent
activation of FX



FIX-FIAV is a zymogen that requires physiological activation



- The inactive FIX-FIAV zymogen is expressed
- Activation is required



FX activation in the absence of FVIII

Studies to show proof of concept of FIX-FIAV *in vitro* and *in vivo*

In vitro, cells



↓
FIX protein
FVIII-independent
activity

Wt mice



↓
FIX protein

FIX-FIAV
protein



↓
Hemostasis in
human plasma

HemA mice



↓
FIX protein
FVIII-independent
activity

Cynomolgus Macaques



↓
FIX protein
Safety / tolerability

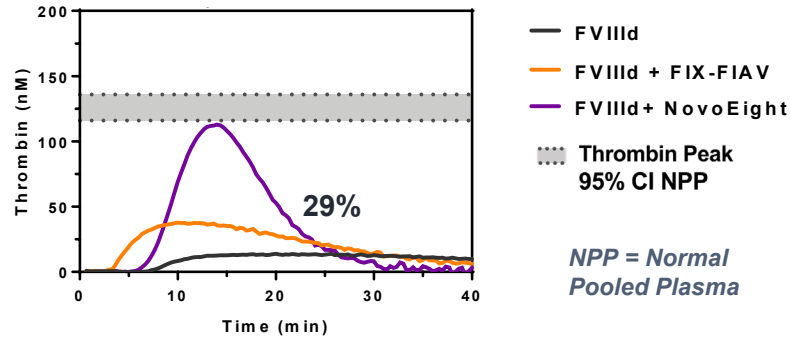
FIX-FIAV shows 32% of FVIII-independent activity in APTT and thrombin generation assay



One stage clotting assay (APTT)

FIX variant	FVIII independent activity (%)
WT	< 6
FIAV	32 ± 6

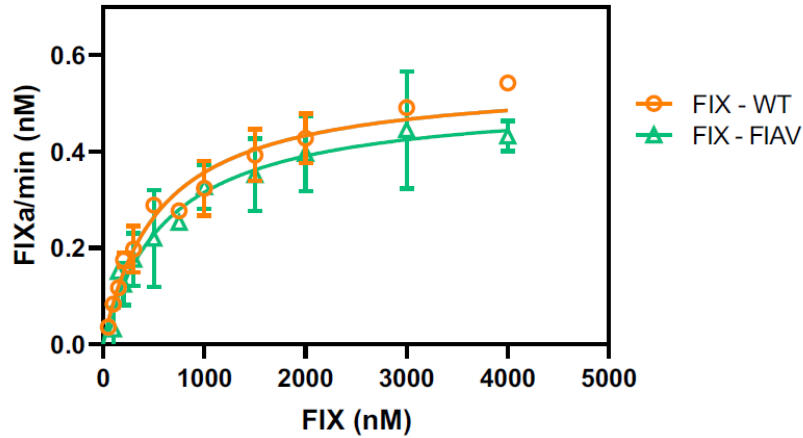
Thrombin generation assay



- FIX-FIAV (5 µg/ml) shows 32% and 29% of FVIII-independent activity by APTT and thrombin generation relative to a FVIII standard
- FIX-FIAV thrombin generation curve overlaps with the normal curve

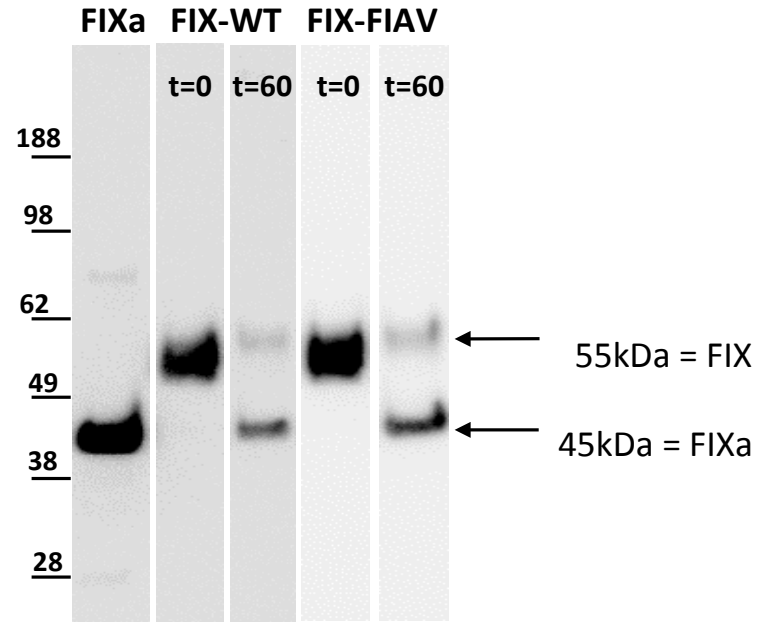
FIX-FIAV shows similar physiological activation as FIX-WT

TF/FVIIa activation of FIX variants



	FIX - WT	FIX - FIAV
kcat		
Best-fit values		
Et	= 50.00	= 50.00
kcat	0.01103	0.01027
Km	548.4	630.6
Vmax	= 0.5516	= 0.5135

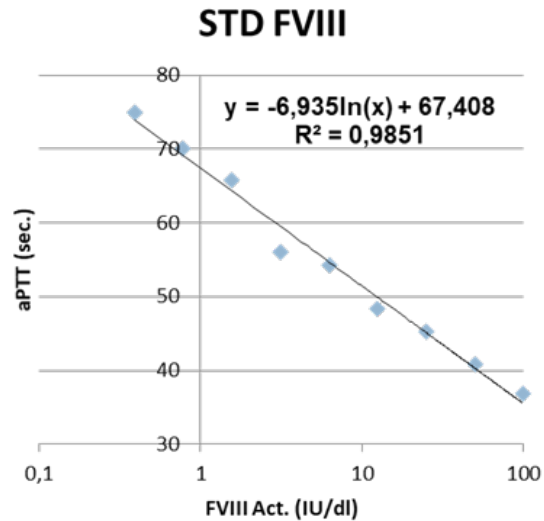
Western blot



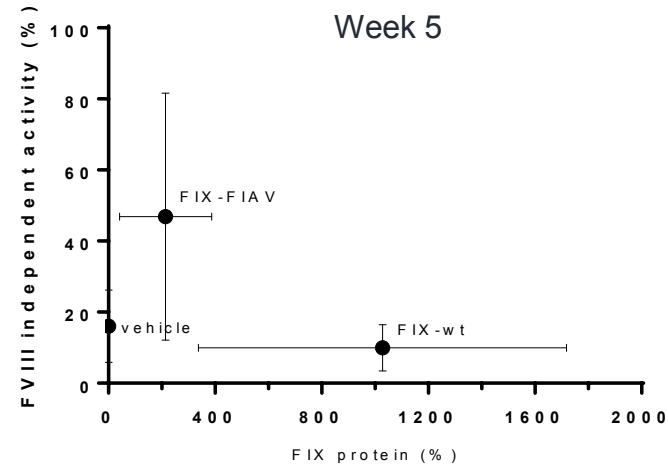
FVIII-independent activity upon AAV injection in hemophilic mice



n=10, male
FVIII KO mice
IV dose 5×10^{13} gc/kg



FVIII-independent activity vs FIX protein

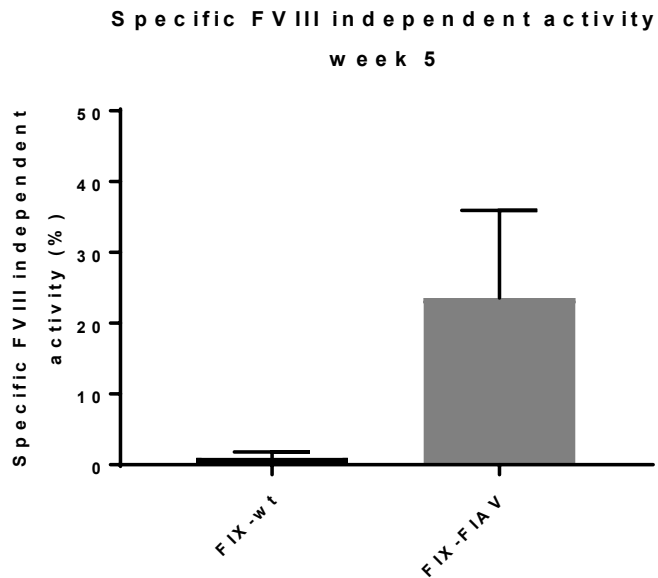


- FIX-FIAV shows FVIII independent activity in hemophilic mice
- Measured in APTT assay

FIX protein level by ELISA; FVIII activity by APTT

FIX-FIAV shows a therapeutic meaningful FVIII-independent activity in hemophilic plasma

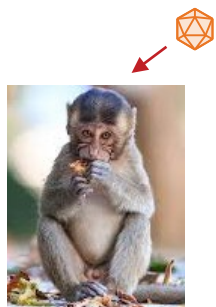
- Normalisation of the FVIII-independent activity to 100% of FIX protein
- ~**24%** of FVIII-independent activity in hemophilic mice



Summary efficacy AMT-180

- Recombinant FIX-FIAV
 - **29%** FVIII-like activity in thrombin generation assay
 - **32%** FVIII like-activity in clotting assay
- AMT-180 in hemophilic mice
 - **24%** FVIII-like activity in clotting assay
- AMT-180 expected to show clinical meaningful efficacy (per 100% protein)

FIX-FIAV expression in NHPs expected to translate to therapeutically relevant FVIII independent activity in humans



Male Cynomolgus macaque

n=2

IV, 9×10^{13} gc/kg

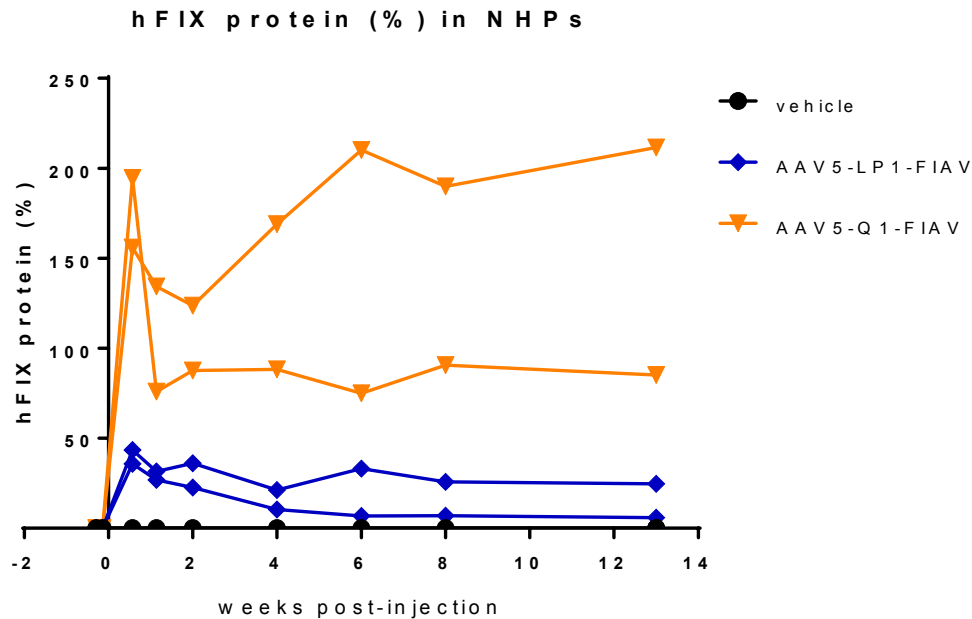
adapted delivery

1 vehicle treated NHP

1) AAV5-LP1-FIAV

2) AAV5-Q1-FIAV

Q1= a proprietary liver specific promoter



8-fold increased protein expression using Q1

Safety assessments

Thrombogenicity

- No elevation of coagulation activation markers: TAT + D-dimer levels in AAV-injected mice and NHPs
- Histopathological examination of the NHP organs did not show signs of thrombus formation

Immunogenicity (poster PB0301)

- *In silico* and *in vitro* assessment of immunogenicity potential
- FIX-FIAV poses a very low immunogenicity risk compared to FIX-wt

Conclusions

- **AMT-180 is expected to prevent bleeds**
 - Sufficient thrombin generation
 - Clot formation (APTT)
- **Safety assessments:**
 - No thrombogenicity in animal models to date (normal activation)
 - Low predicted immunogenicity risk (poster PB0301)
- **Designed for patients with hemophilia A with and without inhibitors**

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