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Ying Poi Liu[1], Vanessa Zancanella[1], Betty Au[1], Paula Montenegro-Miranda[1], Martin de Haan[1], Viola J.F. Strijbis[2], Mettine H.A. Bos[2], Karin Huber[3], Joachim Schwäble[3], Erhard Seifried[3], Pavlina Konstantinova[1], Sander Van Deventer[1]

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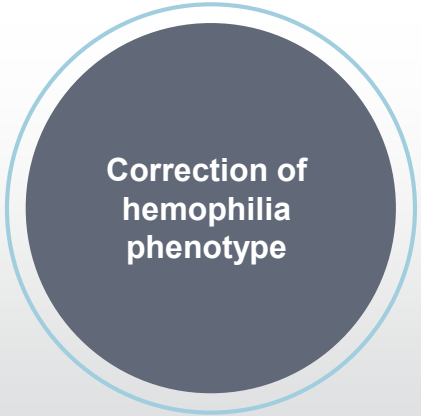

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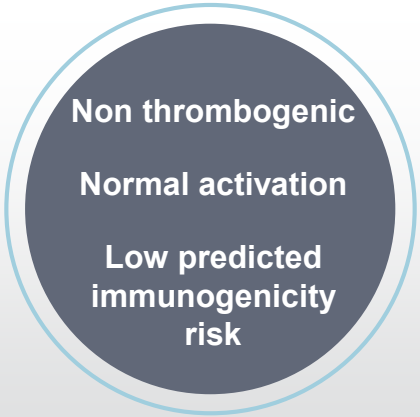

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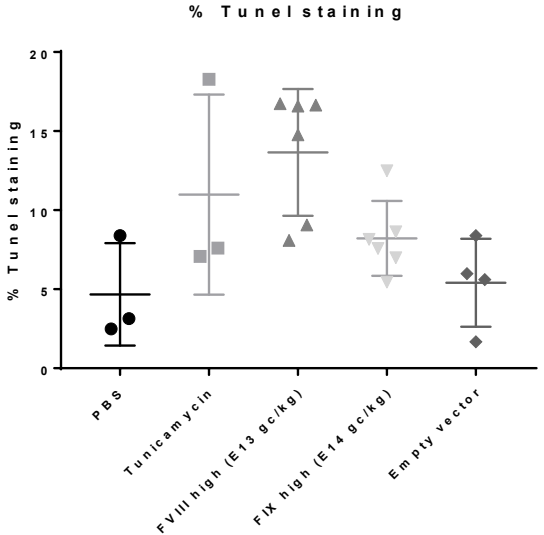

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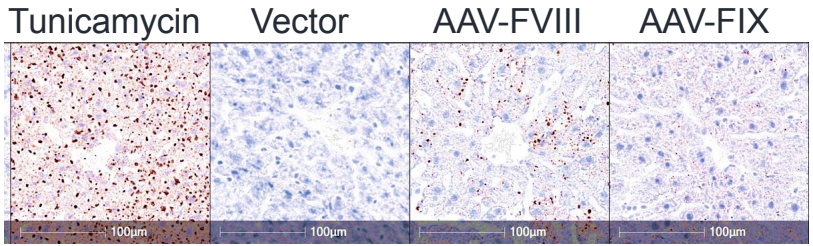

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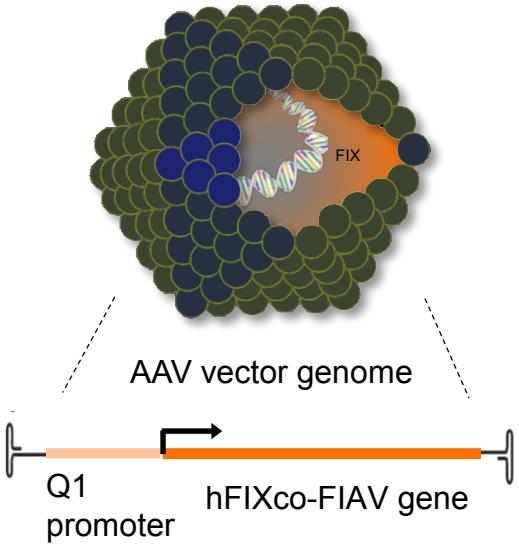

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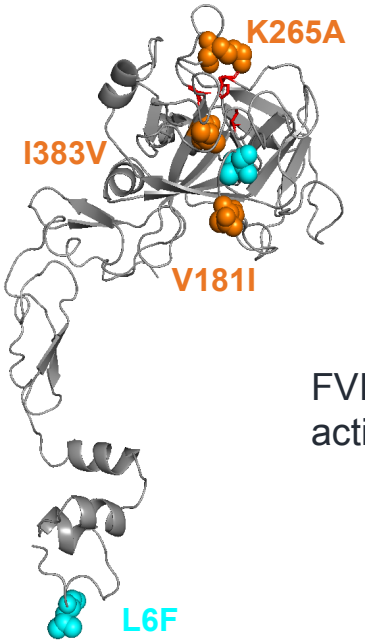

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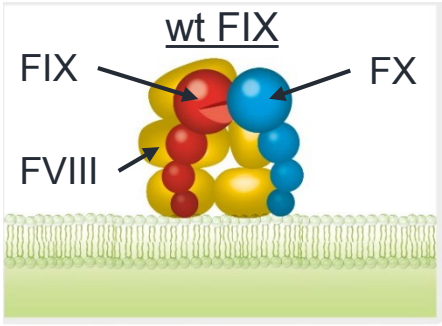

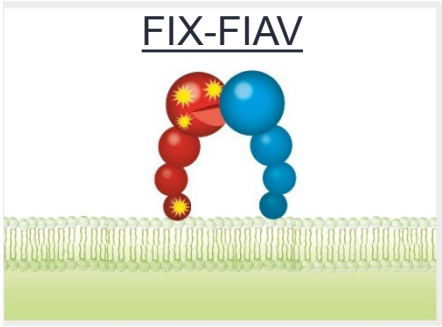

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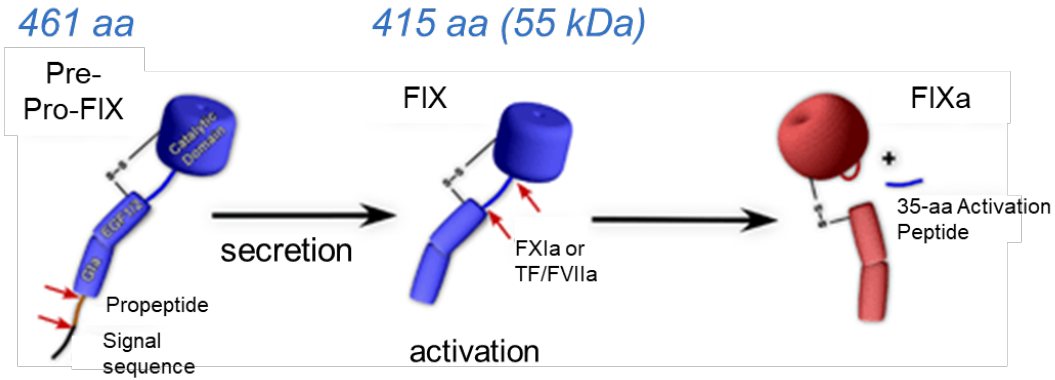

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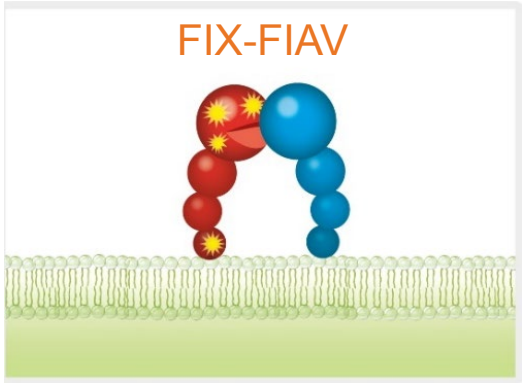

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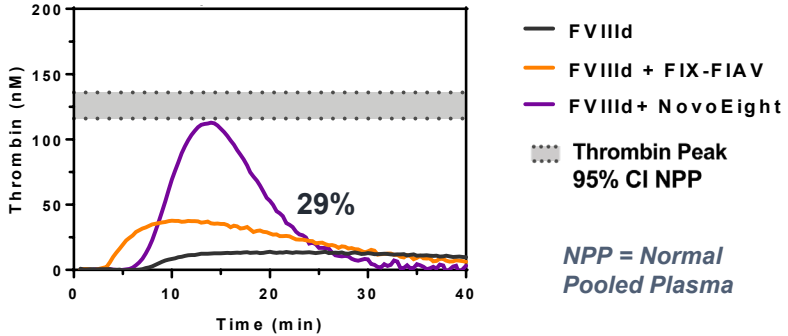

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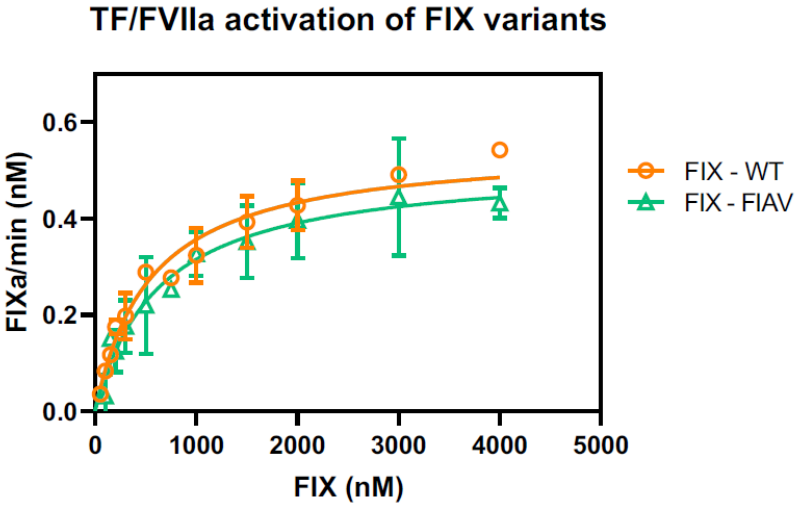

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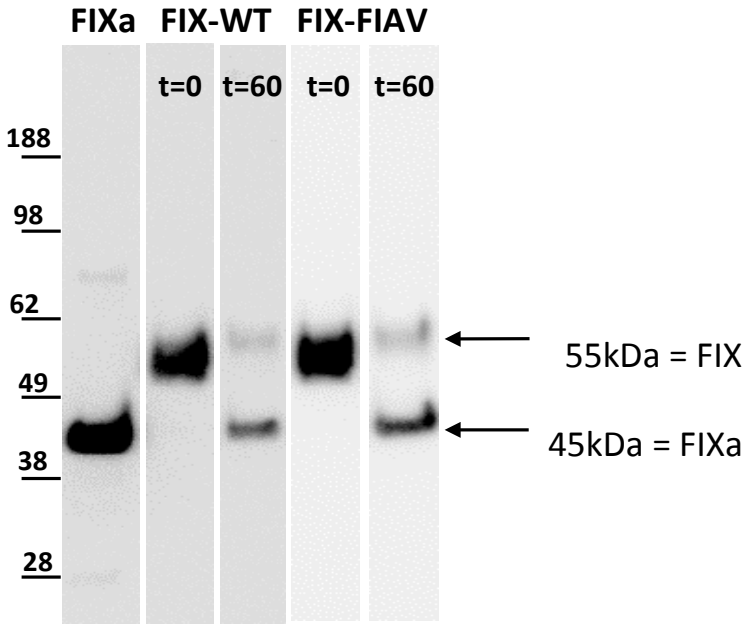

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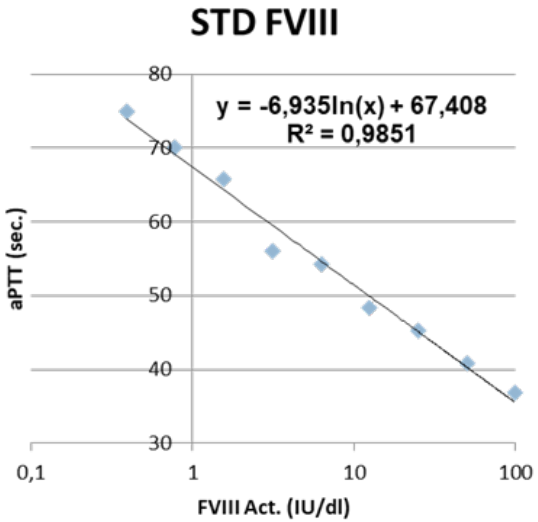

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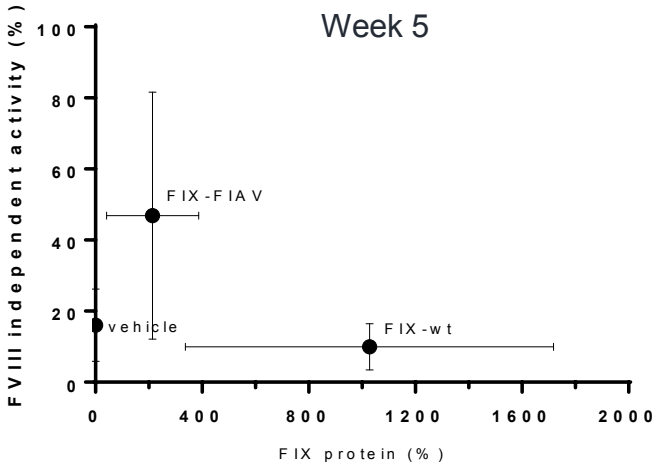

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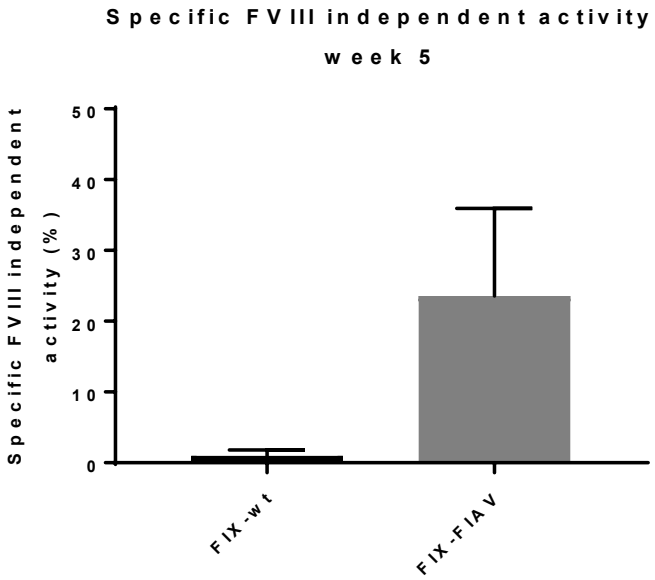

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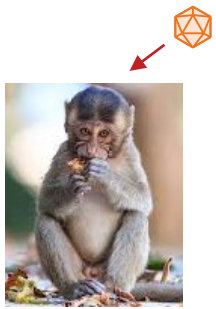

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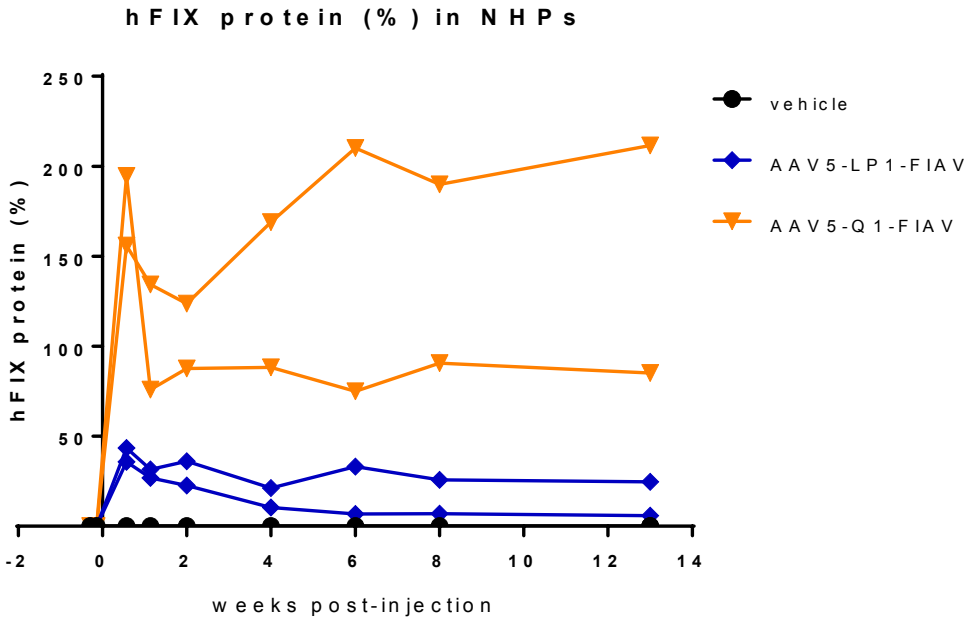

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Research
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Vanessa Zancanella
Tom van der Zon

Immunology
Nikki Timmer
Valerie Ferreira

Non Clinical
Martin de Haan
Paula Miranda
Srijana Tripathi
Corina van der Kruijssen

Vector and process development
Erich Ehlert
Tamar Grevelink
Mustafa Kyamil
Richard van Logtenstein
Maroeska Oudshoorn
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Mark van Veen
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Analytical development
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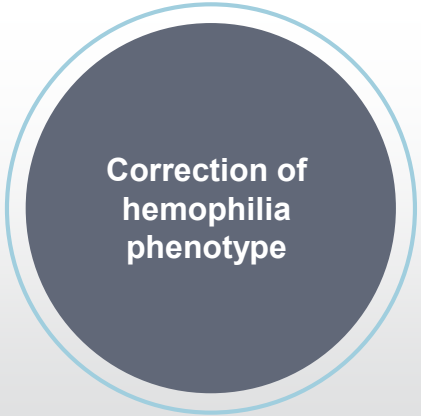

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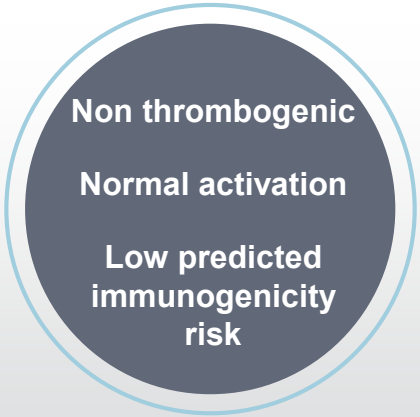

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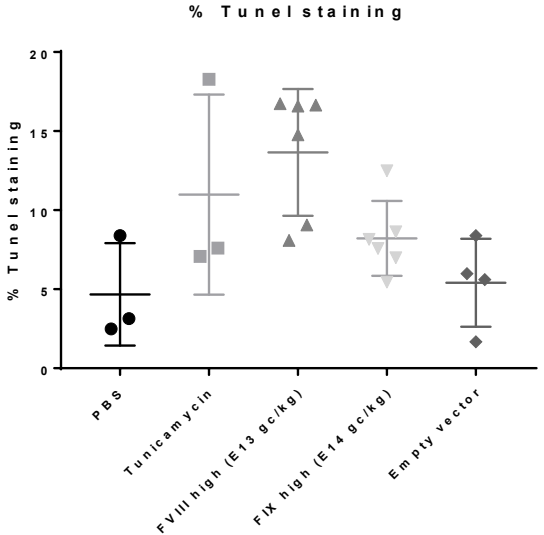

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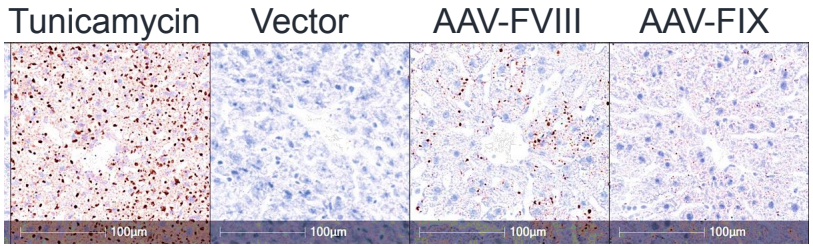

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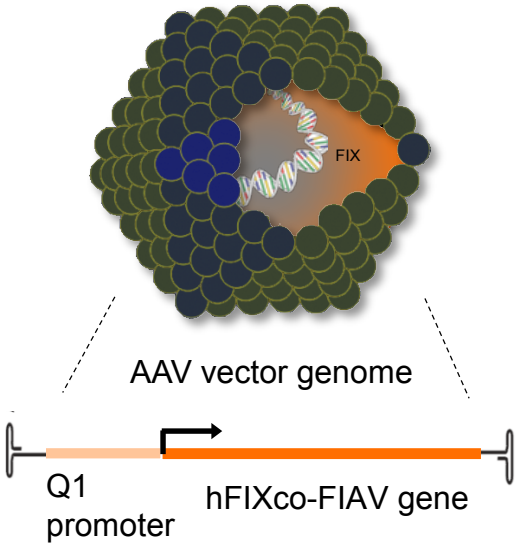

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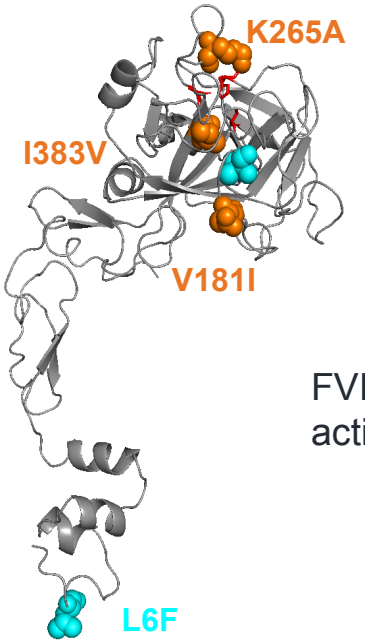

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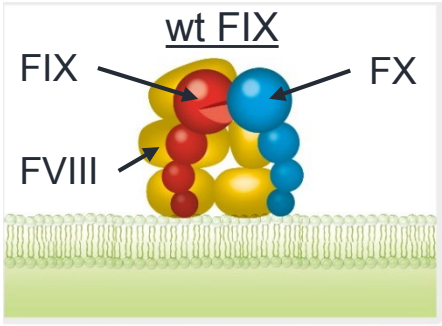

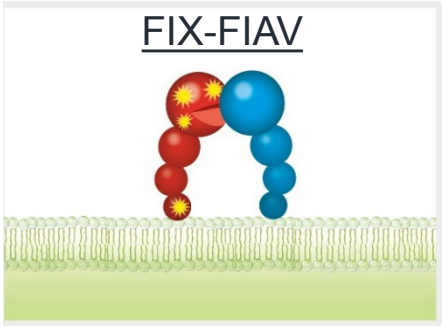

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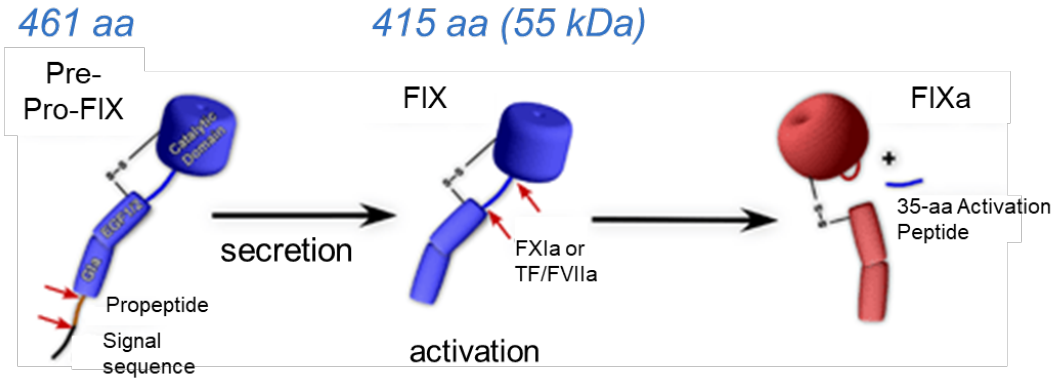

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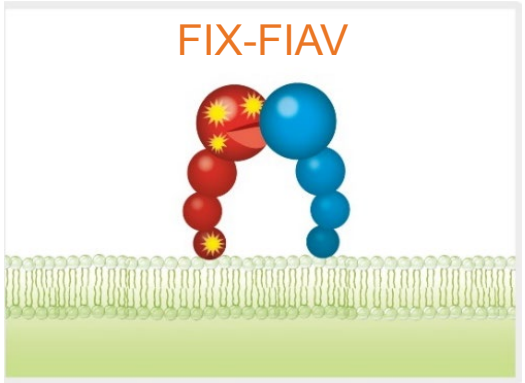

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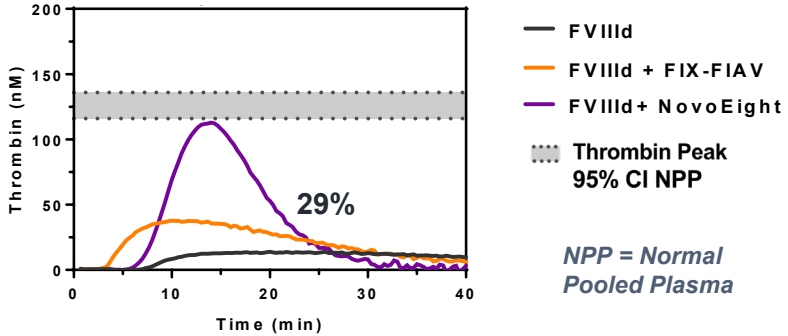

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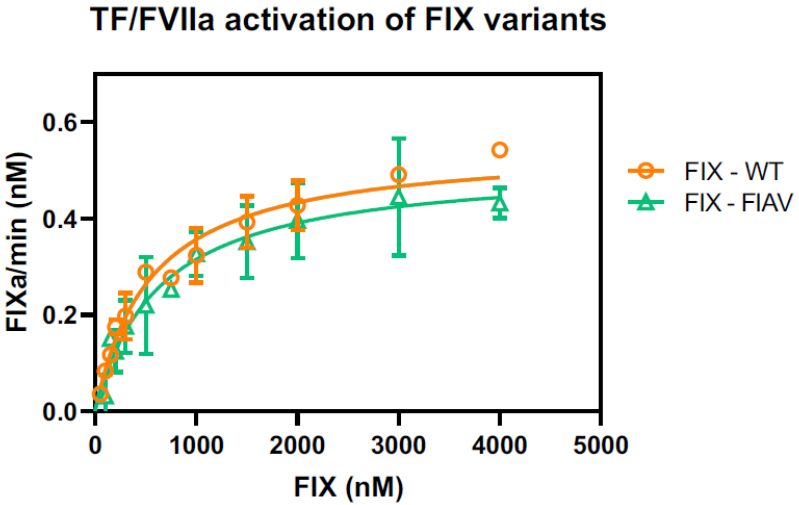

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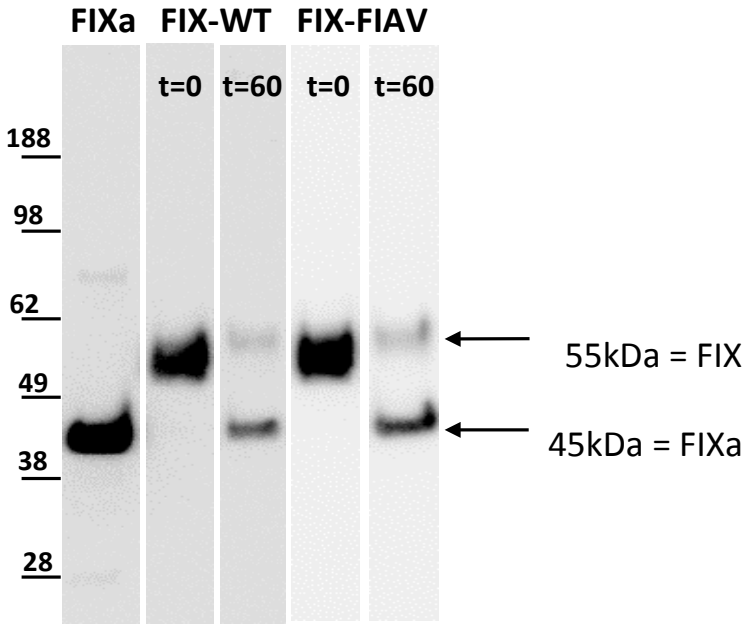

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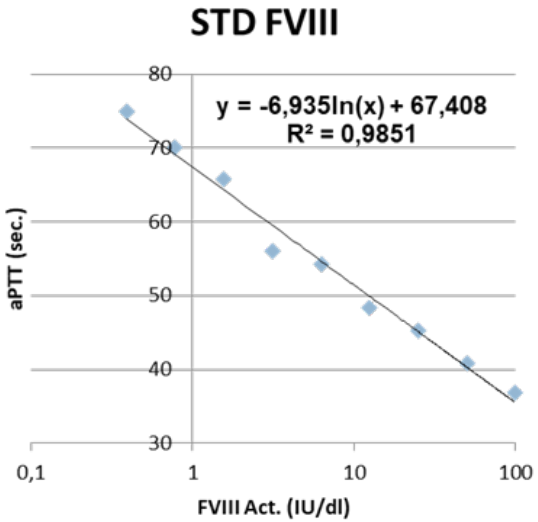

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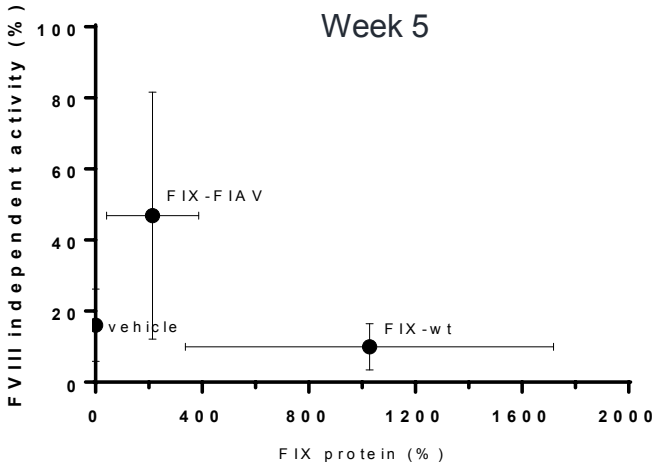

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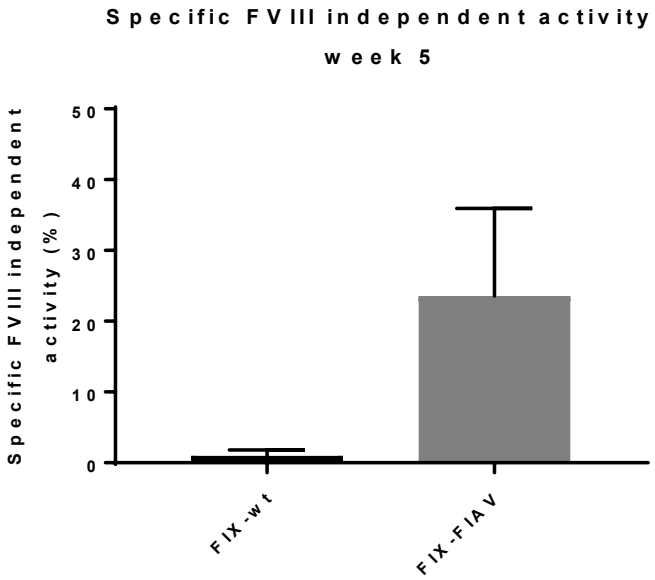

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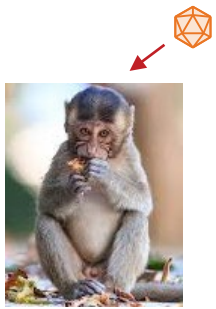

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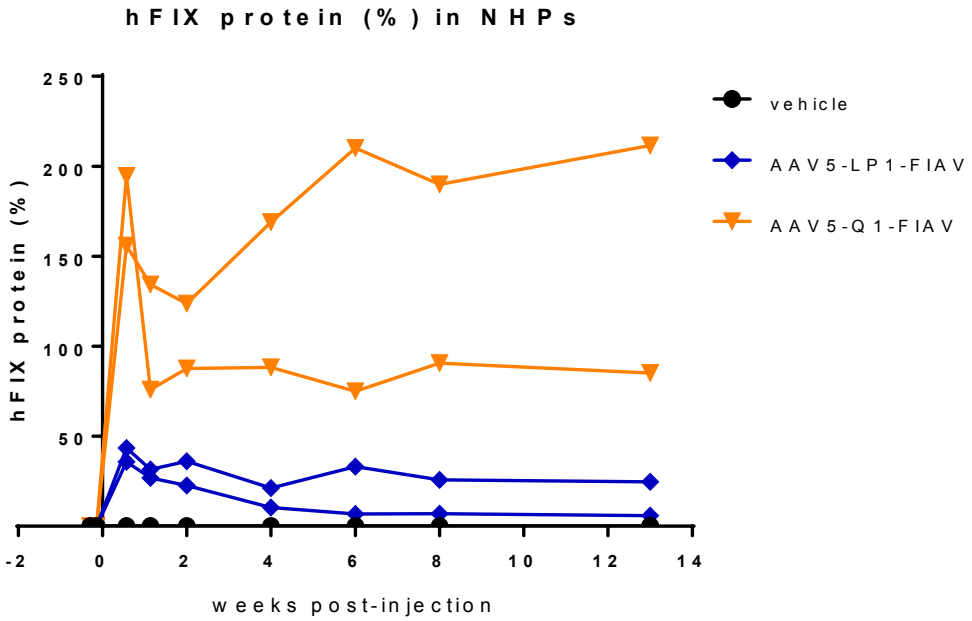

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Jolanda Liefhebber
Andrew McCreary
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Stable Expression of FIX and Maintained Reductions in Bleeding and Factor IX Consumption Following AMT-060 Gene Therapy with up to 3.5 Years of Follow Up in Adults with Severe or Moderate-Severe Hemophilia B

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Unmet needs in hemophilia B

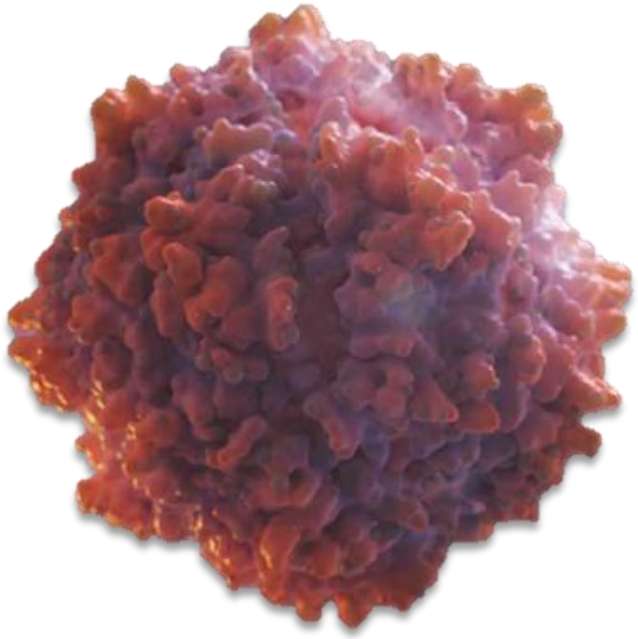
- *Significant unmet needs remain with the current standard of care factor IX (FIX) prophylaxis[1,2]:*
  - **Bleeding risk** *due to fluctuating levels of protection*
  - **Cumbersome treatment** *with frequent infusions and lifestyle restrictions*
  - **Treatment adherence issues** *and resulting suboptimal clinical outcomes*
  - **Quality of life** *and pain*
  - *Accrual of* **joint damage**

Steady state FIX levels in the mild to non-hemophilic ranges offer the potential to address these unmet needs

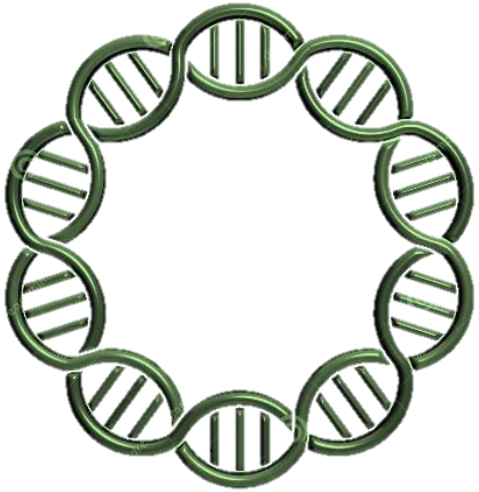
1. VandenDriessche T and Chuah MK. Hum Gene Ther. 2017;28(11):1013-1023; 2. Bauer KA. Am J Manag Care. 2015;21(6 Suppl):S112-22.

Introduction: gene therapy for hemophilia B: AMT-060

AAV5 capsid



Liver-specific promoter & human FIX gene



AMT-060 – wildtype

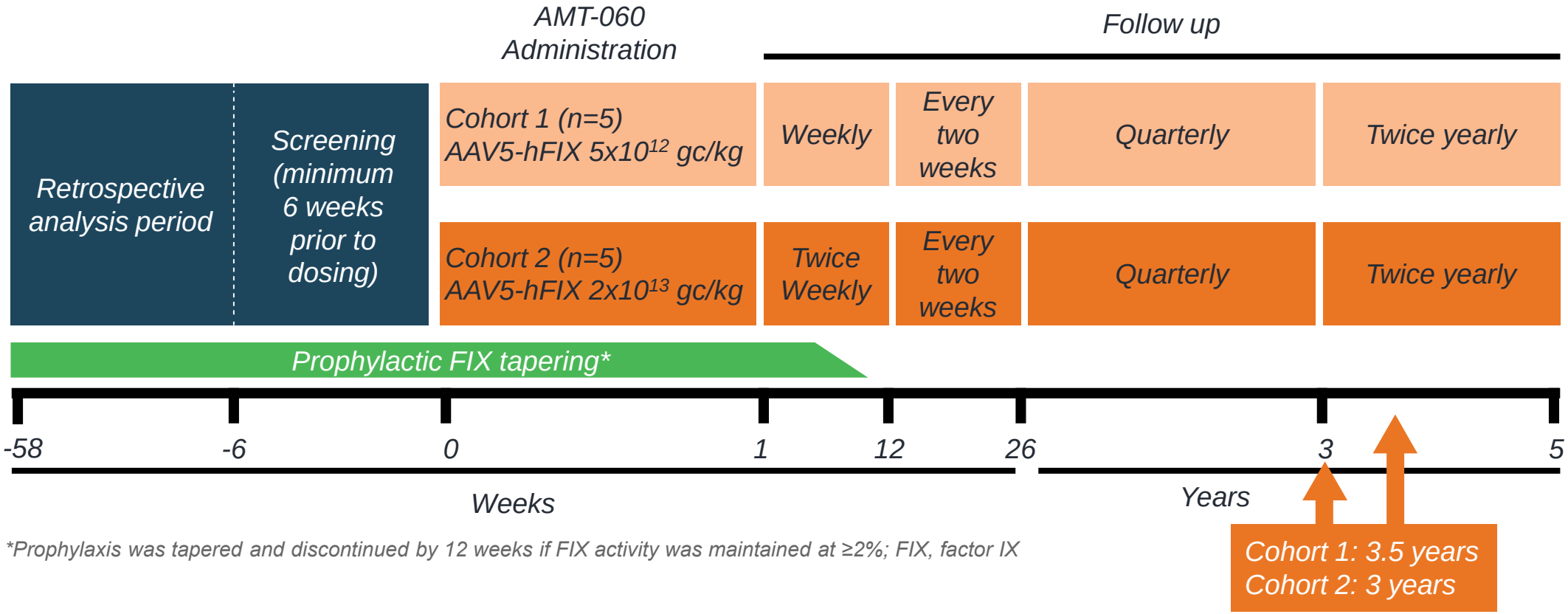
- *Low prevalence of pre-existing neutralizing antibodies able to impact clinical outcomes*[1,4]
- *Previously tested in humans without sign of cellular immune activation*[2]

- *WT hFIX (codon optimized)*
- *Clinically demonstrated* **safe and durable**[3] *increases in FIX activity with meaningful* **improvements in clinical outcomes**[3]

1. Boutin et al, Human Gen Ther 2010; 21(6):704-12. 2. D'Avola et al, Journal of Hepatology 2016; doi: http://dx.doi.org/10.1016/j.jhep.2016.05.012. 3. Nathwani et al. NEJM 2014; 371:1994-2004. 4. Majowicz et al. Mol Ther 2018; 26:458

AMT-060 Phase I/II study design

- *Multi-national, open-label, dose-escalating study (NCT02396342)[1,2]*
- *10 adult males with severe/moderately severe hemophilia B[1,2]*
- *Results previously reported to 2.5 years[2]*



*Prophylaxis was tapered and discontinued by 12 weeks if FIX activity was maintained at ≥2%; FIX, factor IX

1. Miesbach et al. Blood 2018;131:1022-31; 2. Leebeek et al. Poster presented at ASH, December 2018

Baseline characteristics[1]

Variable		Cohort 1 (N=5)	Cohort 2 (N=5)
Age (years)		*69 (35-72)*	*35 (33-46)*
Weight (kg)		*85 (71-89)*	*84 (71-96)*
FIX use[a]	*Prophylaxis, IU/week*	*4000 (2000–8000)*	*4000 (4000-10,500)[b]*
	Annualized mean, IU/year	*354,800*	*173,200*
Mean bleeds in the year prior to enrollment, n	*Total*	*14.4*	*4.0[c]*
	Spontaneous	*9.8*	*3.0*
	Traumatic	*2.8*	*1.0*
	Unknown	*1.8*	*0.0*
Hemophilia joint health scores[d]		*27 (2-49)*	*6 (0-17)*
HIV positive status, n		*1*	*0*
Prior hepatitis C infection, n		*4*	*2*
AAV5 NAb⁺ *(luciferase assay)[2]*		**3**	**0**

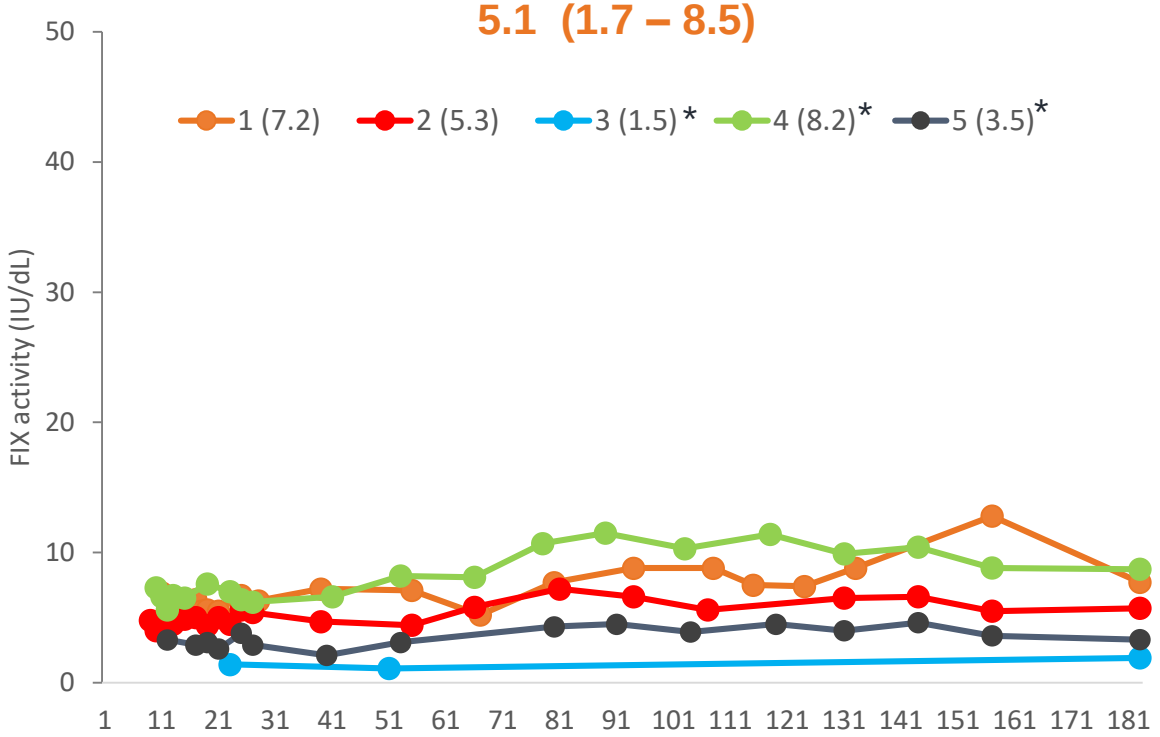
Values are median (min-max) unless otherwise stated. N=number. [a]QOD used as 3.5 x per week for calculations. [b]1 participant in Cohort 2 received on-demand treatment and is therefore not included; [c]Historical bleed data missing for 1 participant in Cohort 2 who is therefore not included; [d]Joint status was assessed using the Haemophilia Joint Health Score version 2·1.6 FIX, factor IX; n, number of participants; HIV, human immunodeficiency virus; NAb, neutralizing antibody

1. Table modified from Miesbach et al. Blood 2018;131:1022-31; 2. Majowicz et al. Mol Ther 2018; 26:458

Sustained dose-dependent increases in FIX activity

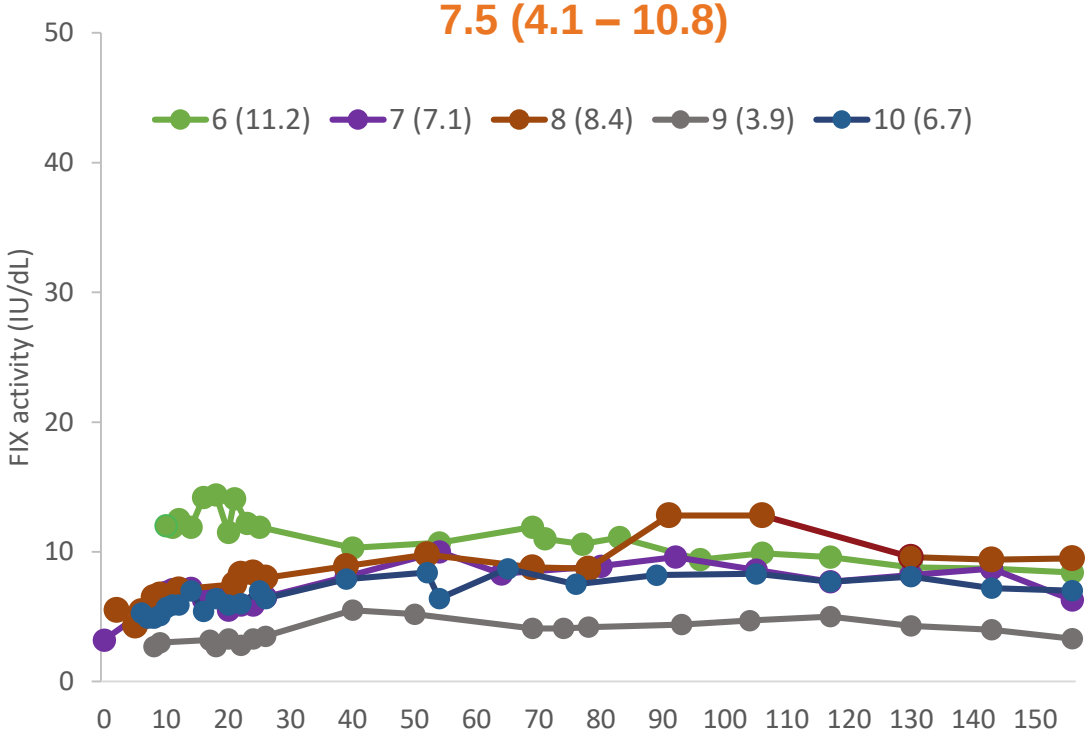
Cohort 1

Steady state mean FIX activity (95%CI): 5.1 (1.7 – 8.5)



Cohort 2

Steady state mean FIX activity (95%CI): 7.5 (4.1 – 10.8)

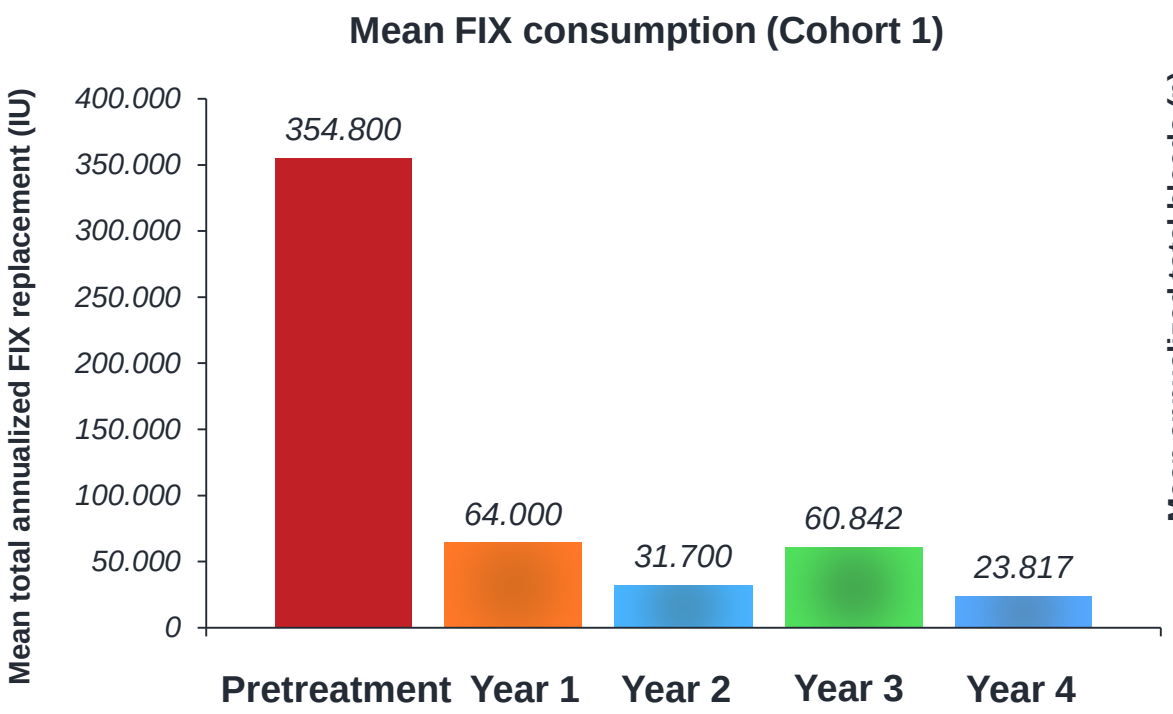


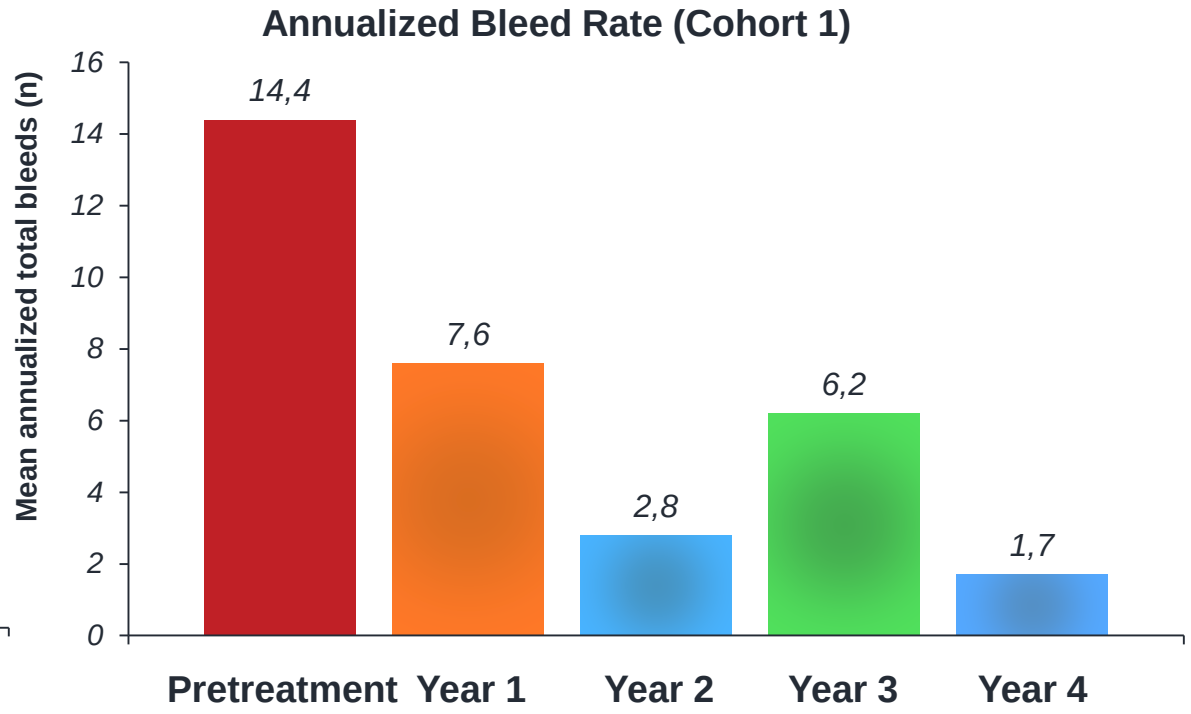
Weeks following AMT-060 treatment

FIX activity levels correlated approximately 1:1 with FIX protein expression

*Values in parentheses represent mean FIX activity over time. Only values at least 10 days after last FIX concentrate administration are included. FIX prophylaxis was continued after AMT-060 and tapered between Weeks 6 and 12 *Patient retrospectively tested positive for AAV5 neutralizing antibodies using the luciferase-based assay. 3 patients were presumed cross-reactive matter positive. FIX, factor IX; CI, confidence interval; IU, international units*

Reductions in FIX use and bleeds sustained over long term follow up (Cohort 1)

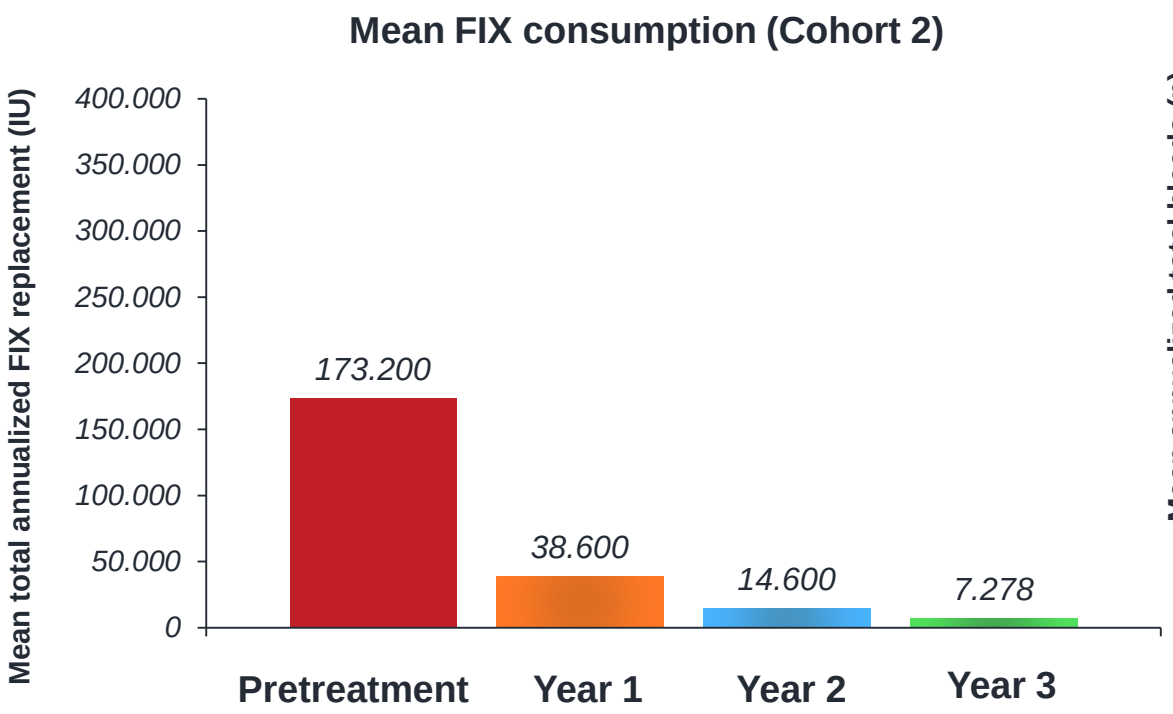


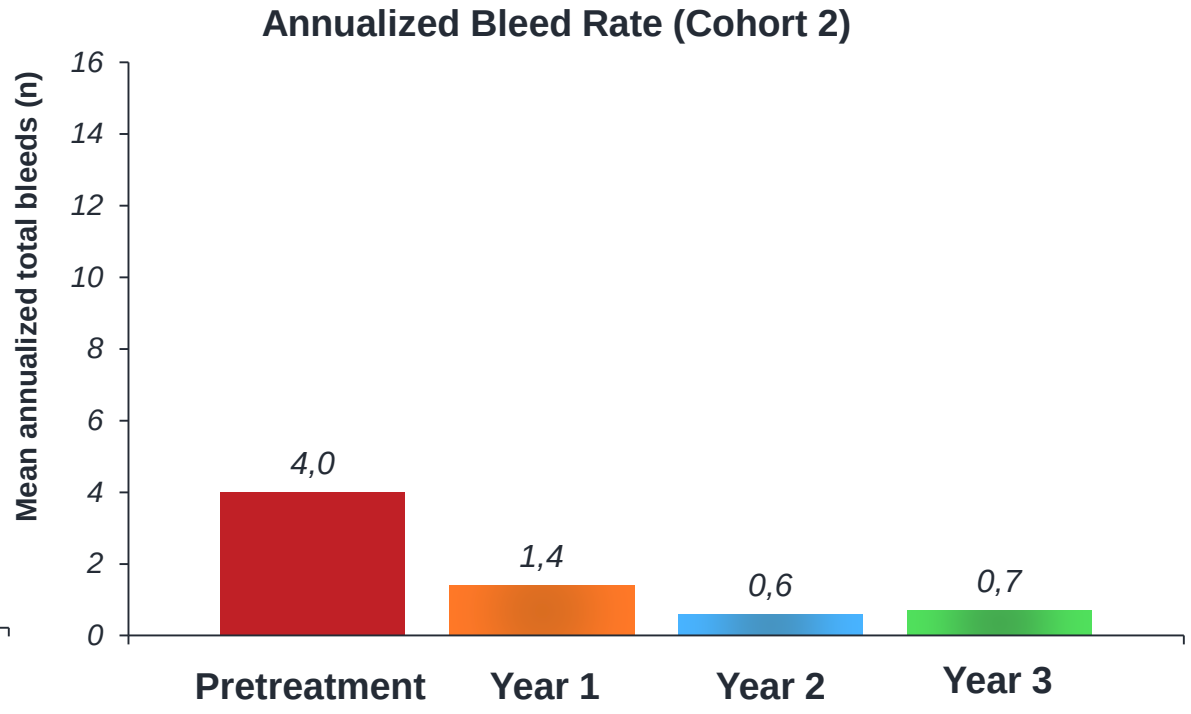


Reduction relative to pre-AMT-060	FIX use	Bleeds
Year 1	*82%*	*47%*
Year 2	*91%*	*81%*
Year 3	*83%*	*57%*
Year 4	*93%*	*88%*

Mean FIX consumption excludes surgical procedures

Reductions in FIX use and bleeds sustained over long term follow up (Cohort 2)





Reduction relative to pre-AMT-060	FIX use	Bleeds
Year 1	*78%*	*65%*
Year 2	*92%*	*85%*
Year 3	*96%*	*83%*

Mean FIX consumption excludes surgical procedures

Treatment Emergent Adverse Events considered possibly / probably related to treatment (TRAE)

TRAE	n (E) Cohort 1 (N=5)	n (E) Cohort 2 (N=5)
Any TRAE	**4 (5)**	**5 (10)**
Liver enzyme increased	*1 (1)*	*2 (3[a])*
Pyrexia	*1 (1)*	*2 (2)*
Anxiety	*1 (1)*	*1 (1)*
Drug ineffective	*1 (1)*	*0*
Joint swelling[*]	*1 (1)*	*0*
Palpitations	*0*	*1 (1)*
Headache	*0*	*1 (1)*
Prostatitis	*0*	*1 (1)*
Rash	*0*	*1 (1)*

TRAE, treatment emergent adverse event reported as possibly/probably related to treatment by the investigator; FIX, factor IX; n, Number of participants with events; (E), number of events; [a]2 events reported in the same participant; [*]TRAE reported in last 12 months

Serious AE

- *1 participant: short, self-limiting fever in first 24 hours post-AMT-060*
- *2 participants (1 in Cohort 1, 1 in Cohort 2): mild, asymptomatic elevations in liver enzymes*

Overall

- *1 new TRAE[*] was observed during the last 12 months of observation post-treatment*
- *No participants developed FIX inhibitors*

Conclusions

- *The* **safety profile** *of AMT-060 remains positive*
  - *No development of FIX inhibitors*
  - *No new clinically significant AEs, ALT elevation or capsid-specific T-cell activation since last report*

- **Stable, durable FIX activity over 3.5 years**

- **Long-term clinical benefit** *in all participants*
  - *Reductions in bleeds sustained over time in both cohorts*
  - *All participants who discontinued prophylaxis remain prophylaxis-free*
  - *Annualized FIX consumption decreased by 87% across the duration of follow up (78-96% per year) compared to pre-treatment*

ALT, alanine aminotransferase; AE, adverse event; FIX, factor IX;

Next steps: Phase IIb and Phase III with AMT-061

- *The **Phase IIb AMT-061 study** (NCT03489291) in 3 participants with FIX activity ≤1% and anti-AAV5 NAbs showed at 36 weeks post treatment:[1]*
  - *AMT-061 was **well-tolerated** with no serious AEs*
  - ***Sustained FIX activity** up to 54.1%*
    - *Mean FIX activity 45.0% at 36 weeks (n=3)*
    - *Suggests **anti-AAV5 NAbs** may not be a barrier for AAV5 gene therapy[2]*
  - ***No bleeds** or associated use of factor replacement therapy*
  - ***No loss of FIX activity** or requirement for immunosuppression*
- *The **Phase 3 HOPE-B AMT-061 study** (NCT03569891) is enrolling*
  - ***First patient treated early 2019***
  - *Expected to enroll approximately 55 participants with severe hemophilia B*
  - *Those with pre-existing AAV5 NAbs will not be excluded*

hFIX, human Factor IX; Nab, neutralizing antibodies; HOPE B, Health Outcomes with Padua gene: Evaluation in Hemophilia B

1. Giermasz et al. Oral presentation at ISTH on July 6th 2019. 2. Majowicz et al. Mol Ther - Methods Clin Develop 2019. DOI : 10.1016/j.omtm.2019.05.009.

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Acknowledgments

We thank the participating patients and their families

- **Academic Medical Center Amsterdam**: *M. Coppens, L. Landman, M. van Maarseveen, K. Nooij, M. Kemper, C. Ris – Stalpers.*
- **Azienda Ospedaliera Universitaria Careggi Florence**: *G. Castaman.*
- **Erasmus Medical Center**: *F. Leebeek, M. Kruip, A. G. Mulders, E. van der Graaf, R. Bouamar, C. Bakker.*
- **Fondazione IRCCS Cà Granda Ospedale Maggiore Milan**: *F. Peyvandi.*
- **Rigshospitalet Copenhagen**: *P. Kampmann, E. Funding, R. Duus Müller, R. Svensgaard, C. Nielsen, Mette Nordahl Rahbek.*
- **University Hospital Frankfurt am Main**: *W. Miesbach, J. Schwäble, S. Gundermann, K. Scholz, H. Bönig, E. Seifried.*
- **University Medical Center Groningen**: *K. Meijer, F. Yspeerd, K. Thedinga, M. Voskuilen, B. Molmans, M. Segers, B. Waarts.*
- **University Medical Center Utrecht**: *R. Schutgens, P. van der Valk, E. Beers, D. Dekker, M. van Haaften-Spoor, S. Oortwijn-De Loo, A. Braem-Enneman, M. Timmer, H. Aanstoot.*
- **Vivantes Klinikum Berlin**: *R. Klamroth, C. Kubicek-Hofmann, A. Orlovic, Y. Limberg.*

No evidence of germline transmission of vector DNA following intravenous administration of AAV5-hFIX to male mice

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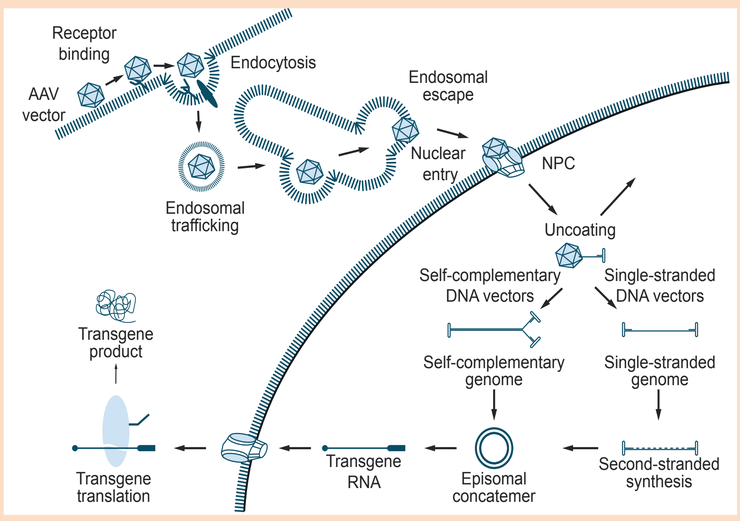
[1]uniQure Biopharma B.V.

PB0303

BACKGROUND

- Concerns exist regarding the possibility that gene transfer using viral vectors may lead to vertical germline transmission of the vector DNA to the next generation[1-4]
- Recombinant adeno-associated viral (AAV) vectors are commonly used to deliver genes to human cells
- The risk of germline transmission is limited by the following properties:
  - The AAV vector genome persists in the nucleus as an episome and does not require integration into the host DNA for transcription (**Figure 1**)[5,6]
  - AAVs are not capable of replication

Figure 1. AAV vector genomes remain episomal

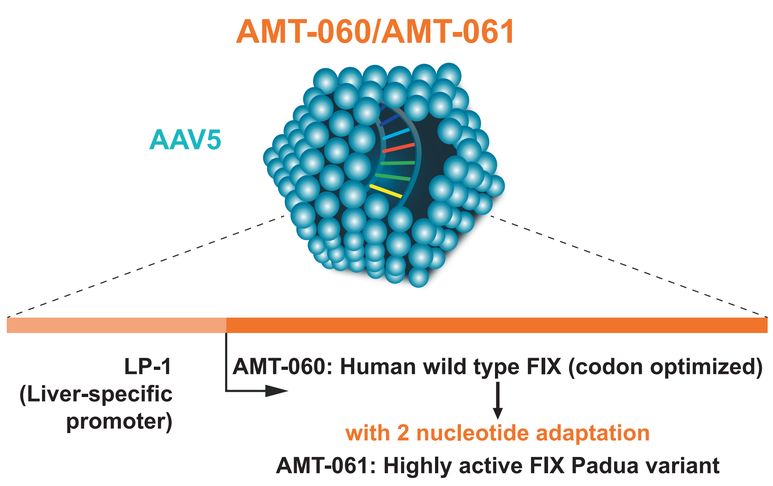


Reproduced from Salganik et al[6]

STUDY AIMS

- To investigate the possibility of germline transmission in mice following intravenous (IV) administration of AMT-060
- AMT-060 and AMT-061 (a modified version of AMT-060) (**Figure 2**) are potential gene therapies for moderate/severe hemophilia B, currently being studied in clinical trials[7-9]
- Since hemophilia B predominantly occurs in male patients, germline transmission through sperm was investigated in mice in a GLP compliant study, according to current gene therapy guidelines (EMEA/273974/2005)

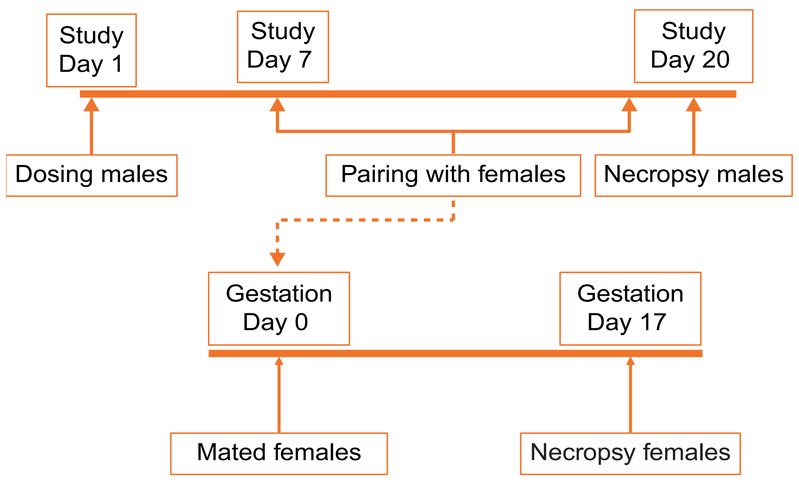
Figure 2. Structure of AMT-060/061



STUDY DESIGN

- Male C57Bl/6 mice each received a single IV infusion of vehicle control (n=5) or 2.3 x 10^{14} gc/kg AMT-060 (n=15) via the tail vein on Day 1 (**Figure 3**)
- After 6 days, each mouse was paired with 2 untreated female mice daily until confirmation of mating by a copulation plug, or for a maximum of 11 days
- A 6-day period between AMT-060 treatment and start of mating was chosen to maximize the chances of vector transmission to offspring
- Males were sacrificed 20 days post-treatment; the seminal vesicle, epididymis, testes and a sperm sample were collected
- Females were necropsied on Day 17 of gestation; the uterus, placenta and fetuses were collected. Each fetus was examined for viability and externally visible abnormalities
- Tissue samples of 5 animals per sex were analyzed for vector DNA by quantitative (Q)PCR

Figure 3. Study design



RESULTS

Pregnancy-related outcomes

- Treatment of male mice with AMT-060 did not affect mating performance, fertility indices or pregnancy performance (**Table 1**)

Table 1. Effects of AMT-060 on fertility and pregnancy outcomes

	Control	AMT-060-treated
Mating performance		
No. males paired	5	15
No. males siring	3	12
Male fertility index (%)	60%	80%
No. females paired	10	30
No. pregnant	5	20
Female fertility index (%)	50%	67%
Pregnancy outcome		
No. pregnant	5	20
Total no. of uterine implants	33	156
Total live implants (%)	29 (88%)	139 (89%)
Total dead implants (%)	4 (12%)	17 (11%)
Mean implants	6.6 ± 2.1	7.8 ± 1.9
Mean live implants	5.8 ± 2.4	7.0 ± 2.0
Mean dead implants	0.8 ± 0.8	0.9 ± 0.9
Mean fetal weight (g)	0.85 ± 0.04	0.89 ± 0.09
No. of fetuses with external abnormalities (%)	0 (0%)	2 (1%)

Observations in male mice

- There were no clinical signs that were considered related to treatment with AMT-060
- Body weight, body weight changes (**Table 2**), and food consumption were comparable between control and treated male mice
- Macroscopic evaluation at necropsy did not indicate any abnormalities in either group

Table 2. Effects of AMT-060 on body weight development in male mice

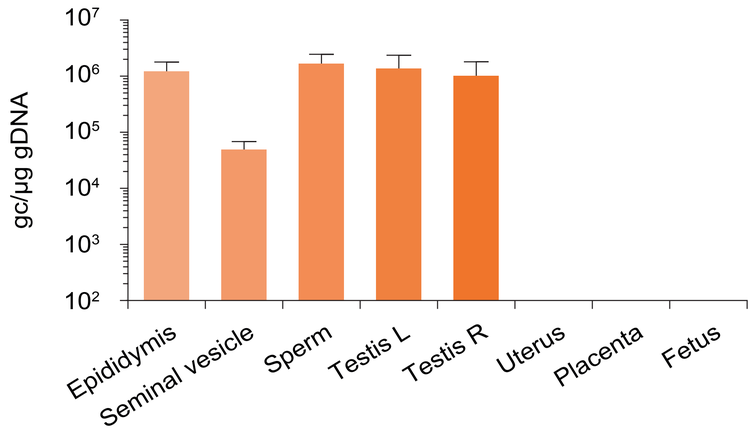
Group		Study day					
		-4	1	4	11	18	Change from Day 1 to 18
Control	mean	22.0	22.6	23.0	23.8	25.0	2.3
	SD	1.6	1.9	1.8	2.3	1.4	0.9
	n	5	5	5	5	5	5
Treated	mean	22.9	23.6	24.4	24.9	26.2	2.6
	SD	1.5	1.4	1.5	1.3	1.4	0.7
	n	15	15	15	15	15	15

SD, standard deviation

Vector DNA germline transfer and biodistribution

- QPCR showed high levels of vector DNA in all male reproductive tissues 20 days after AMT-060 treatment (**Figure 4**)
- In contrast, vector DNA was below the lower limit of quantification (100 gc/µg of genomic DNA) in uterus, placenta and fetuses (**Figure 4**)

Figure 4. Biodistribution of AAV5 vector DNA in mouse tissues



gc, genome copies; gDNA, genomic DNA

CONCLUSION

- Treatment of male mice with AMT-060 was not associated with vertical transmission of AAV5 vector DNA to offspring
- Vector DNA was detected in male reproductive tissues (epididymis, seminal vesicle, sperm, and testes), but not in reproductive tissues (uterus and placenta) from untreated females following mating with treated males
- AMT-060 treatment had no effect on reproductive parameters
- Results lend support to the current view that the (human) risk of germline transmission following gene therapy with AAV vectors is low[1-6]

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8. Leebeek FWG et al. Oral presentation at ISTH on Saturday, 6 July 2019, 13:00-14:15
9. Giermasz A et al. Oral presentation at ISTH on Saturday, 6 July 2019, 13:00-14:15

DISCLOSURES

All authors are uniQure biopharma B.V. employees. Writing support, funded by uniQure B.V., was provided by Jackie Read of GK Pharmacomm Ltd.

Presented at the 27th International Society on Thrombosis and Haemostasis (ISTH) congress, Melbourne, Australia, July 6-10, 2019

Low predicted immunogenicity risk associated with FIX variants that can promote coagulation in the absence of FVIII: *in vitro* and *in silico* assessments

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PB0301

INTRODUCTION

- Human factor IX (FIX) variants, such as hFIX-FIAV and hFIX-IDAV, can promote coagulation independently of Factor VIII.[1]
- hFIX variants can ameliorate the bleeding phenotype in hemophilia A mice[1] and could be an attractive approach for development as a gene therapy to treat people with hemophilia A.
- One concern in using coagulation factor mutants is potential immunogenicity.
- hFIX-FIAV and hFIX-IDAV were analyzed to determine the potential for immunogenicity via antigen presentation of epitopes via major-histocompatibility complex (MHC) Class I and II.
- The aim of this research was to determine the risk of immunogenicity of hFIX variants by *in silico* and *in vitro* analyses.

METHODS

STEP 1: *In silico* assessment (ABZENA)

- Wild-type human FIX (wt-hFIX), hFIX-FIAV and hFIX-IDAV amino acid sequences were evaluated for their immunogenic potential.
- Peptides spanning the entire sequence were tested as 9mer peptides in one amino acid increments.
- Algorithms used to screen for potential T cell epitopes by identifying linear motifs of 9-10 amino acids that bind to MHC Class I or II.[2]
  - Class I: Immune Epitope Database (IEDB) peptide library.
  - Class II: iTope™ (MHC Class II binding prediction) and T cell epitope database (TCED™).

Step 2: T cell epitopes assay (PROIMMUNE)

- Ability of each candidate peptide to bind to MHC alleles and stabilize the MHC peptide complex.[3]
- Determination of on and off rate binding properties (strength and stability) for candidate peptides.

RESULTS

Step 1: *In silico* analysis

- **MHC class I**: Four moderate affinity peptides identified in the hFIX-FIAV and hFIX-IDAV variant sequences by in silico analysis underwent Step 2 analysis (**Table 1**).
- **MHC-class II**: No binding peptides were identified in the hFIX-FIAV or hFIX-IDAV variant sequences.
  - No difference in MHC Class II predicted immunogenicity between wt-hFIX and hFIX variants.
  - No further analyses on MHC Class II predicted immunogenicity performed (**Table 1**).

Table 1. Immunogenicity testing

	Step 1 *In silico* analysis		Step 2 T cell epitopes assay
MHC Class I	Four potential moderate affinity non-germline epitopes identified	→	Candidate peptides assessed for binding to various MHC Class I alleles
MHC Class II	Zero non-germline epitopes identified	✱	Step 2 testing not performed

Step 2: T cell epitope analysis

- The quantitative and qualitative binding properties of the MHC class I binding peptides were determined for a selection of the most frequent human leukocyte antigen (HLA) alleles in the general population.
- Three out of the four peptides identified were excluded as potential epitopes (**Table 2**).
  - Peptide 4 showed binding to two MHC Class I alleles A*02:01 and B*35:01
  - Allele frequency in North American Caucasian population:
    - A*02:01: 45.0%
    - B*35:01: 10.7%

Table 2. REVEAL® scores showing the level of incorporation of variant peptides to different MHC class I alleles

Allele	FIX-FIAV Peptide 1 RYNSGK**F**EE	FIX-FIAV FIX-IDAV Peptide 2 QSFNDFTR**I**	FIX-FIAV FIX-IDAV Peptide 3 SFNDFTR**I**V	FIX-FIAV FIX-IDAV Peptide 4 N**A**YNHDIAL	Positive control
A 01:01	0.8	0.5	0.5	0.4	+100
A 02:01	0.6	32.9	0.5	**+132.8**	+100
A 03:01	2.4	**+70.3**	**+77.7**	44.0	+100
A 11:01	1.0	0.9	1.8	1.7	+100
A 24:02	15.2	0.6	0.4	1.3	+100
A 29:02	1.2	1.3	0.6	0.9	+100
B 07:02	0.3	0.3	0.2	16.4	+100
B 08:01	0.3	0.3	0.4	**+59.8**	+100
B 14.02	0.0	0.1	0.0	15.2	+100
B 15.01	0.2	0.8	0.3	11.1	+100
B 27.05	0.6	0.1	0.1	0.0	+100
B 35:01	0.4	0.4	0.1	**+62.8**	+100
B 40: 01	0.2	0.1	0.1	0.0	+100

The modified amino acid in each peptide sequence is underlined. Positive control was a known T-cell epitope peptide with very strong binding. REVEAL score for each MHC-peptide complex calculated by comparison to the binding (on-rate) of the positive control at the latest time point.

- Peptide 4 was determined as posing an extremely low risk for the two alleles due to:
  - Poor stability of the MHC/peptide complexes (A*02:01).
  - Weak binding of the peptide to MHC (B*35:01) (**Table 3**).

Table 3. Binding and on- and off-rate data for Peptide 4

MHC Class I allele	On-rate $T_{½}$ (h)	Off-rate $T_{½}$ (h)	Kinetic score	R score
A*02:01	8.90	0.20	0.02	0.02
Positive control	12.72 ± 2.09	>120	9.43 ± 0.16	9.43
B*35:01	45.90	>120	2.61	1.08
Positive control	375.17 ± 124.83	>120	0.32 ± 0.33	0.32

Kinetic scores are calculated by dividing the off-rate by the on-rate. Higher kinetic scores indicate better epitopes. R scores provide an overall rating for each peptide. The higher the R-score, the better the epitope. R scores ≥45% of the positive control warrant further investigation.

CONCLUSIONS

- hFIX-FIAV and hFIX-IDAV variants are not associated with a significant risk of immunogenicity.
- hFIX-FIAV has been selected for further development.

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Presented at the 27th International Society on Thrombosis and Haemostasis (ISTH) congress, Melbourne, Australia, July 6-10, 2019