HEMOPHILIA AND GENE THERAPY

Prof Dr Frank W.G. Leebeek
Dept Hematology
Erasmus MC
Rotterdam
The Netherlands

ECAT FOUNDATION

Erasmus MC
University Medical Center Rotterdam

Hemophilia : a bleeding disorder

Deficiency of coagulation factor VIII (hemophilia A)

Deficiency of coagulation factor IX (hemophilia B)

Prevalence: 1: 10.000

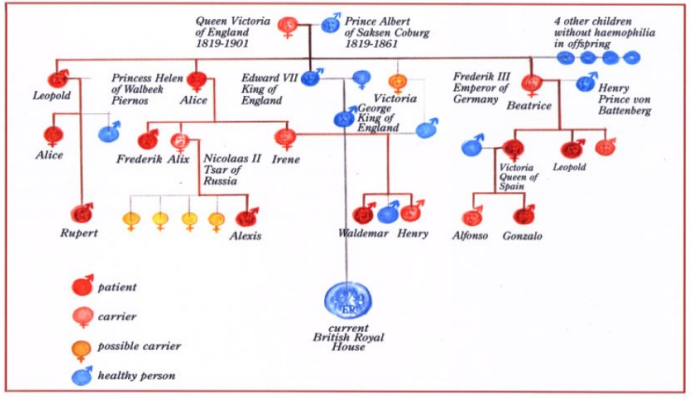
X-linked disease males are affected, females are carrier

Caused by a mutation/deletion/inversion in FVIII or FIX gene

Hemophilia A:B 6:1

Severity:

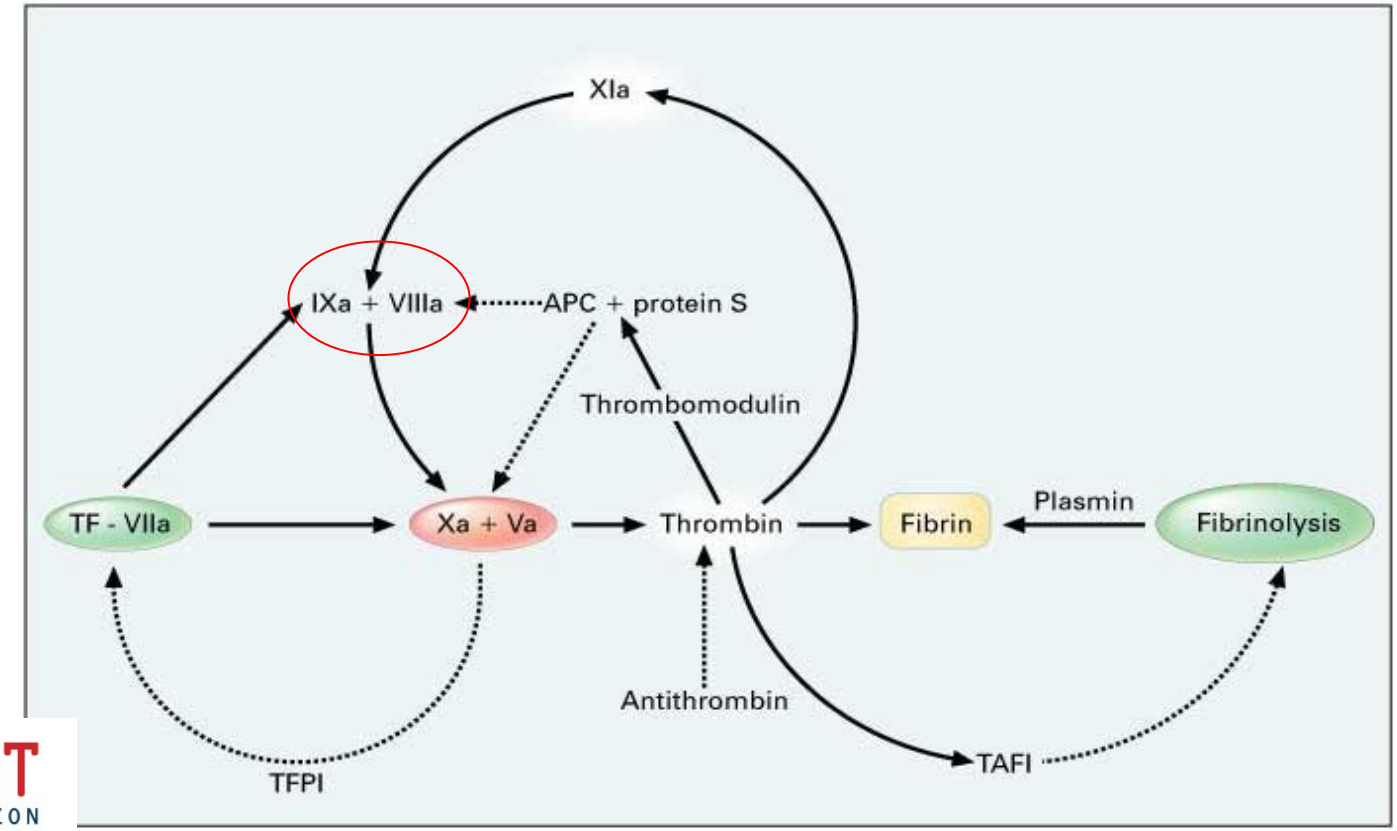
< 1%	(severe)
1 - 5%	(moderate)
> 5%-40%	(mild)



ECAT FOUNDATION

Erasmus MC

Secondary hemostasis



ECAT FOUNDATION

Erasmus MC

Hemophilia: a bleeding disorder

Joint bleeding

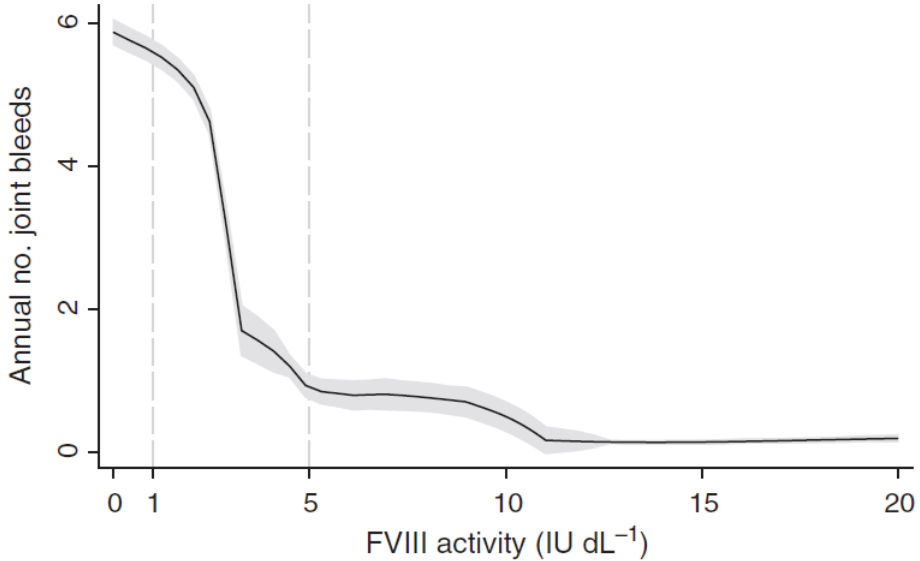
Muscle bleeding

Intracranial bleeding

Bleeding after trauma, surgery or interventions

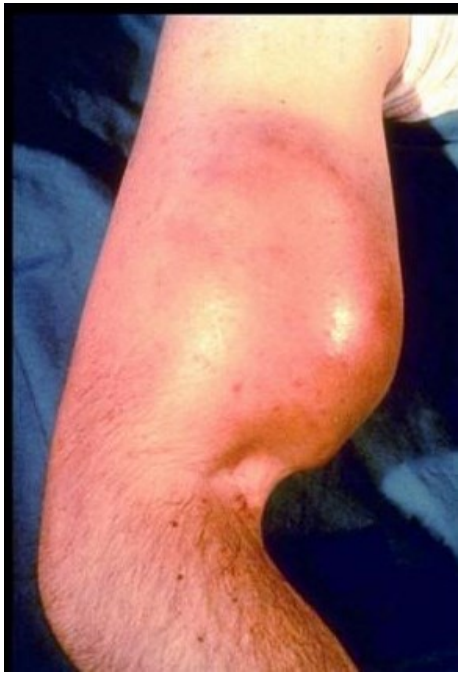
Severe hemophilia:	spontaneous bleeding
Moderate/mild hemophilia:	most bleedings after trauma or interventions

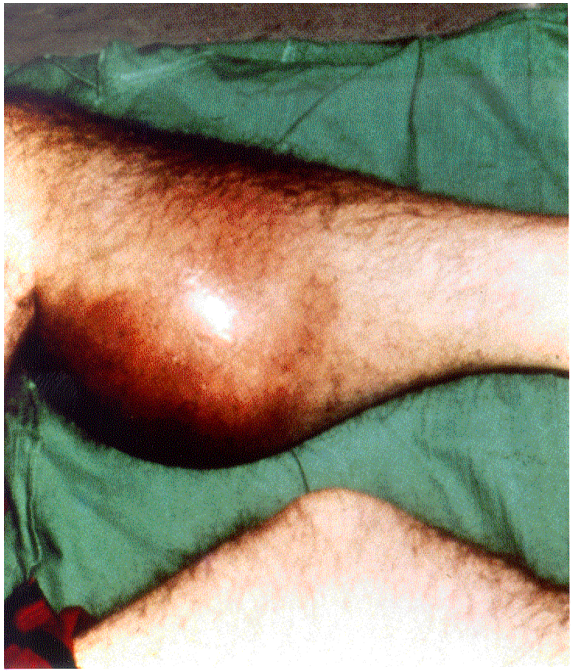
Annual joint bleeds and FVIII level



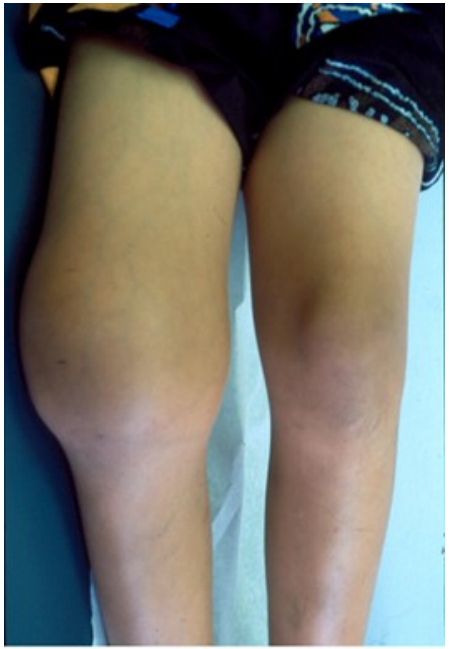
Den Uijl, et al. Haemophilia 2011

Muscle bleeds



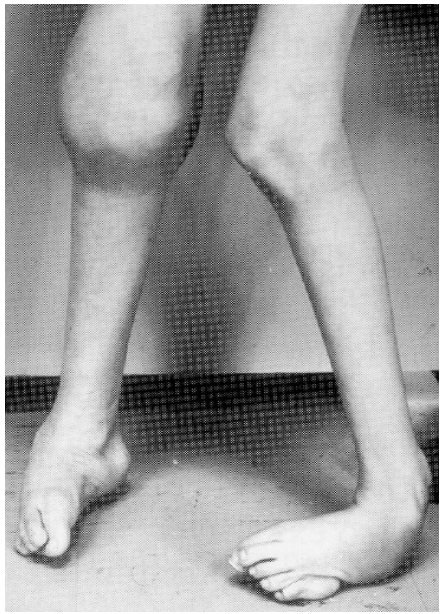


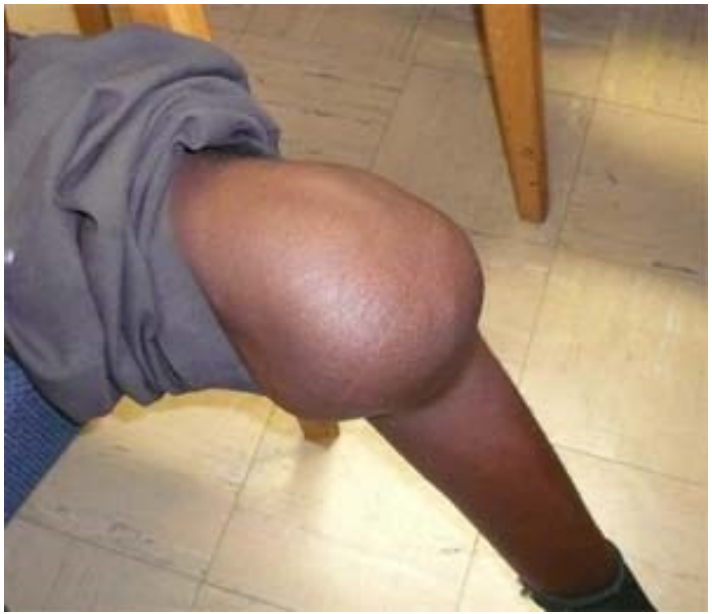
Joint bleed



knee bleed

Long term damage: arthropathy





Current hemophilia treatment: prophylaxis

Aim: to prevent joint bleeds and hemophilia arthropathy

- Hemophilia A : F VIII 2 - 4 x / week intravenous administration
- Hemophilia B : F IX 1 - 2 x / week intravenous administration

- Emicizumab: MAb mimicks FVIII activity once/ 2 weeks s.c

Drawbacks of current hemophilia treatment

- Regular intravenous infusions 1-4 x/week life long
- Bleedings still occur, fear for bleeding
- No cure of the disease
- Several complications may occur due to factor infusion
  - Inhibitors, infections, allergic reactions
- High costs of coagulation factor treatment or MAb treatment
- 75% of the world hemophilia population is not treated

Aim of gene therapy in Hemophilia

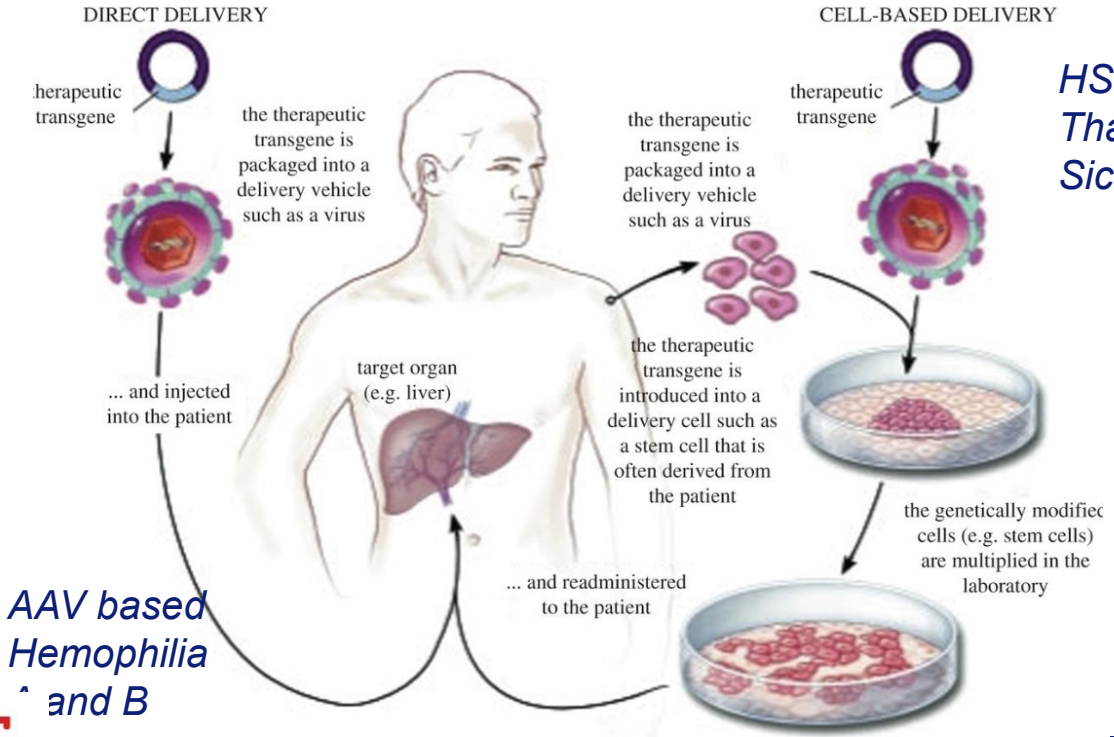
Adding the *FVIII* or *FIX* gene leads to expression of FVIII or FIX

Reduction of spontaneous bleedings and arthropathy

No regular prophylaxis necessary

Improvement of quality of life

Gene therapy



HSC based
Thalassemia
Sickle cell

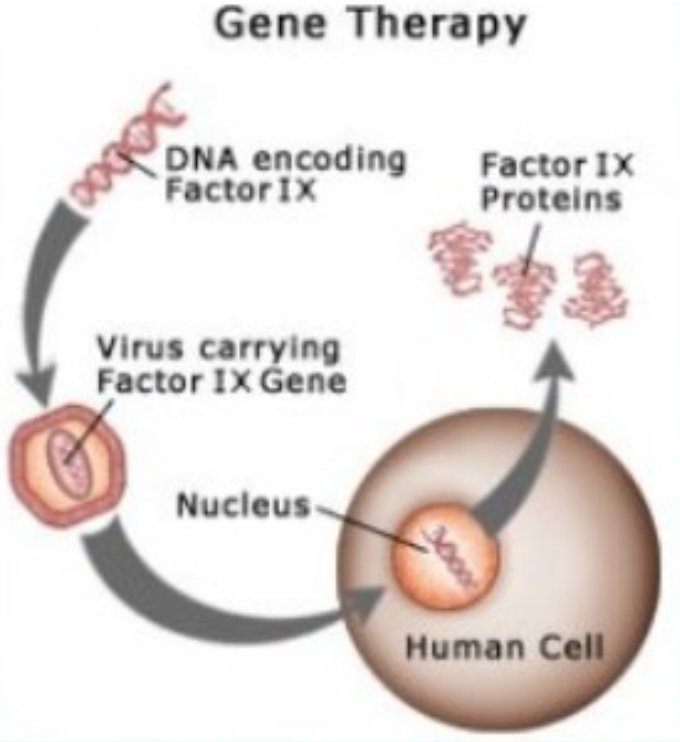
AAV based
Hemophilia
A and B

ECAT FOUNDATION

Erasmus MC

Swystun LL, et al. Circ Res 2016

Mode of action of in vivo gene therapy



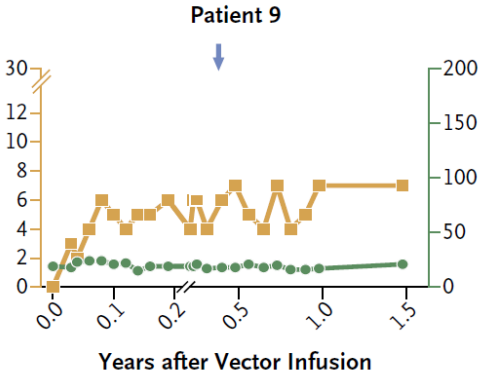
First AAV-8 gene therapy trial

The NEW ENGLAND JOURNAL of MEDICINE

N ENGL J MED 371;21 NEJM.ORG NOVEMBER 20, 2014

Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B

A.C. Nathwani, U.M. Reiss, E.G.D. Tuddenham, C. Rosales, P. Chowdary, J. McIntosh, M. Della Peruta, E. Lheriteau, N. Patel, D. Raj, A. Riddell, J. Pie, S. Rangarajan, D. Bevan, M. Recht, Y.-M. Shen, K.G. Halka, E. Basner-Tschakarjan, F. Mingozzi, K.A. High, J. Allay, M.A. Kay, C.Y.C. Ng, J. Zhou, M. Cancio, C.L. Morton, J.T. Gray, D. Srivastava, A.W. Nienhuis, and A.M. Davidoff



ECAT FOUNDATION

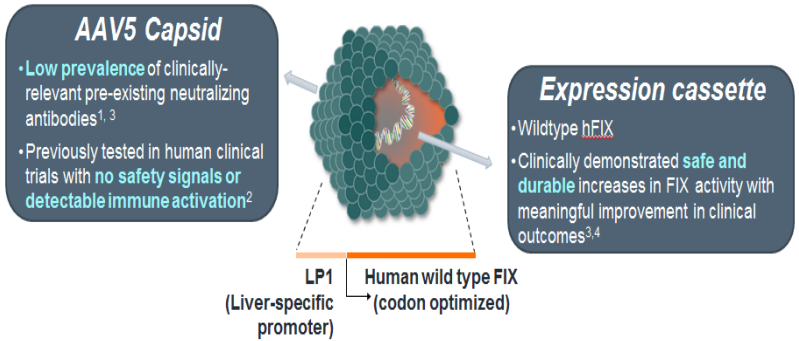
Erasmus MC

blood®
Regular Article

GENE THERAPY

Gene therapy with adeno-associated virus vector 5-human factor IX in adults with hemophilia B

Wolfgang Miesbach,[1] Karina Meijer,[2] Michiel Coppens,[3] Peter Kampmann,[4] Robert Klamroth,[5] Roger Schutgens,[6] Marco Tangelder,[7] Giancarlo Castaman,[8] Joachim Schwäble,[9] Halvard Bonig,[9,10] Erhard Seifried,[9] Federica Cattaneo,[11] Christian Meyer,[12] and Frank W. G. Leebeek[13]



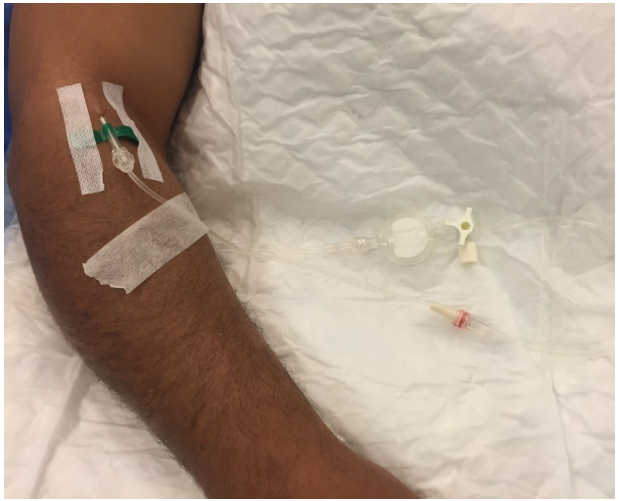
AMT-060 administration

Locally prepared in pharmacy

250 mL total volume infusion

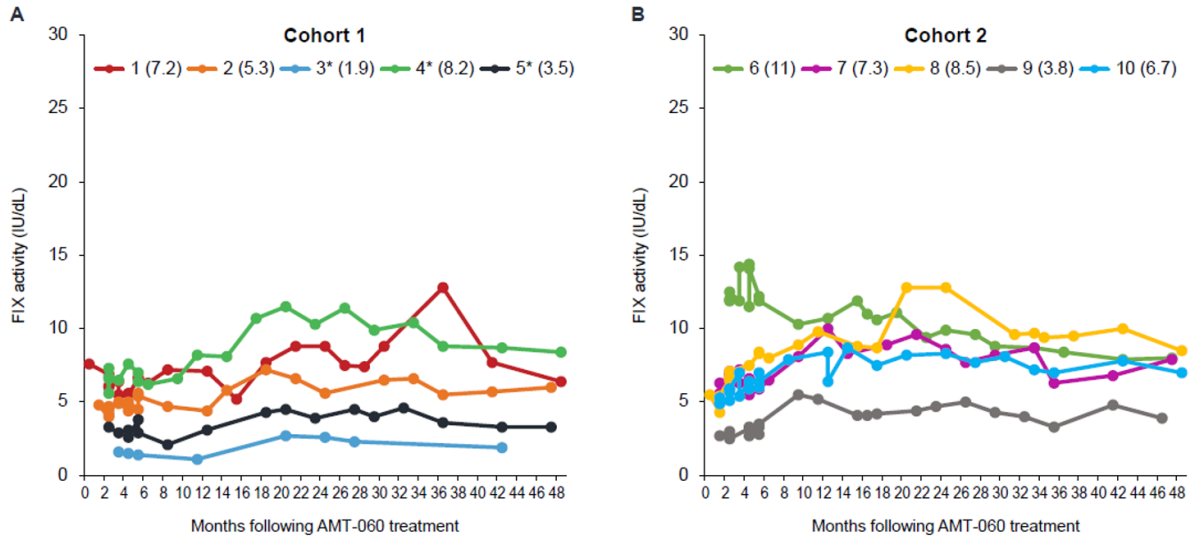
30 minute IV infusion

24 hour observation in hospital



- 10 patients treated in Phase I/II trial
- 6 treated in the Netherlands (ErasmusMC, UMCG, UMCU, AMC)

Stable long-term expression of FIX

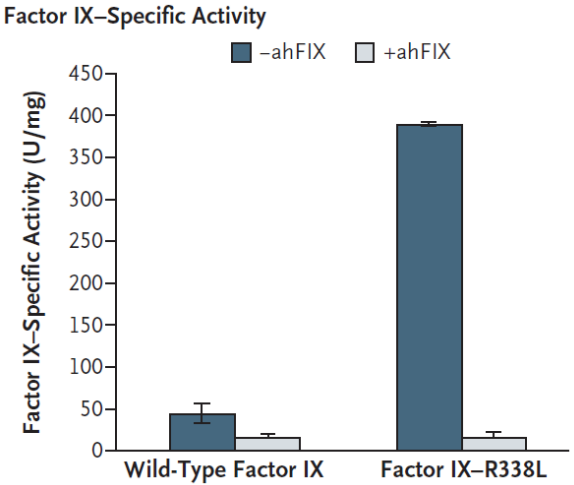


Leebeek, FWG, et al, submitted

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

X-Linked Thrombophilia with a Mutant Factor IX (Factor IX Padua)



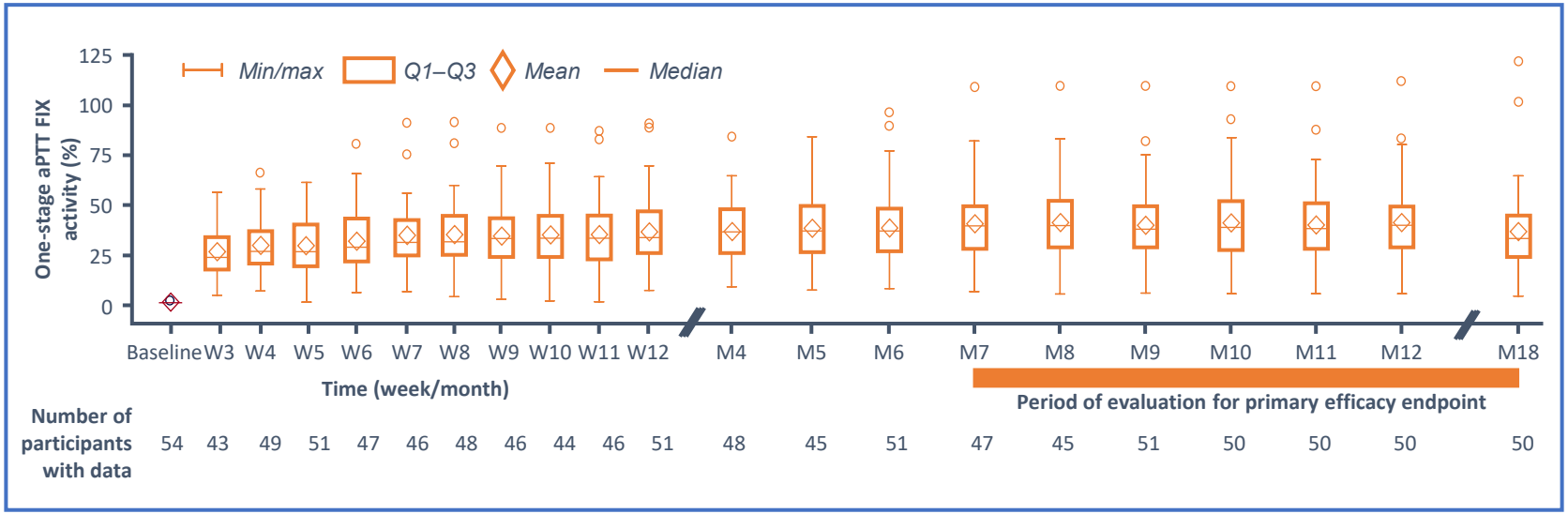
R338L FIX mutant
High specific activity
4-6 fold higher activity

ECAT FOUNDATION

Erasmus MC

Simioni P, et al , NEJM 2009

Sustained FIX activity levels up to 18 months



Uncontaminated central laboratory data (the visit did not occur within 10 days of exogeneous FIX use). FIX levels beginning with the Week 3 assessment were used in the analysis. Subjects with 0 uncontaminated central-laboratory post-AMT-061 values had change from baseline assigned to zero for this analysis and had their post-baseline values set equal to their baseline value. Baseline FIX was imputed based on subject's historical hemophilia B severity documented on the case record form. If the patient had documented severe FIX deficiency (FIX plasma level <1%), their baseline FIX activity level is imputed as 1%. If the subject had documented moderately severe FIX deficiency (FIX plasma level ≥1% and ≤2%), their baseline FIX activity level was imputed as 2%.

Mean (SD) FIX activity
6 months: 39.0 IU/dL (18.7); 18 months: 36.9 IU/dL (21.4)

ECAT FOUNDATION

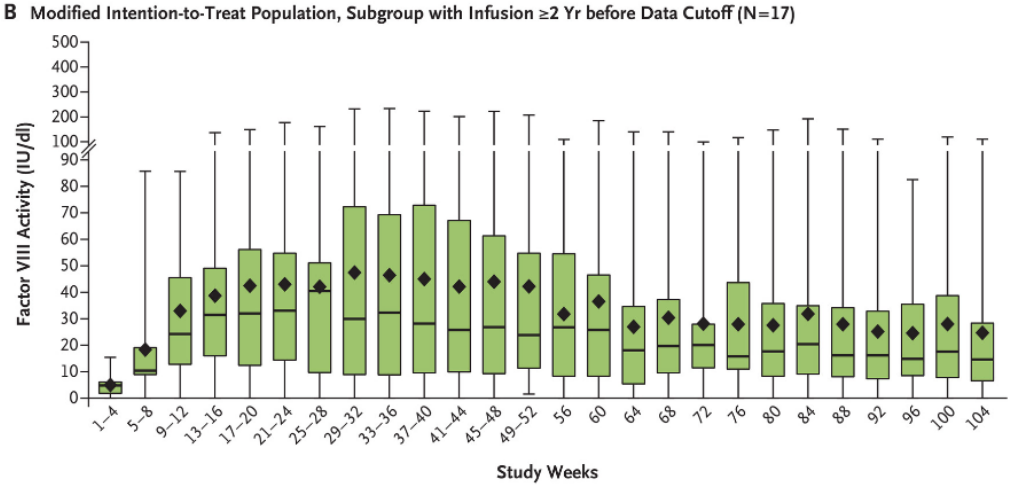
Erasmus MC

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 17, 2022 VOL. 386 NO. 11

Valoctocogene Roxaparvovec Gene Therapy for Hemophilia A

M.C. Ozelo, J. Mahlangu, K.J. Pasi, A. Giermasz, A.D. Leavitt, M. Laffan, E. Symington, D.V. Quon, J.-D. Wang, K. Peerlinck, S.W. Pipe, B. Madan, N.S. Key, G.F. Pierce, B. O'Mahony, R. Kaczmarek, J. Henshaw, A. Lawal, K. Jayaram, M. Huang, X. Yang, W.Y. Wong, and B. Kim, for the GENEr8-1 Trial Group*



MAIN OUTCOMES:

Increase of FVIII to mean of 42 IU/dL

Variable response

Decrease of bleeding by 84%

FVIII levels decreased over time

At 5 years 50% of patiënts had FVIII levels > 5 IU/dL

ALT elevations in 85%

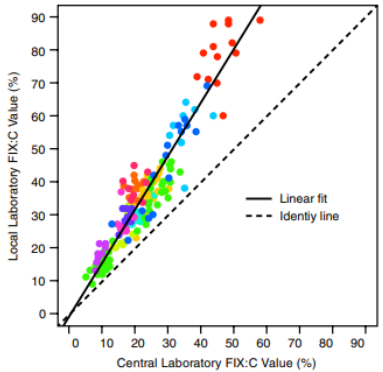
Approved by EMA 2022

FIX measurements after gene therapy

Parameter	FIX:C APTT One-stage Clotting Assays				FIX:C Chromogenic Assay
Manufacturer	Siemens Healthcare Diagnostics	Instrumentation Laboratory	Diagnostica Stago	Diagnostica Stago	ROSSIX
Instrument	BCS XP	ACL TOP	STA-R Evolution	STA-R Evolution	BCS XP
Reagent	Actin FSL	HemosIL SynthASil	STA-C.K. Prest	STA-PTT Automate	ROX Factor IX Kit
Activator	Ellagic acid	Silica	Kaolin	Silica	NA
% CAP-accredited laboratories (2017)	23%	26%	5%	34%	NA

Factor levels after gene therapy with fidanacogene elaparvovec

Lower levels locally vs centrally measured



ECAT FOUNDATION

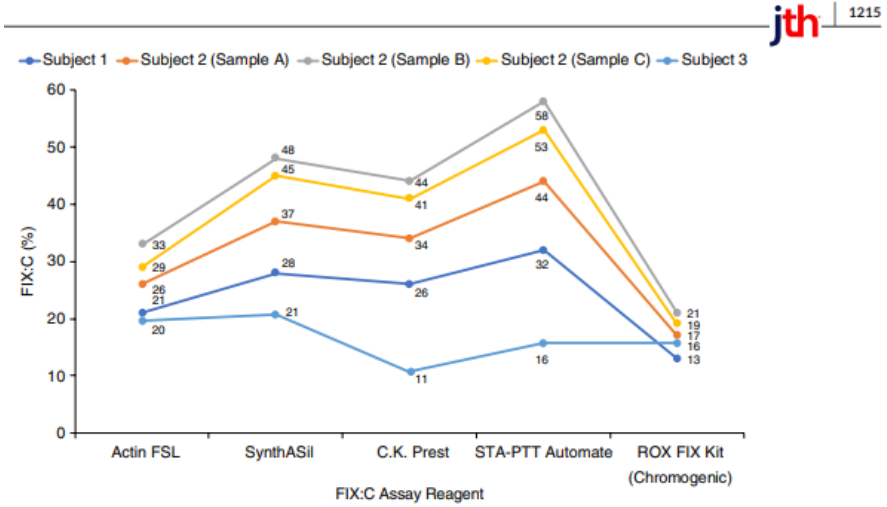
Erasmus MC

Robinson M et al. JTH 2021

Variability in FIX assays

Results for individual assays differed

Chromogenic assay results are lower than one-stage (APTT-based) levels



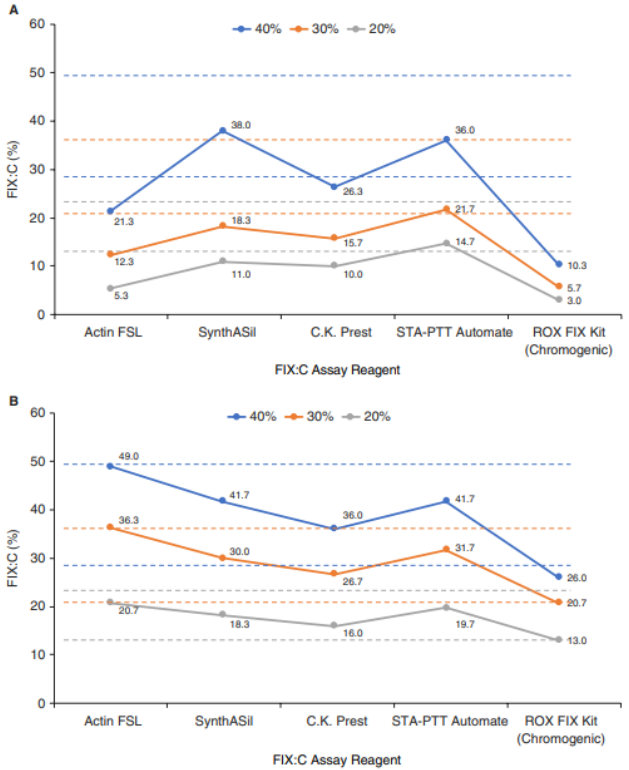
Spiking experiments

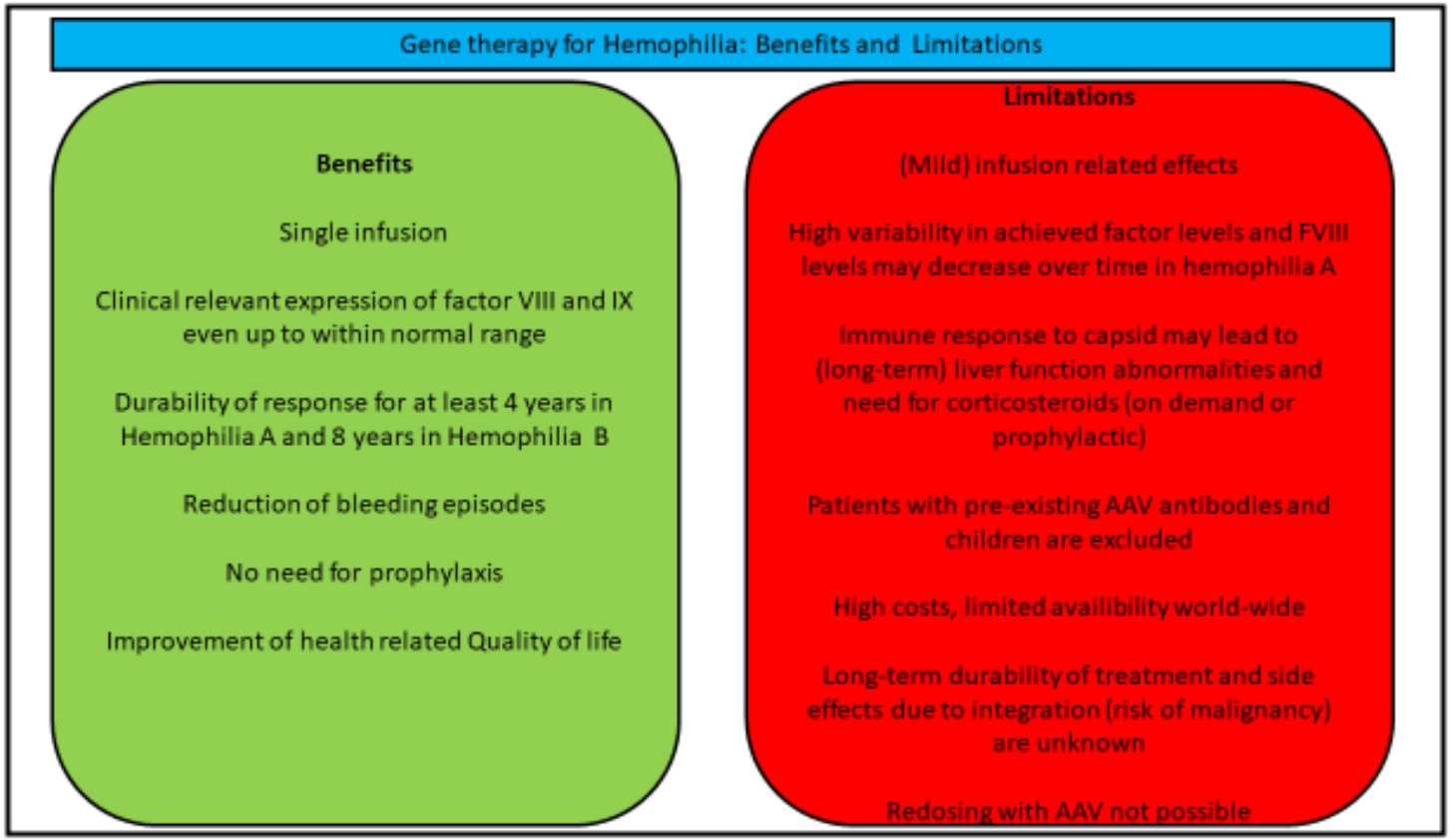
FIX levels differ between various assays

Spiking with FIX Padua: lower levels measured with various assays

Spiking with FIX WT: less variability between assays

At present, one-stage clotting assays are used in clinical practice to measure the labeled potency of approved FIX therapeutics and to monitor individuals undergoing therapy




Gene therapy for Hemophilia: Benefits and Limitations
Benefits
Single infusion
Clinical relevant expression of factor VIII and IX even up to within normal range
Durability of response for at least 4 years in Hemophilia A and 8 years in Hemophilia B
Reduction of bleeding episodes
No need for prophylaxis
Improvement of health related Quality of life
Limitations
(Mild) infusion related effects
High variability in achieved factor levels and FVIII levels may decrease over time in hemophilia A
Immune response to capsid may lead to (long-term) liver function abnormalities and need for corticosteroids (on demand or prophylactic)
Patients with pre-existing AAV antibodies and children are excluded
High costs, limited availibility world-wide
Long-term durability of treatment and side effects due to integration (risk of malignancy) are unknown
Redosing with AAV not possible

Conclusions

AAV-based gene therapy for hemophilia A and B is feasible, safe and effective

FVIII and FIX levels in the normal range may be achieved

High variability in factor levels between various assays

CSA results lower than OSA

FVIII gene therapy (Roctavian) received conditional marketing authorisation throughout EU 24-08-2022