HÄMATOLOGIE HEUTE
KÖLN

Gentherapie zur Behandlung der Hämophilie

Bridging the gap between the present and the future

Complete human genome sequenced (2000)

...CGGAGGCTAGGCTAGTAGGTGTGATAGGTAACGGATGATCG...

3000.000.000 base pairs

C. Venter *B. Clinton* *F. Collins*

"With this profound new knowledge, humankind is on the verge of gaining immense, new power to heal. It will revolutionize the diagnosis, prevention and treatment of most, if not all, human diseases."

President Bill Clinton 26 June 2000

Nature 405, 983–984; 2000

GENETIC DISEASES

Just 1 point-mutation in 3.000.000.000 bp can cause a potentially life-threatening disorder (*e.g.*: hemophilia, Duchenne muscular dystrophy, cystic fibrosis, …)

➡ **Equivalent to 1 typing error in 15 *Encyclopaedia Britannicae***

GENE THERAPY: PROVEN EFFICACY IN PATIENTS *(Anno 2019)*

HEREDITARY BLINDNESS (LEBER AMAUROSIS)

approved for US market

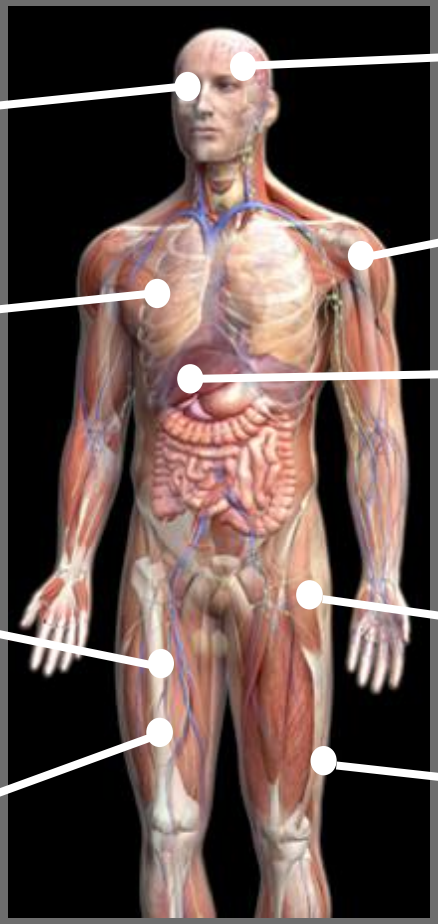
CANCER (MELANOMA, LYMPHOMA)

approved for US market

IMMUNE DEFECTS (SCID)

approved for EU market

ANAEMIA (β-THALASSEMIA)



NEURODEGENERATION (ADRENOLEUKO-DYSTROPHY)

SPINAL MUSCULAR ATROPHY

BLOOD CLOTTING DEFECTS (HAEMOPHILIA)

METABOLIC DISORDERS (LIPOPROTEIN LIPASE DEFICIENCY)

approved for EU market

GRAFT vs. HOST DISEASE

approved for EU market

FDA announces first US gene therapy approval for cancer treatment

By Michael Nedelman, CNN

Updated 1707 GMT (0107 HKT) August 30, 2017

HEALTH

The New York Times

F.D.A. Approves Second Gene-Altering Treatment for Cancer

By DENISE GRADY OCT. 18, 2017

"Tonight I'm launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes.

And to give us all access to the personalized information we need to keep ourselves and our families healthier."

President Barack Obama
2015 State of the Union Address | January 20, 2015

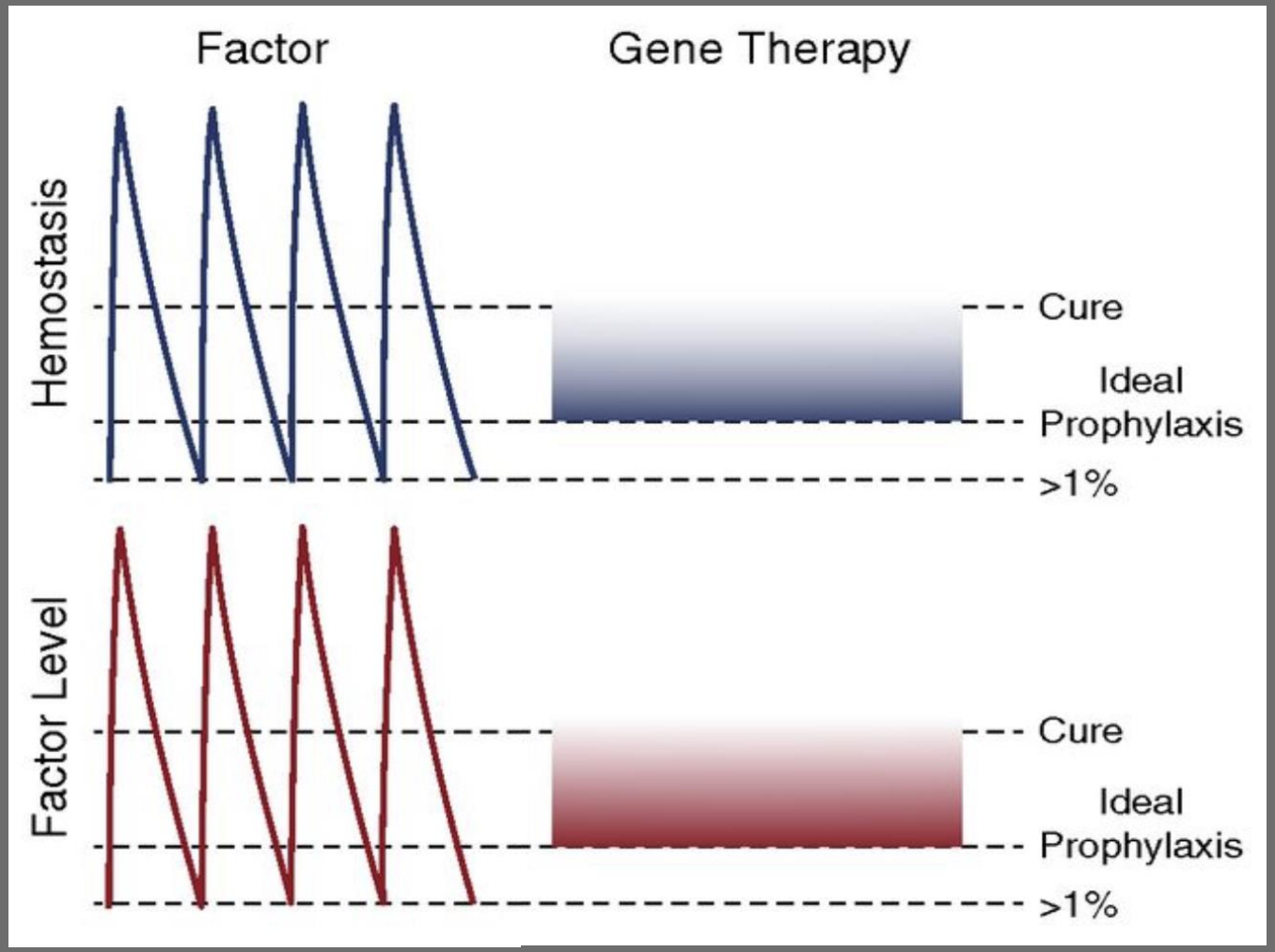
APPROVED CANCER GENE THERAPY

Please excuse
Emily from
school — she was
with me!

THE PRESIDENT

GENE THERAPY FOR HEMOPHILIA vs. STANDARD OF CARE

PROPHYLACTIC (3x/week i.v.)
RECOMBINANT CLOTTING FACTORS

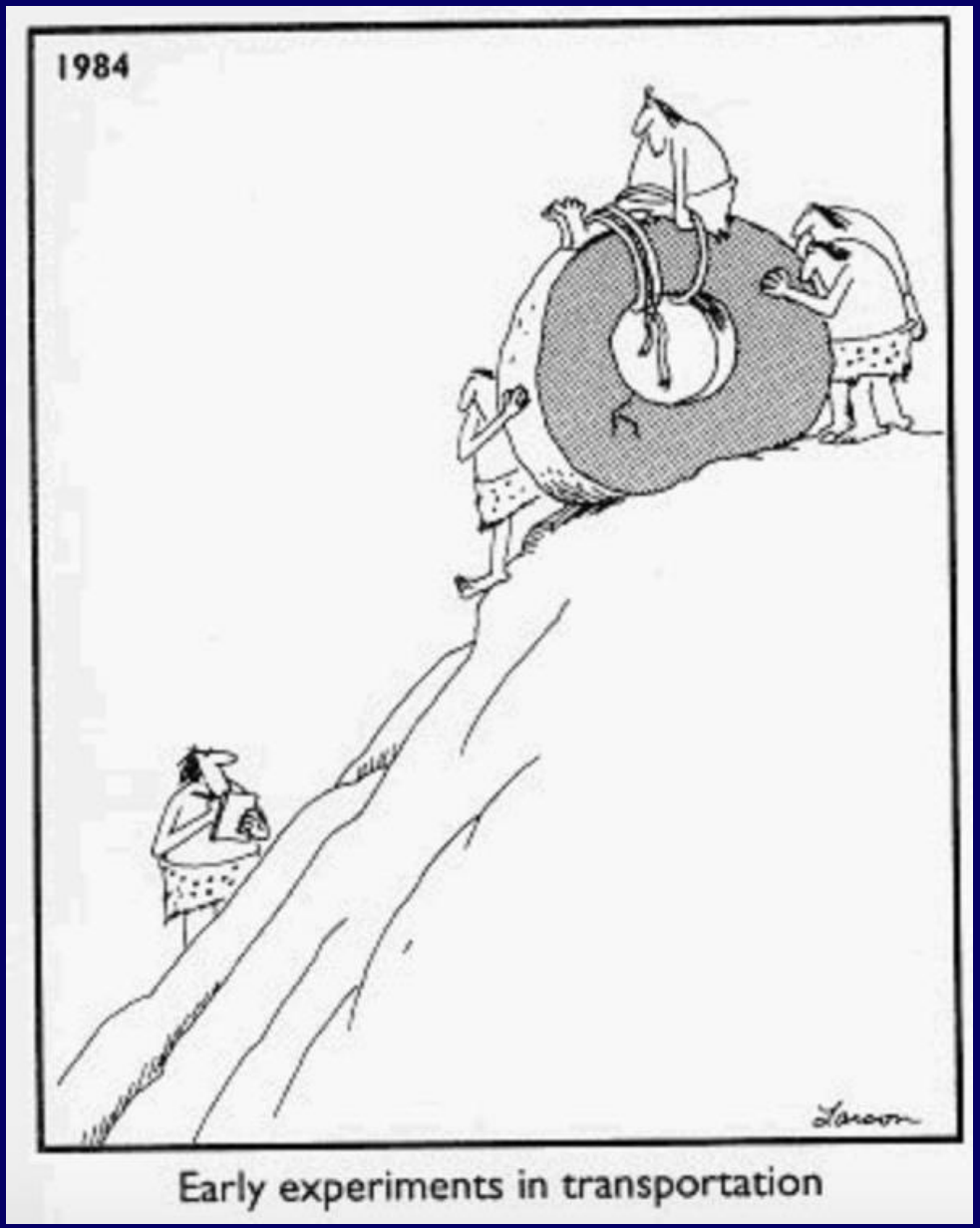


Adapted from Arruda et al. Blood. 2017 Nov 23;130(21):2251-2256.

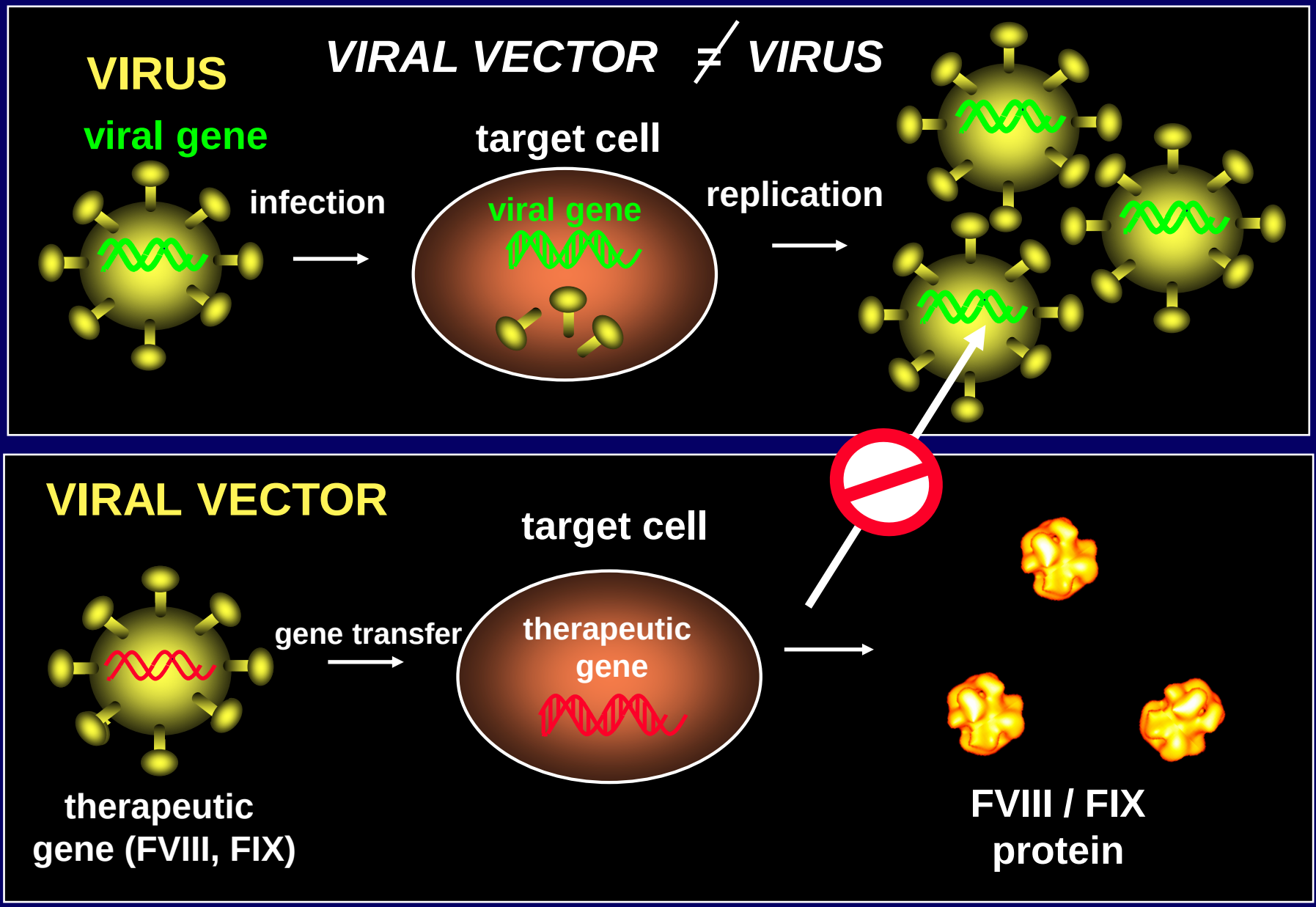
GENE THERAPY: KEY QUESTIONS

- IS GENE THERAPY EFFECTIVE ?
- HOW LONG IS THE EFFECT OF GENE THERAPY EXPECTED TO LAST?
- WHAT ARE THE MAIN SAFETY CONCERNS?
- CAN PATIENTS BE RETREATED?
- CAN WE TREAT PATIENTS WITH INHIBITORS?
- WHAT IS THE RISK OF INHIBITOR DEVELOPMENT AFTER GENE THERAPY?
- CAN WE ULTIMATELY TREAT CHILDREN BY GENE THERAPY IDEALLY BEFORE THE ONSET OF JOINT DISEASE?

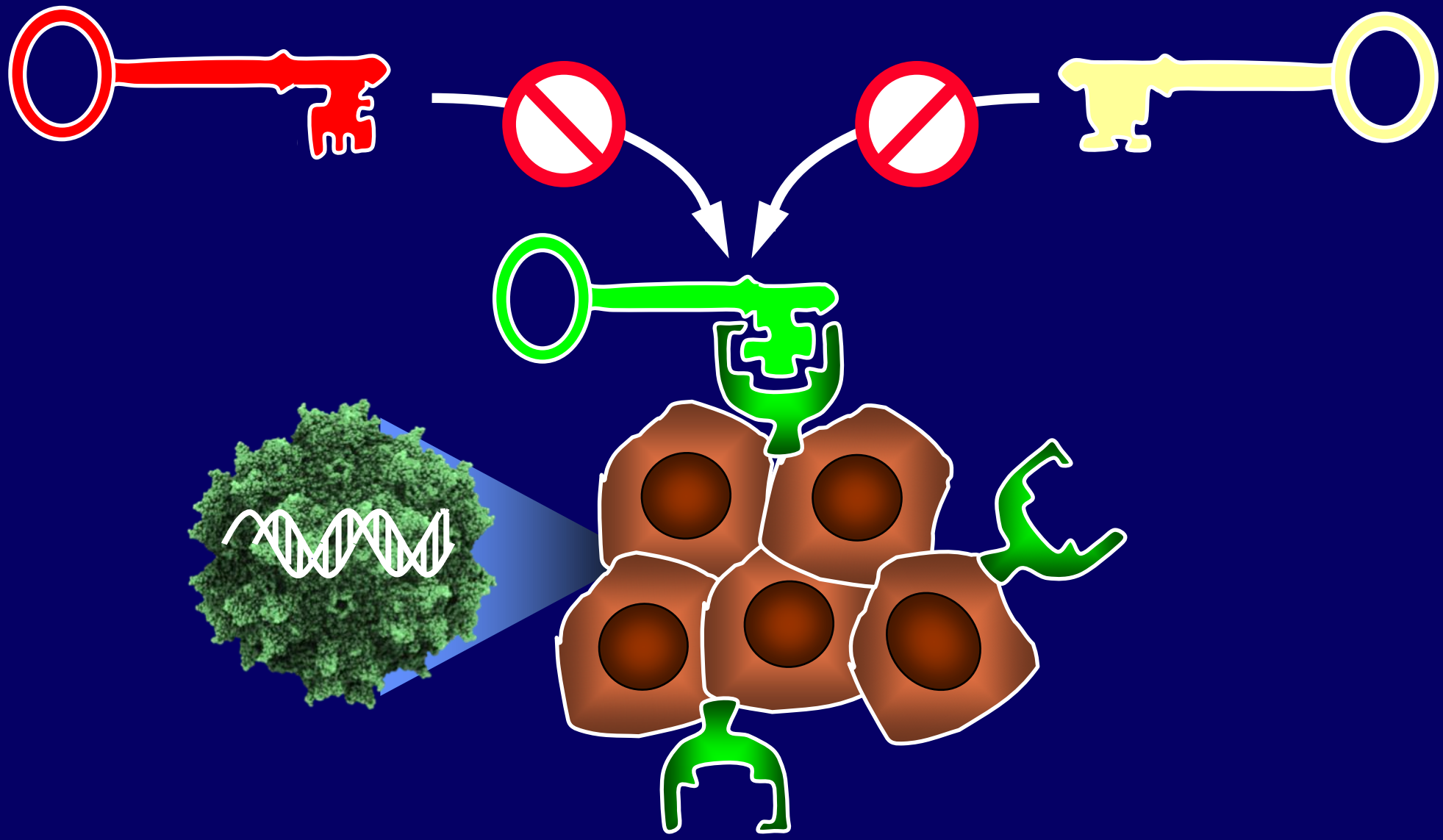
GENE THERAPY MANTRA: "DELIVERY, DELIVERY, DELIVERY !"



GENE THERAPY WITH VIRAL VECTORS

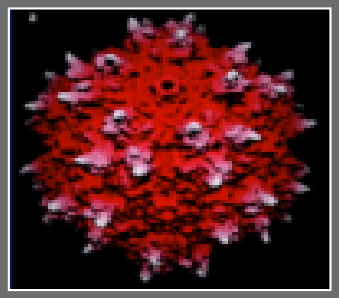


FINDING THE RIGHT 'MOLECULAR KEY' TO ENTER THE TARGET CELLS



FINDING THE RIGHT 'MOLECULAR KEY' TO ENTER THE TARGET CELLS

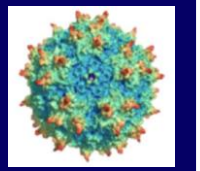
ADENO-ASSOCIATED VIRAL VECTORS (AAV)



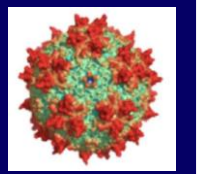
- **Efficacy established in human trials**
- **Sustained expression**
- **Pre-existing immunity**
- **Mostly not integrated**

HEMOPHILIA B
LIVER-DIRECTED GENE THERAPY

CLINICAL GENE THERAPY FOR HEMOPHILIA B



AAV8



AAV5

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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

THROMBOSIS AND HEMOSTASIS

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

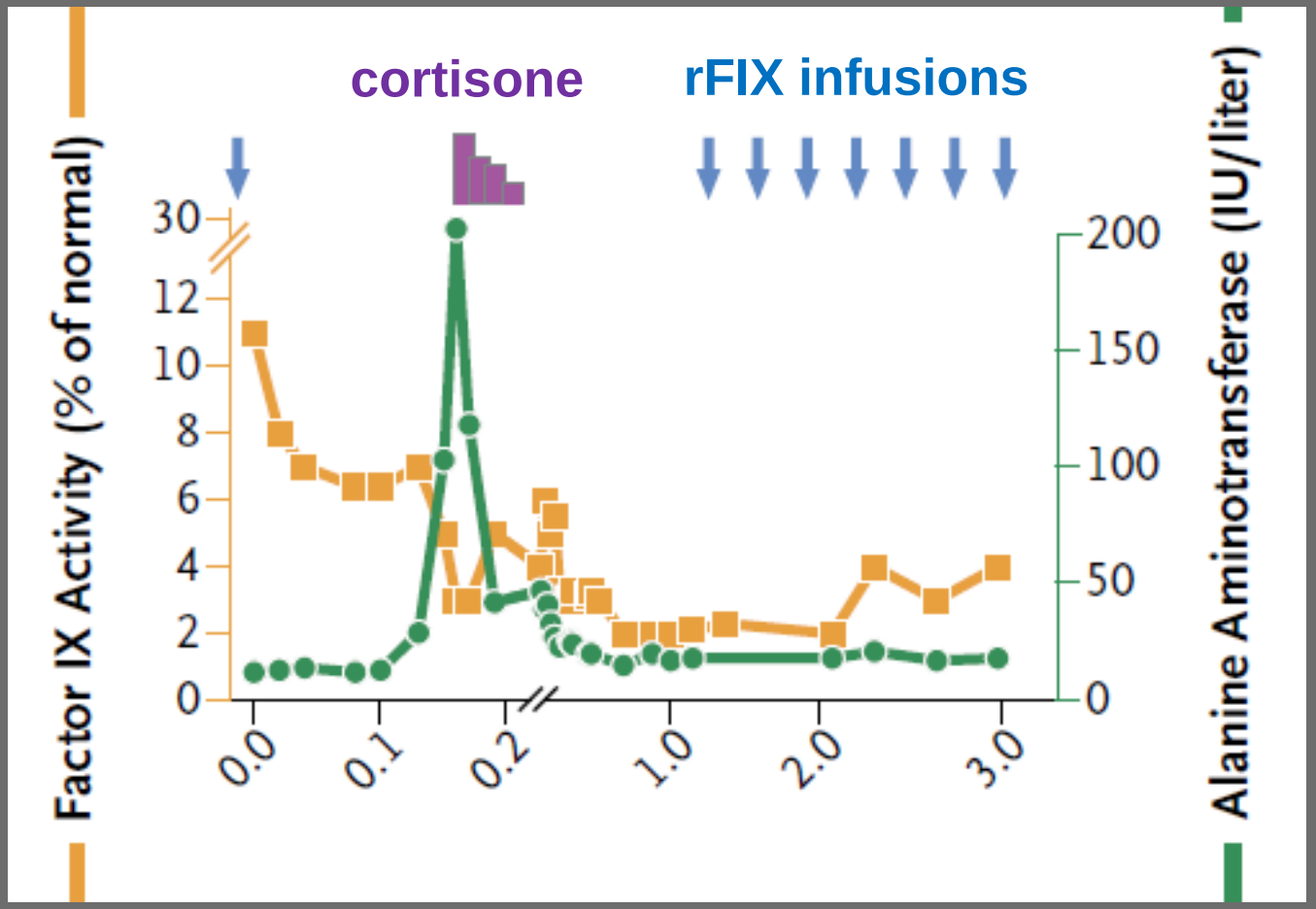
Wolfgang Miesbach,[1] Karina Meijer,[2] Michiel Coppens,[3] Peter Kampmann,[4] Robert Klamroth,[5] Roger Schutgens,[6] Marco Tangelder,[7] Giancarlo Castaman,[8] Joachim Schwäble,[9] Halvard Bonig,[9,10] Erhard Seifried,[9] Federica Cattaneo,[11] Christian Meyer,[7] and Frank W. G. Leebeek[12]

blood

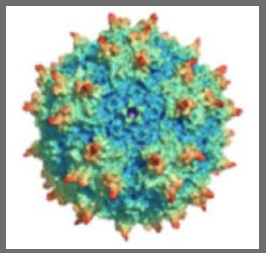
Nathwani et al. *N Engl J Med* 2014;371:1994–2004

Miesbach et al., *Blood.* 2018;131:1022-1031.

CLINICAL GENE THERAPY FOR HEMOPHILIA B



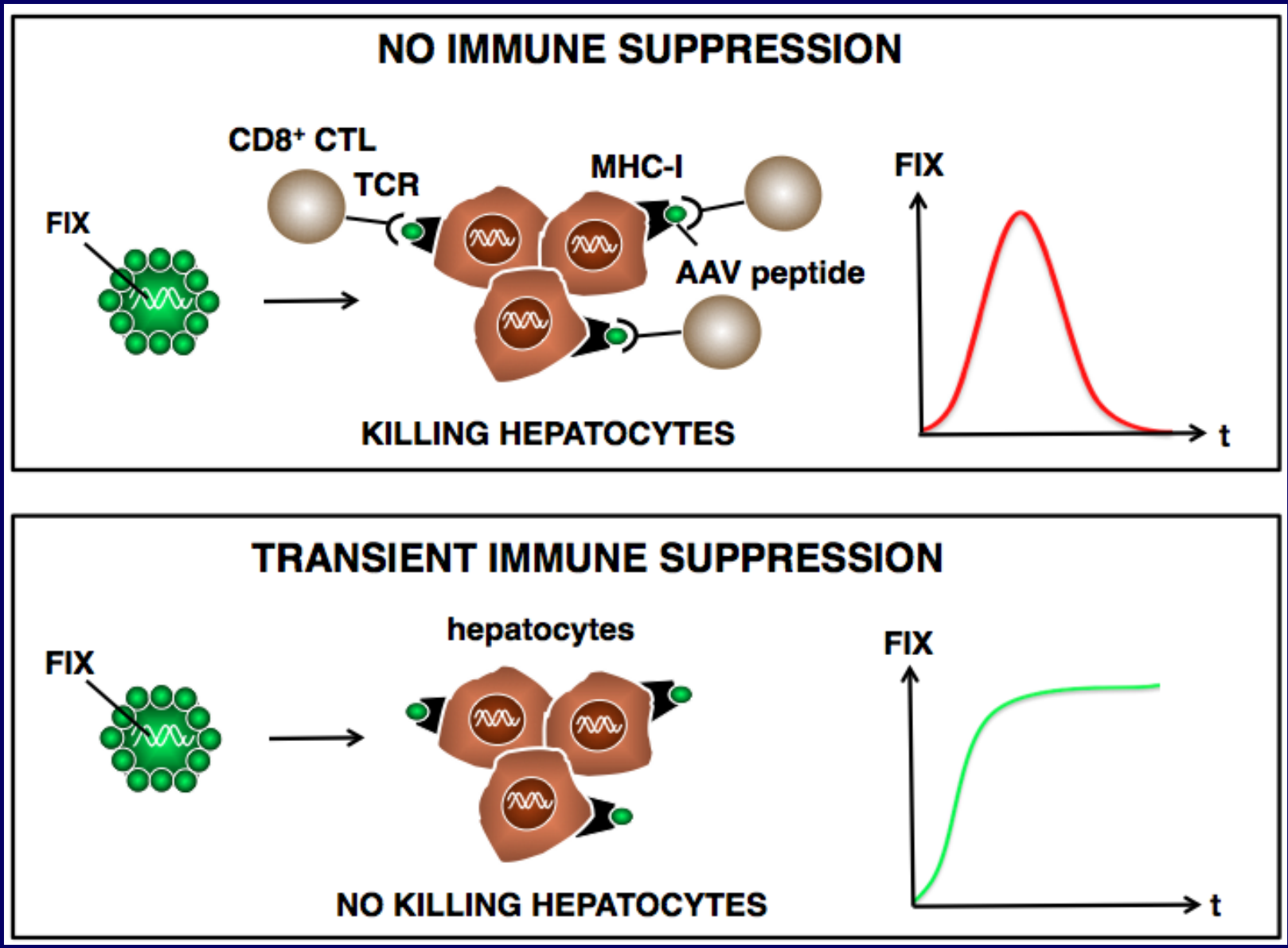
Nathwani et al. *N Engl J Med* 2014;371:1994–2004



AAV8-Factor IX
$2x10^{12}$ vg/kg

AAV8-FIX: SUSTAINED THERAPEUTIC FIX LEVELS (2-5% - about 7 years)
AAV5-FIX : 5% 10X higher vector dose - $2x10^{13}$ vg/kg (Miesbach et al., 2018)

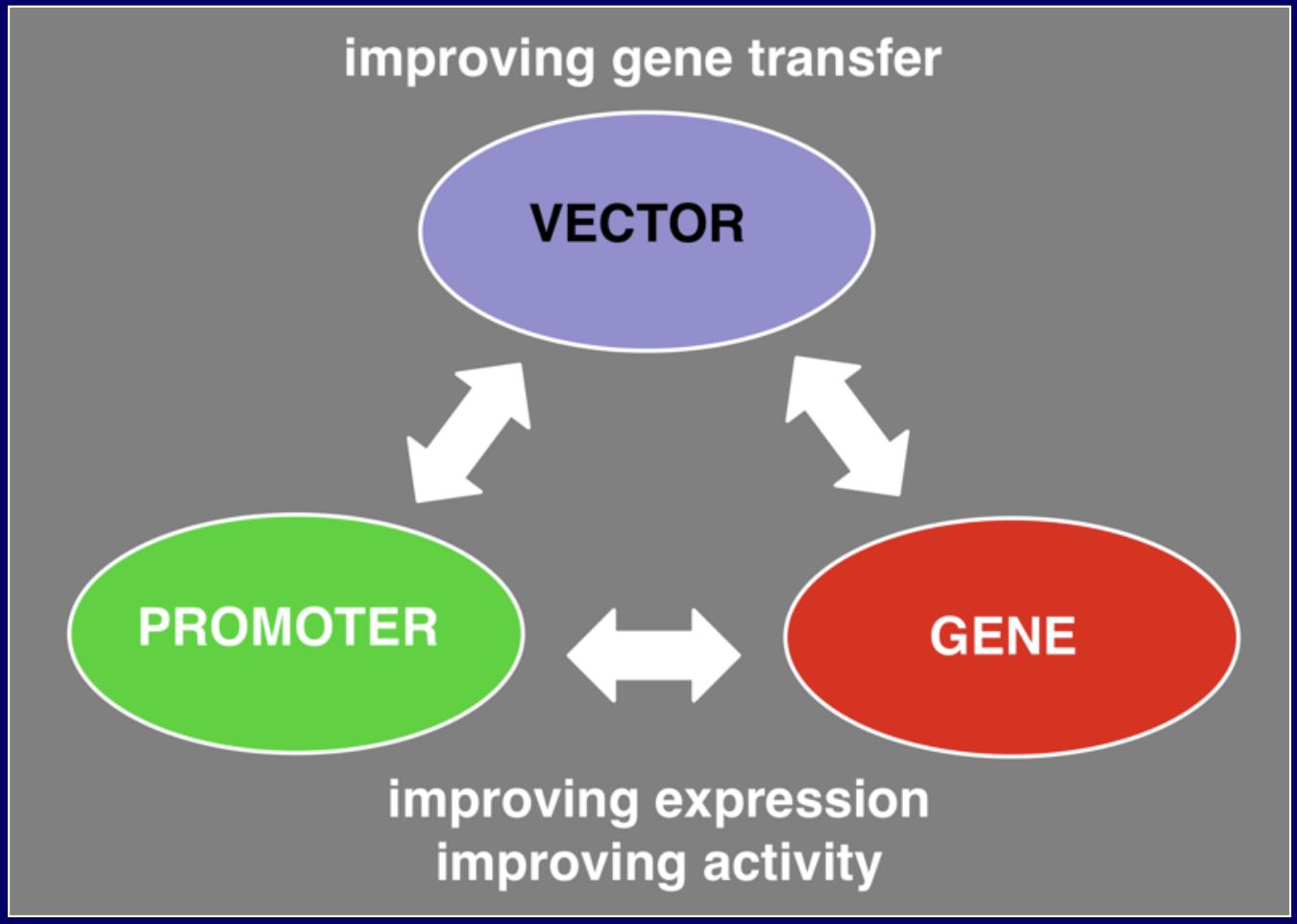
SAFETY ISSUE: IMMUNE RESPONSE



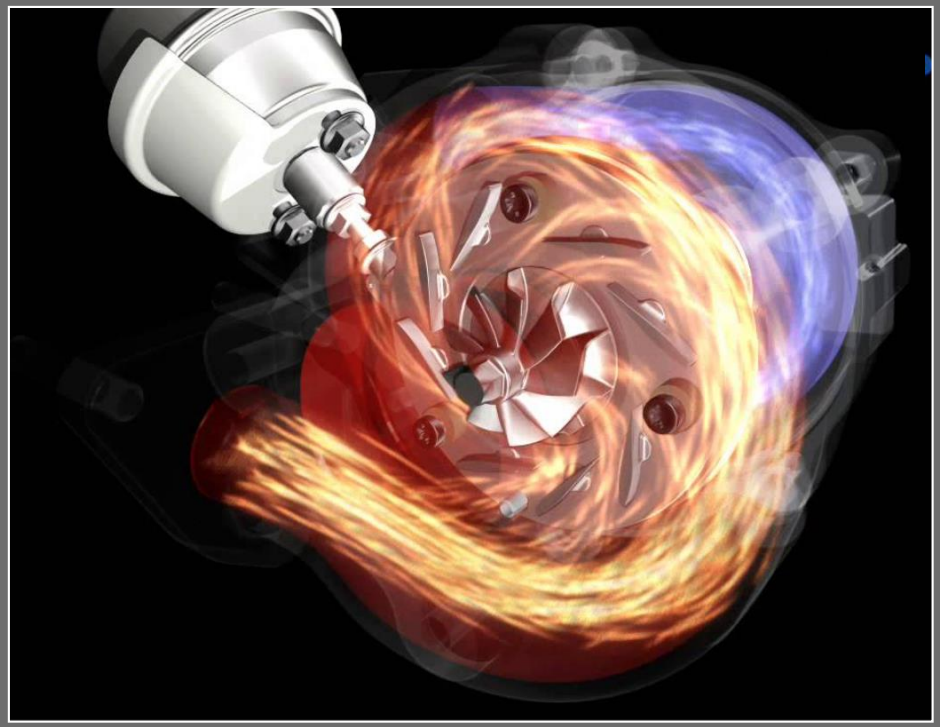
+ OTHER IMMUNE MECHANISMS

NEXT-GENERATION GENE THERAPY

a 3-pronged strategy

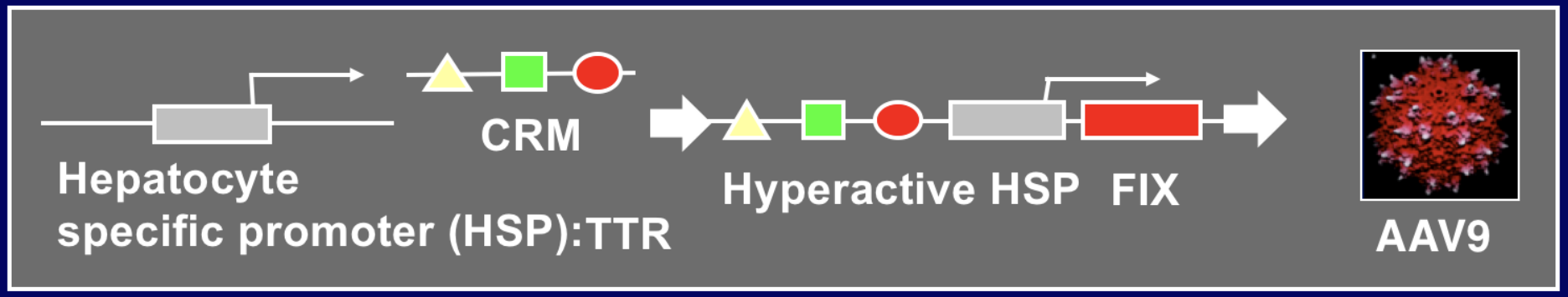


POTENT HEPATOCYTE-SPECIFIC CIS-REGULATORY MODULE (CRM)

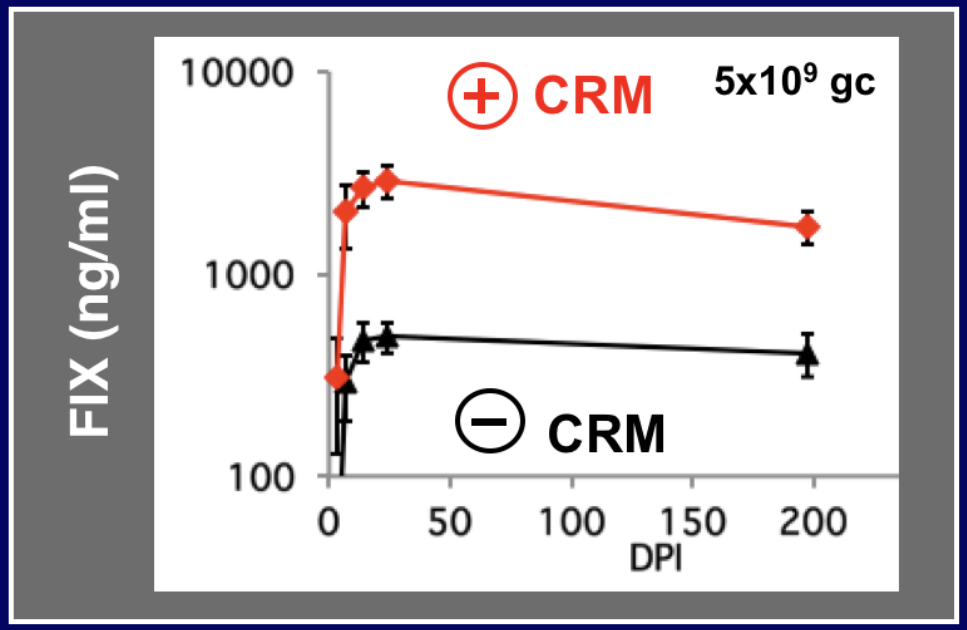


“MOLECULAR TURBOCHARGER” AS EXPRESSION BOOSTER

CRMs: cis-REGULATORY MODULES BOOST EXPRESSION



CRM: cis-regulatory module



ROBUST AND SUSTAINED HEPATOCYTE SPECIFIC FIX EXPRESSION

Michelangelo - Sistine Chapel Ceiling

blood

THROMBOSIS & HEMOSTASIS

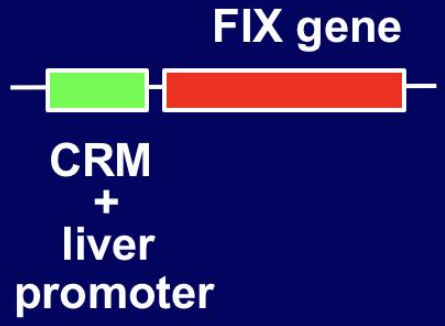
Comment on Nair et al, page 3195

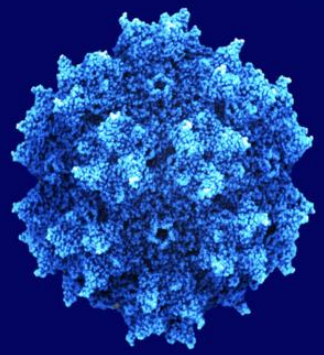
A David promoter with Goliath strength

David M. Markusic[1] and Roland W. Herzog[1] [1]UNIVERSITY OF FLORIDA

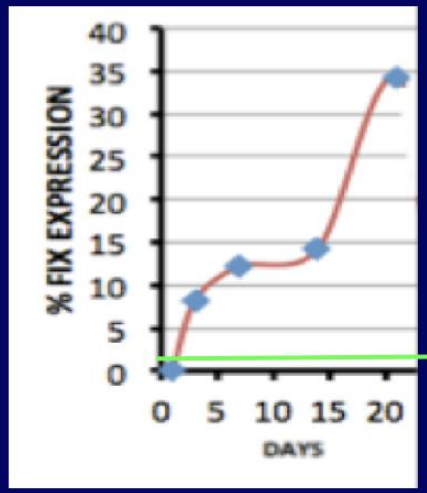
Markusic DM and Herzog RW. *Blood* 2014;123:3068–9.

CRM: PRECLINICAL & TRANSLATIONAL STUDIES





AAV-FIX VECTOR



1% therapeutic threshold

HIGH THERAPEUTIC FIX LEVELS IN RHESUS MONKEYS

CRM: ROBUST HEPATOCYTE SPECIFIC FIX EXPRESSION

blood May 15;123(20):3195-9, 2014.

Computationally designed liver-specific transcriptional modules and hyperactive factor IX improve hepatic gene therapy

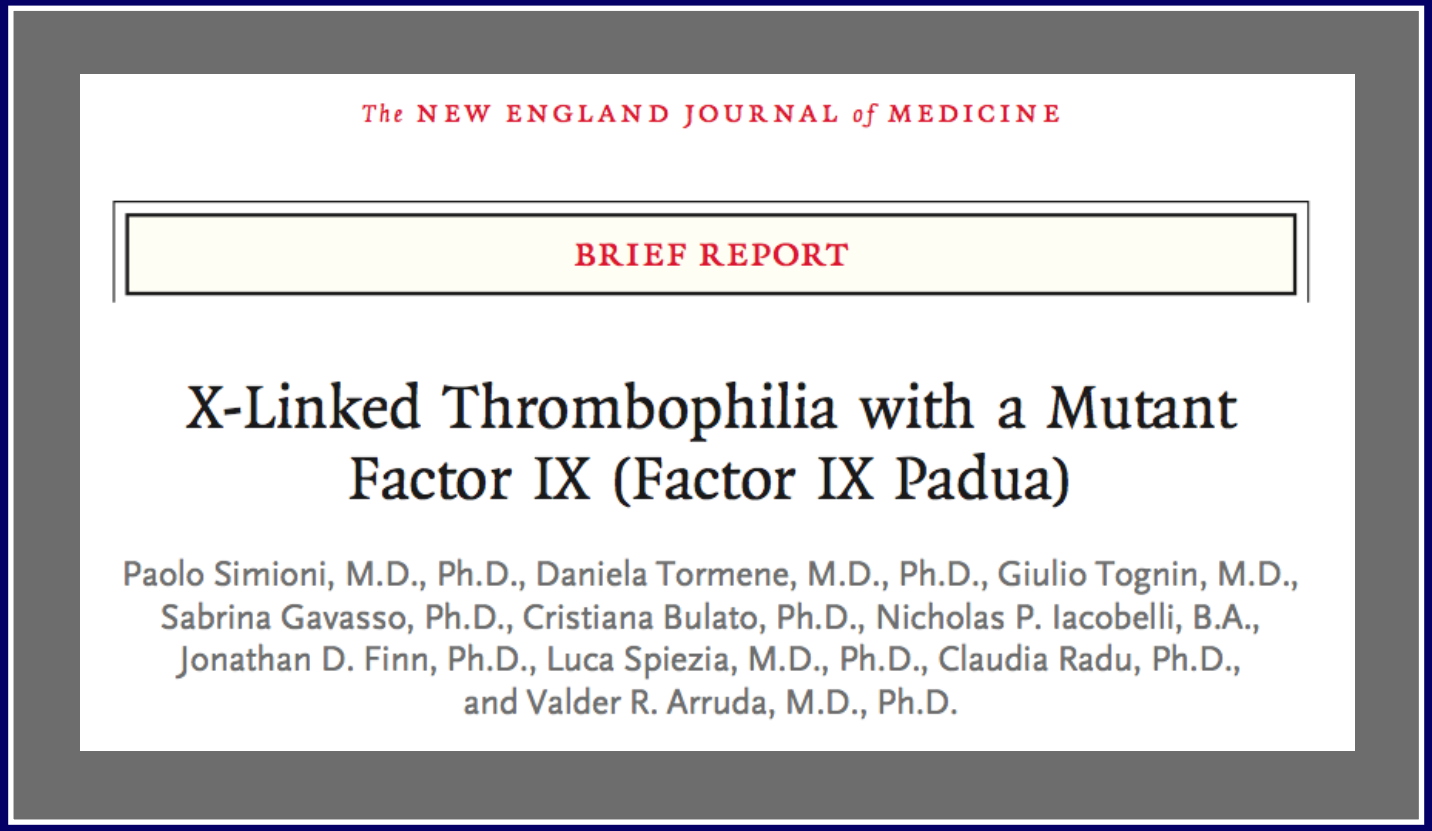
Nisha Nair,[1] Melvin Y. Rincon,[1,2] Hanneke Evens,[1] Shilpita Sarcar,[1] Sumitava Dastidar,[1] Emira Samara-Kuko,[1] Omid Ghandeharian,[1] Hiu Man Viecelli,[3] Beat Thöny,[3] Pieter De Bleser,[4] Thierry VandenDriessche,[1,2] and Marinee K. Chuah[1,2]

Molecular Therapy Sep;22(9):1605-13, 2014.

Liver-Specific Transcriptional Modules Identified by Genome-Wide *In Silico* Analysis Enable Efficient Gene Therapy in Mice and Non-Human Primates

Marinee K Chuah[1,2], Inge Petrus[2], Pieter De Bleser[3], Caroline Le Guiner[4,5], Gwladys Gernoux[4,5], Oumeya Adjali[4,5], Nisha Nair[1], Jessica Willems[1], Hanneke Evens[1], Melvin Y Rincon[1,2], Janka Matrai[1,6,7], Mario Di Matteo[1,2], Ermira Samara-Kuko[1], Bing Yan[6,7], Abel Acosta-Sanchez[6,7], Amine Meliani[8,9], Ghislaine Cherel[10,11], Véronique Blouin[4,5], Olivier Christophe[10,11], Philippe Moullier[4,5], Federico Mingozzi[8,9], Thierry VandenDriessche[1,2]

CAN WE CHANGE THE FIX GENE ITSELF TO BOOST VECTOR PERFORMANCE?



The NEW ENGLAND JOURNAL of MEDICINE

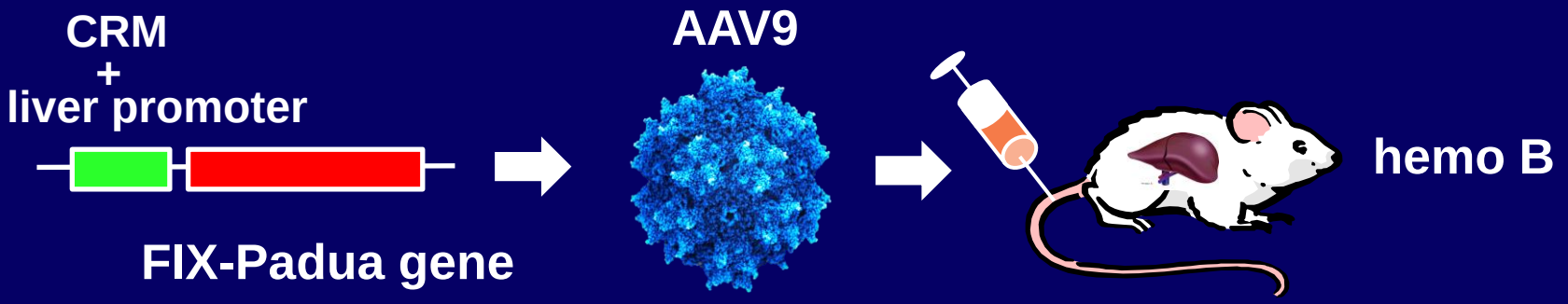
BRIEF REPORT

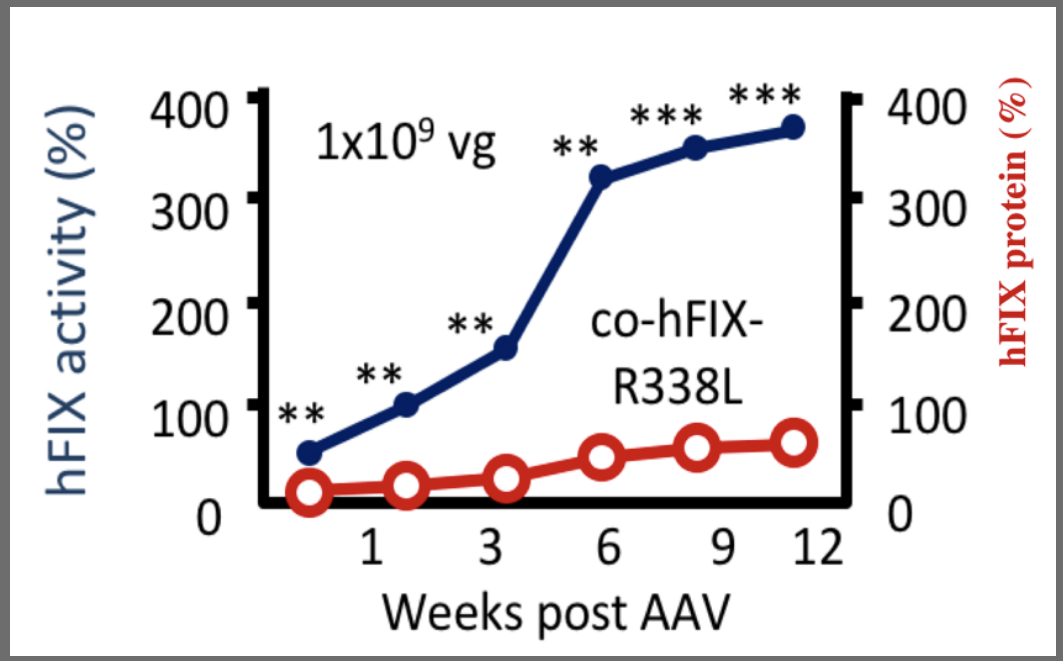
X-Linked Thrombophilia with a Mutant Factor IX (Factor IX Padua)

Paolo Simioni, M.D., Ph.D., Daniela Tormene, M.D., Ph.D., Giulio Tognin, M.D., Sabrina Gavasso, Ph.D., Cristiana Bulato, Ph.D., Nicholas P. Iacobelli, B.A., Jonathan D. Finn, Ph.D., Luca Spiezia, M.D., Ph.D., Claudia Radu, Ph.D., and Valder R. Arruda, M.D., Ph.D.

***Simioni P, et al. N Engl J Med* 2009 Oct 22;361(17):1671-5.**

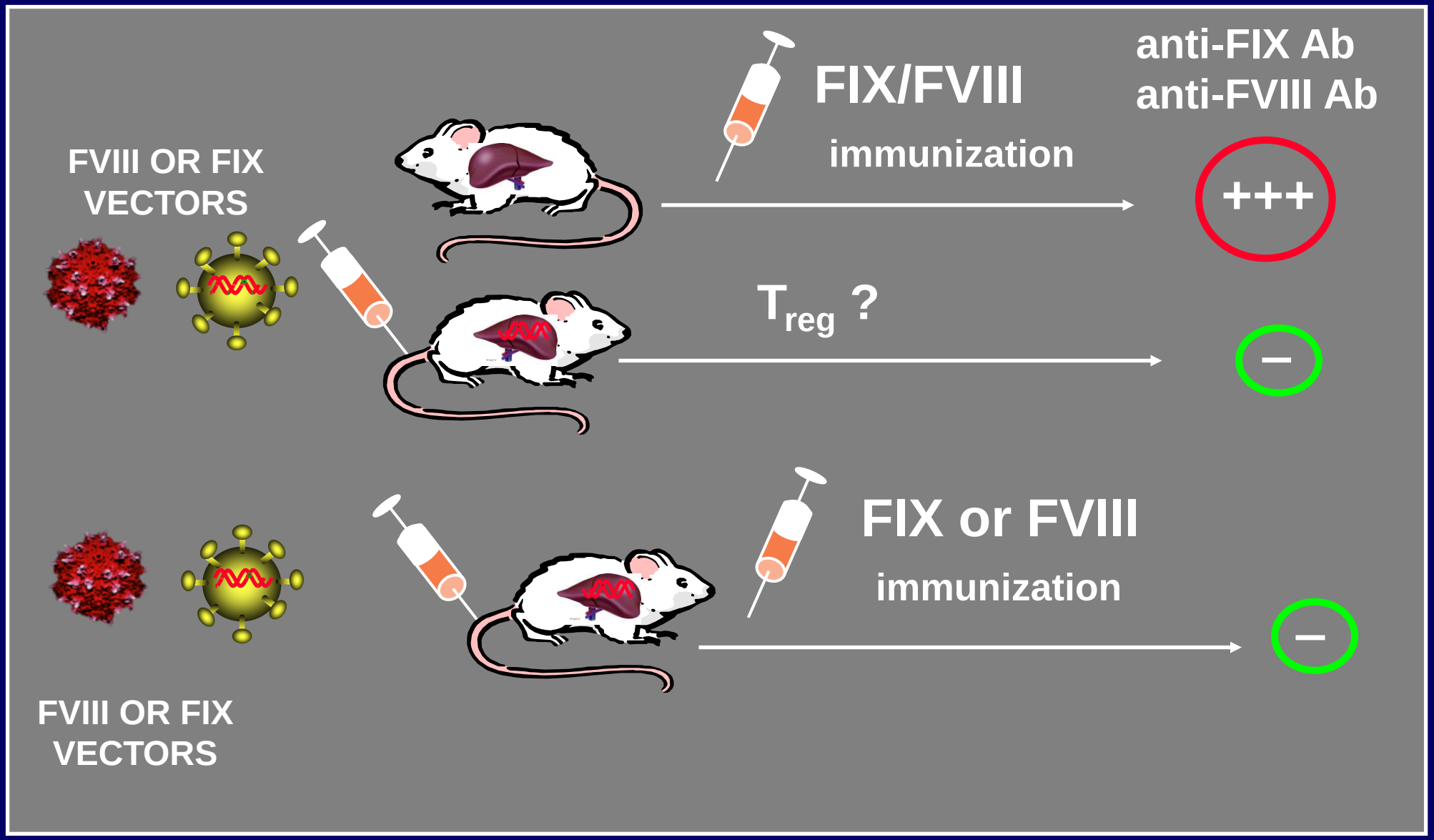
ONE TIME TREATMENT BY GENE THERAPY
SUSTAINED THERAPEUTIC EFFECT WITH LOW VECTOR DOSE





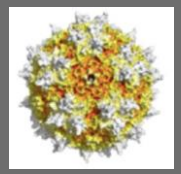
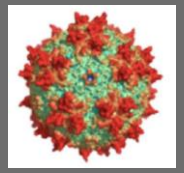
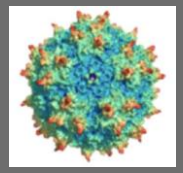
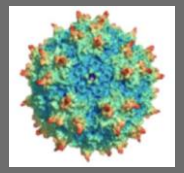
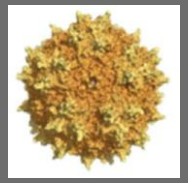
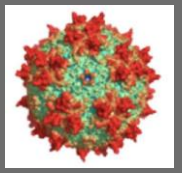
Killing 2 birds with 1 stone: increase expression and activity

IMMUNE TOLERANCE: PROTECTION AGAINST NEUTRALIZING ANTIBODIES



Mingozzi et al., J. Clin. Invest. (2003); Matrai et al. Hepatology (2011);
Cantore, Nair et al. Blood, (2012); Nair et al., Blood, (2014);
Finn et al., Blood (2012); Brown et al., Blood (2007); Follenzi et al., unpublished

GENE THERAPY CLINICAL TRIALS FOR HEMOPHILIA B

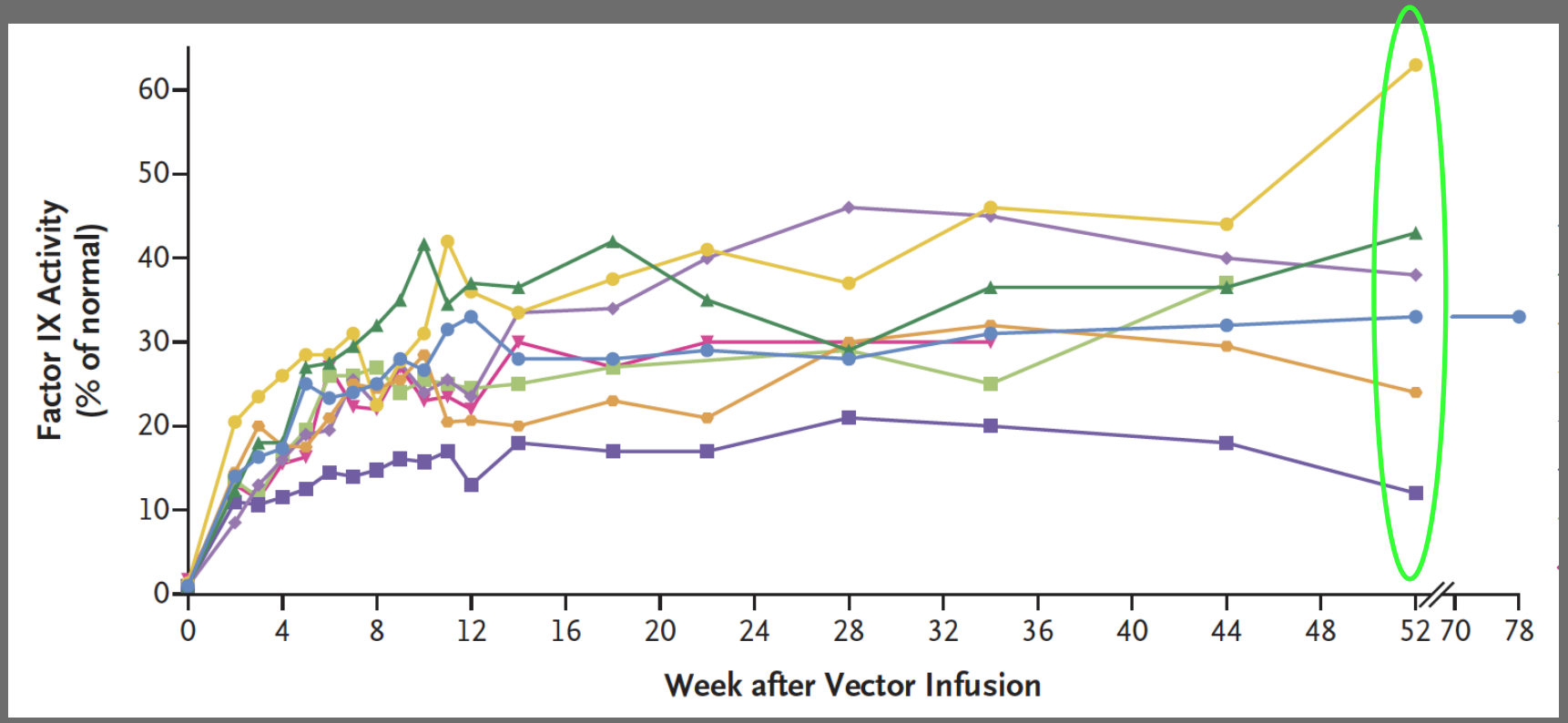
	PHASE III	PHASE III	PHASE I/II	PHASE I/II	PHASE I/II	PHASE I/II
company	*Spark Pfizer*	*UniQure*	*Shire*	*Freeline*	*Sangamo*	*UniQure*
serotype	 Clade E Spk200 SPK-9001	 AAV5 AMT-061	 AAV8 SHP648	 AAV8 FLT180a	 AAV6 SB-FIX	 AAV5 AMT-060
FIX	*coFIX Padua*	*coFIX Padua*	*coFIX Padua*	*coFIX Padua*	*ZFN gene editing in albumin*	*FIX Padua*
promotor	*HSP*	*HSP*	*HSP*	*HSP*	*HSP*	*HSP*

BENCHMARK

Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

December 7, 2017
N Engl J Med 2017; 377:2215-2227
DOI: 10.1056/NEJMoa1708538

Lindsey A. George, M.D., Spencer K. Sullivan, M.D., Adam Giermasz, M.D., Ph.D., John E.J. Rasko, M.B., B.S., Ph.D., Benjamin J. Samelson-Jones, M.D., Ph.D., Jonathan Ducore, M.D., M.P.H., Adam Cuker, M.D., Lisa M. Sullivan, M.D., Suvankar Majumdar, M.D., Jerome Teitel, M.D., Catherine E. McGuinn, M.D., Margaret V. Ragni, M.D., M.P.H., et al.



single i.v. AAV-FIX vector injection $5x10^{11}$ vg/kg

Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

Lindsey A. George, M.D., Spencer K. Sullivan, M.D., Adam Giermasz, M.D., Ph.D., John E.J. Rasko, M.B., B.S., Ph.D., Benjamin J. Samelson-Jones, M.D., Ph.D., Jonathan Ducore, M.D., M.P.H., Adam Cuker, M.D., Lisa M. Sullivan, M.D., Suvankar Majumdar, M.D., Jerome Teitel, M.D., Catherine E. McGuinn, M.D., Margaret V. Ragni, M.D., M.P.H., et al.

December 7, 2017
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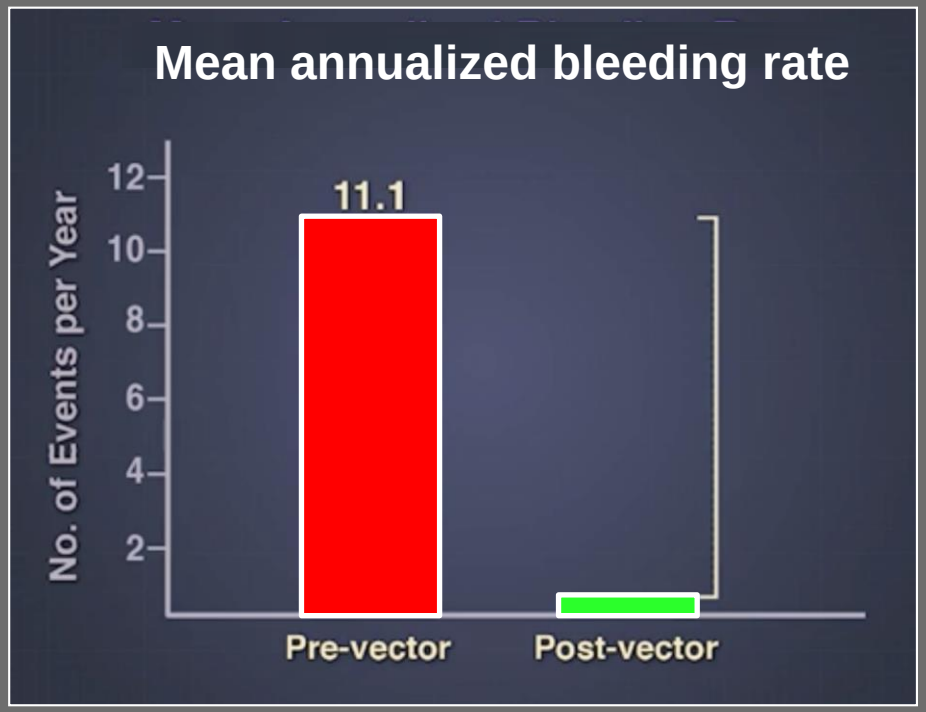
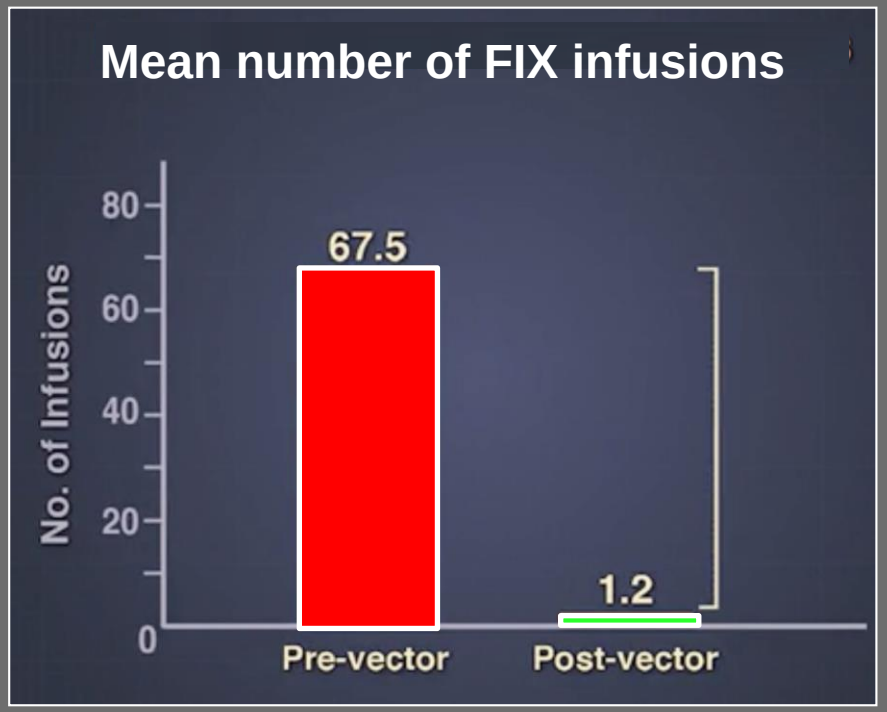




GENE THERAPY FOR HEMOPHILIA A: BOTTLENECKS IN FVIII PRODUCTION

secretion & stabilization

↑

FVIII transport ER to Golgi

FVIII mannose binding to LMAN1

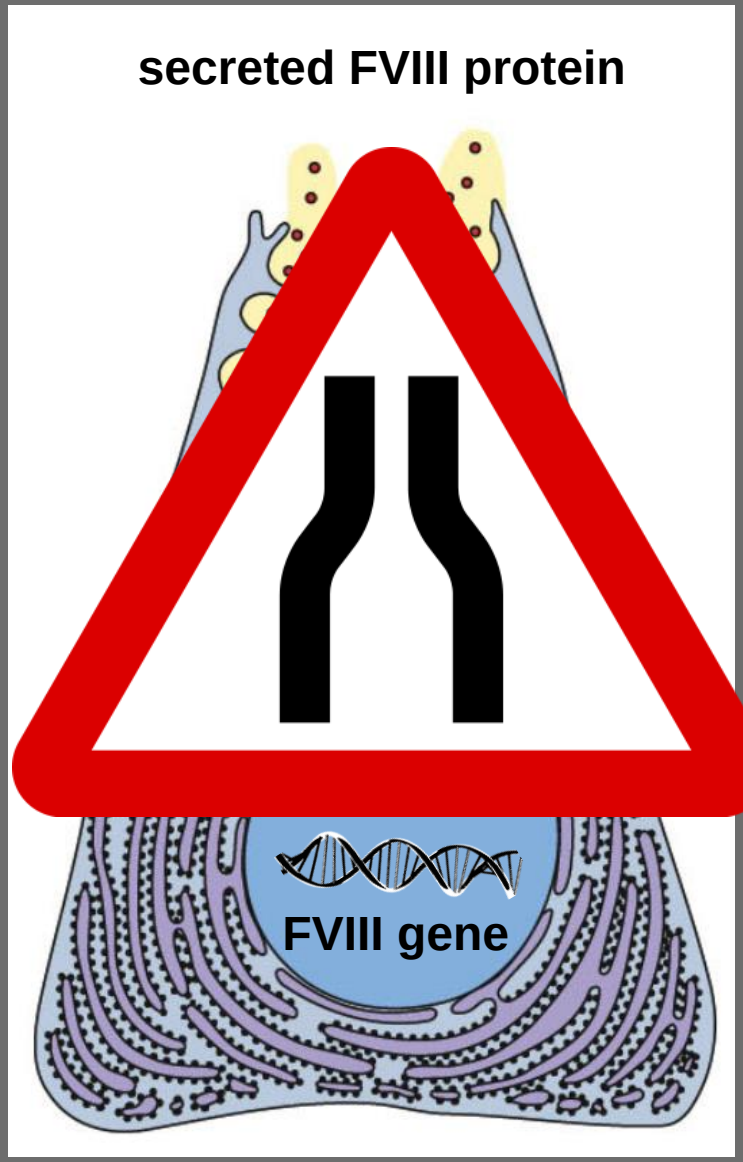
↑

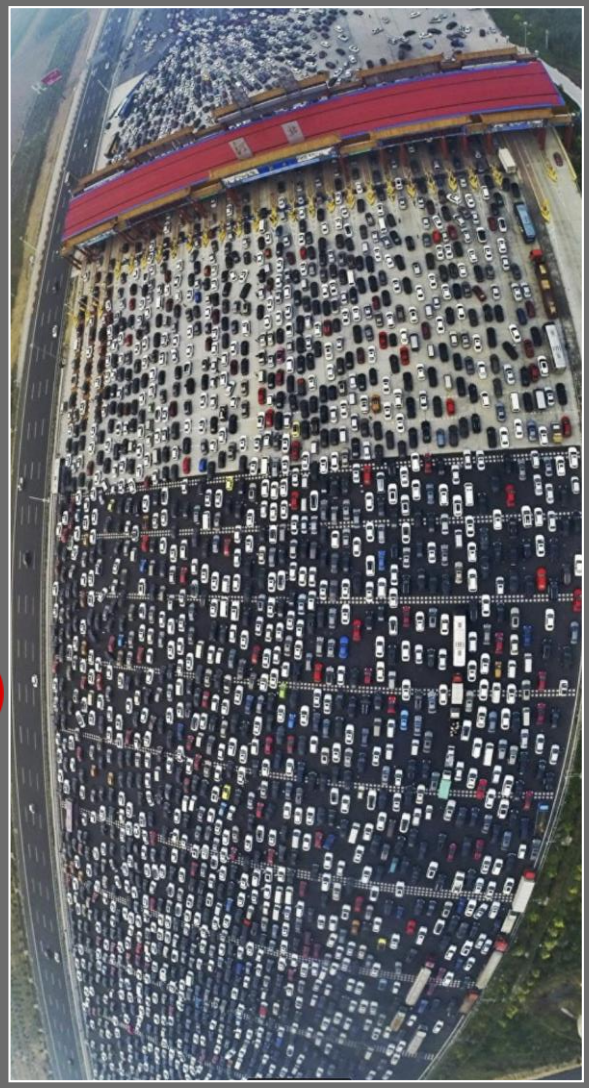
FVIII translation

FVIII binding to BiP ER retention

↑

FVIII transcription



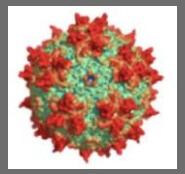
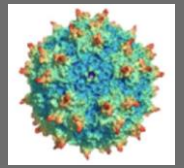
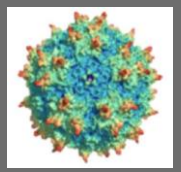
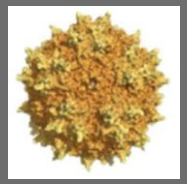
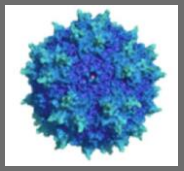
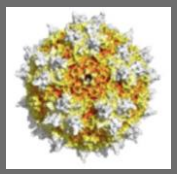


FVIII IN AAV: SIZE MATTERS !!!

AAV-FVIII: Packaging constraints

Dong et al. Hum Gene Ther. 1996 Nov 10;7(17):2101-12.

GENE THERAPY CLINICAL TRIALS FOR HEMOPHILIA A

	PHASE III	PHASE I/II	PHASE I/II	PHASE I/II	PHASE I/II	PHASE I/II
company	*BioMarin*	*Shire*	*Freeline*	*Sangamo Pfizer*	*Dimension Bayer*	*Spark*
serotype	 AAV5	 AAV8	 AAV8	 AAV6	 AAV8	 Clade E Spk200
FVIII	*coFVIIIΔB_{SQ}*	*coFVIIIΔB*	*coFVIIIΔB-V3*	*coFVIIIΔB*	*coFVIIIΔB*	*coFVIIIΔB*
promotor	*HSP (ApoEHCR-AAT)*	*HSP*	*HSP*	*HSP (TTR)*	*HSP*	*HSP*
	BMN 270	SHP 654	AAV8-HLP-hFVIII-V3	SB-525	DTX-201	SPK-8011

BENCHMARK

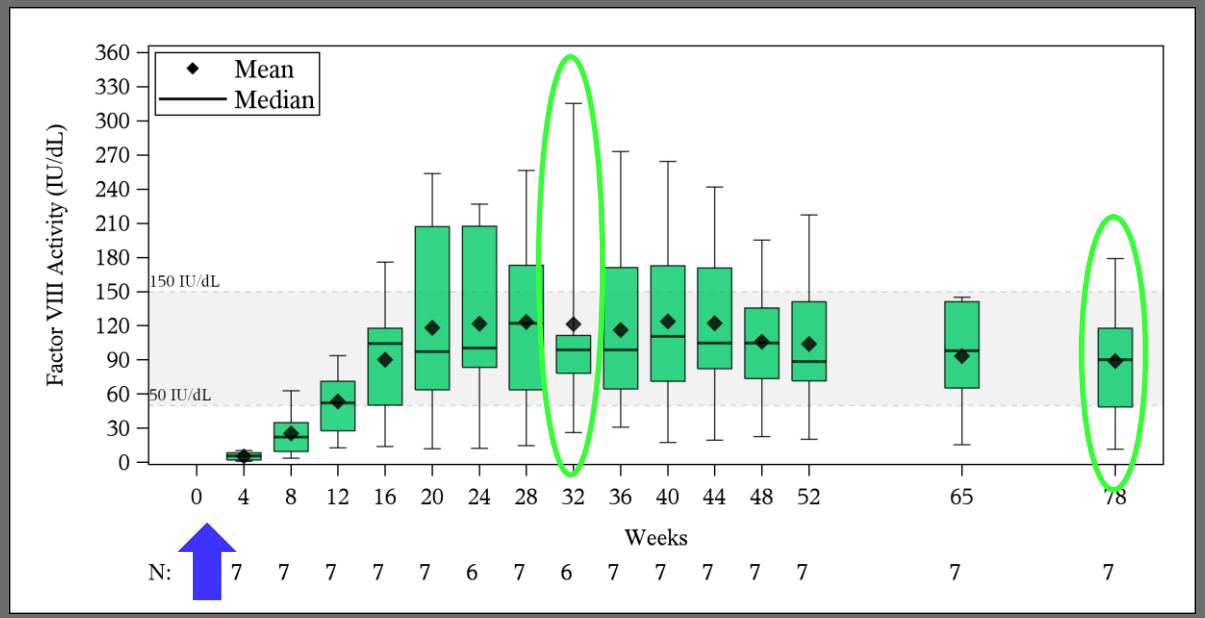
ORIGINAL ARTICLE

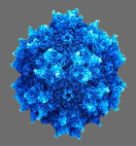
AAV5–Factor VIII Gene Transfer in Severe Hemophilia A

Savita Rangarajan, M.B., B.S., Liron Walsh, M.D., Will Lester, M.B., Ch.B., Ph.D., David Perry, M.D., Ph.D., Bella Madan, M.D., Michael Laffan, D.M., Hua Yu, Ph.D., Christian Vettermann, Ph.D., Glenn F. Pierce, M.D., Ph.D., Wing Y. Wong, M.D., and K. John Pasi, M.B., Ch.B., Ph.D.

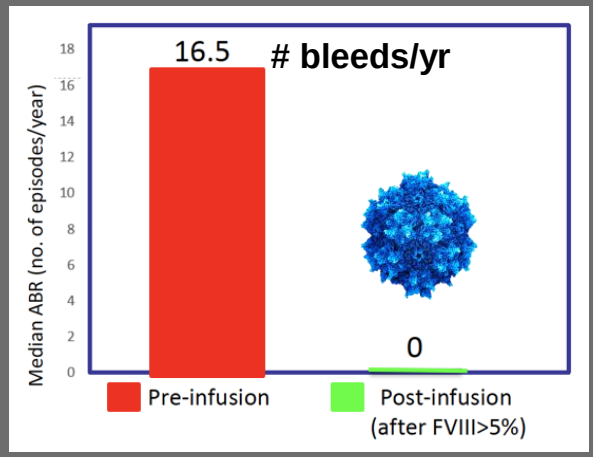
N Engl J Med 2017; 377:2519-2530 | December 28, 2017 | DOI: 10.1056/NEJMoa1708483

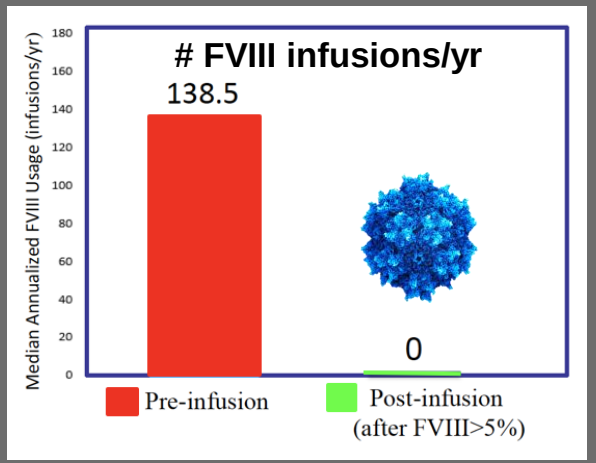
ASH 2017





single i.v. AAV5-FVIII vector injection





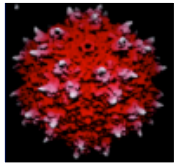
"MILLION DOLLAR QUESTION"
WILL EXPRESSION BE LIFE-LONG IN PATIENTS AFTER GENE THERAPY?

EXPECTATIONS: Long-term expression

Sustained **(life-long)**

Sustained **>10 years** [3]

Sustained

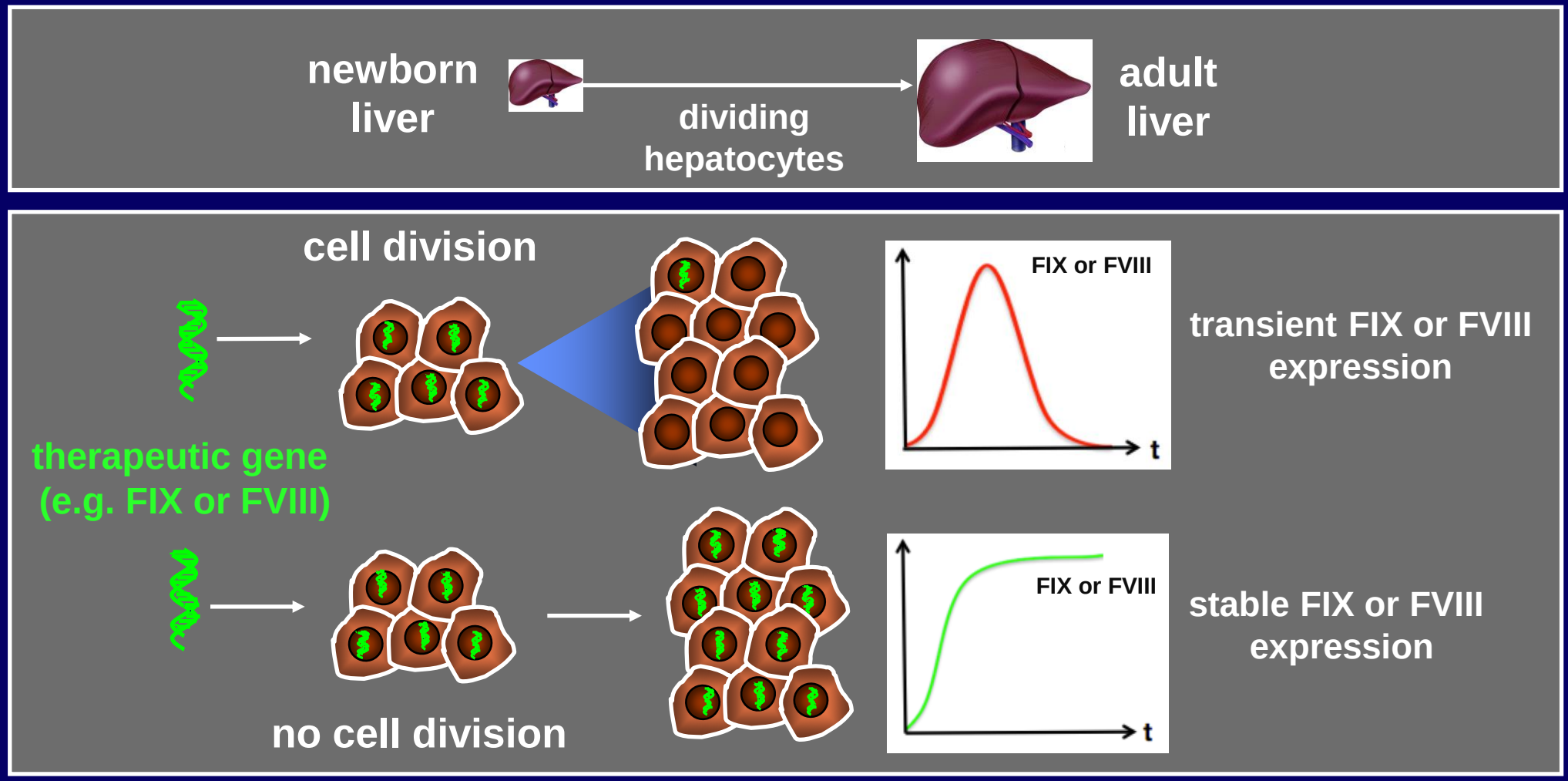


FIX: **>7** years (median)[1]

FIX: 10 years[2]

1. Nathwani et al. *N Engl J Med.* 2014;371(21):1994-2004.
2. Buchlis et al. *Blood*. 2012; 119(13):3038-3041.
3. Nichols et al., Hum Gene Ther Clin Dev. 2015 Mar 1; 26(1): 5–14.

ADVANTAGE GENOMIC INTEGRATION: SUSTAINED EXPRESSION IN DIVIDING CELLS IMPLICATIONS FOR PEDIATRIC PATIENTS



GENE EDITING

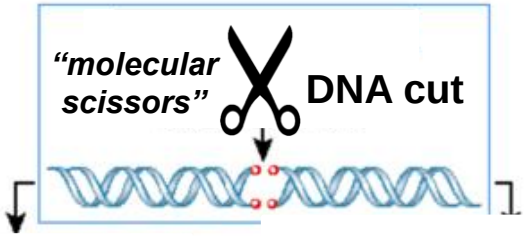
Correcting the mutation directly *in situ* within the defective gene itself

OFF-TARGET

non-desirable

ON-TARGET

desirable


“molecular scissors”
DNA cut

Molecular Therapy
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Efficient *In Vivo* Liver-Directed Gene Editing Using CRISPR/Cas9

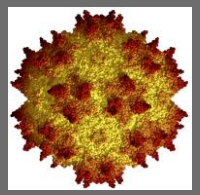
Kshitiz Singh,[1,5] Hanneke Evens,[1,5] Nisha Nair,[1,5] Melvin Y. Rincón,[1,2,3] Shilpita Sarcar,[1] Ermira Samara-Kuko,[1] Marinee K. Chuah,[1,2,4] and Thierry VandenDriessche[1,2,4]

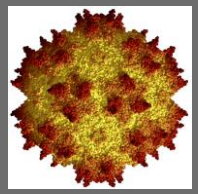
Molecular Therapy (2018)
https://doi.org/10.1016/j.ymthe.2018.02.023

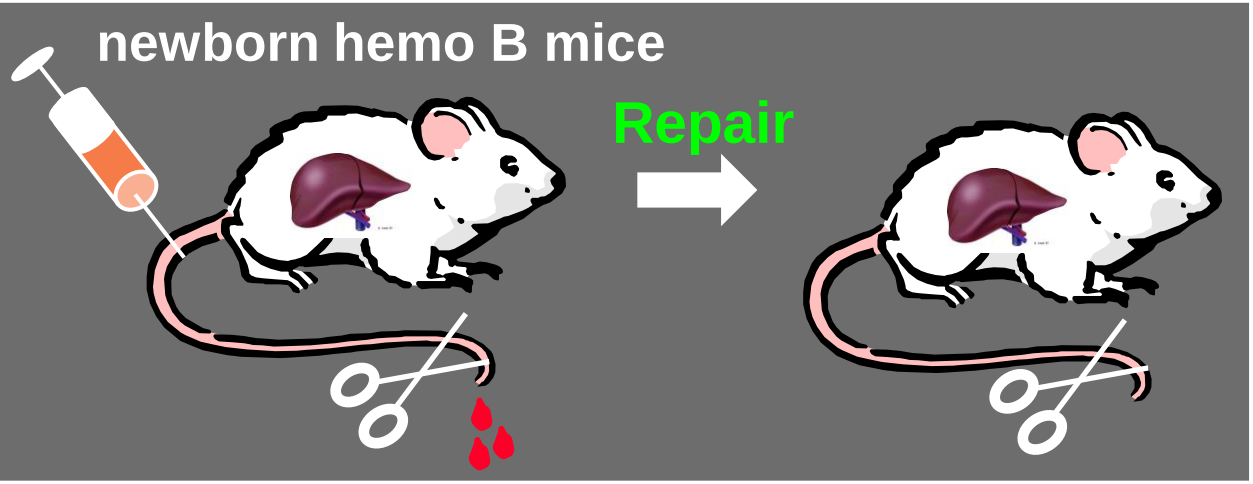
LIVER-SPECIFIC GENE EDITING OF FIX GENE: CRISPR/Cas9

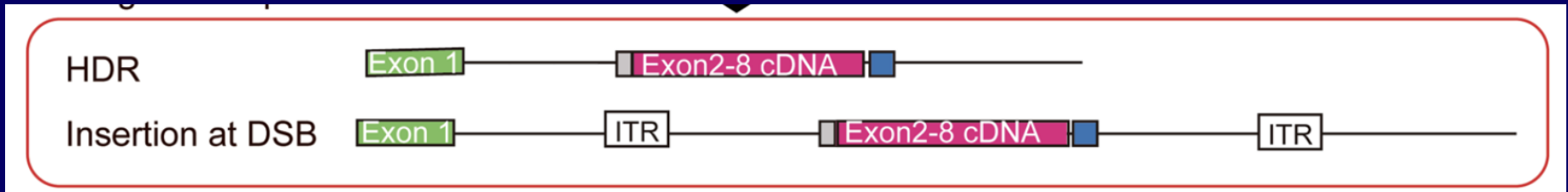
AAV-Cas9-gRNA
($6x10^{10}$ vg)

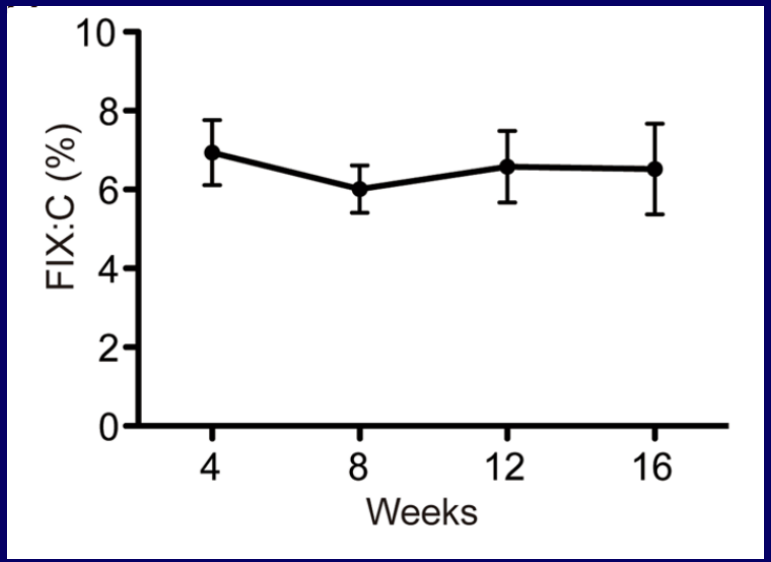
AAV-donor-FIX
($2x10^{11}$ vg)











Ohmuri et al.,
Sci Rep. 7(1): 4159, 2017

GENE THERAPY: KEY QUESTIONS

- **IS GENE THERAPY EFFECTIVE?**
  YES, hemophilia and other genetic diseases; cancer
- **HOW LONG IS THE EFFECT OF GENE THERAPY EXPECTED TO LAST? > 7 years – lifelong? LV vs AAV vs gene-editing**
- **WHAT ARE THE MAIN SAFETY CONCERNS?**
  hepatitis/inflammation, but can be controlled (cortisone); integration
- **CAN PATIENTS BE RETREATED?**
  Vector-antibodies preclude vector readministration (possible solutions)
- **CAN WE TREAT PATIENTS WITH INHIBITORS?**
  Some animal studies show tolerance induction and dissappearance of inhibitors
- **WHAT IS THE RISK OF INHIBITOR DEVELOPMENT AFTER GENE THERAPY?**
  All patients pre-screened for lack thereof
- **CAN WE ULTIMATELY TREAT CHILDREN BY GENE THERAPY IDEALLY BEFORE THE ONSET OF JOINT DISEASE?**
  Likely integrating vector or gene editing needed / AAV readministration ?

CONCLUSIONS

- **Sustained FIX (>1% 8 yrs; 30-40%) and FVIII (50%; 2 yrs) expression**
- **Correction of bleeding, discontinuation prophylaxis**
- **Diminished factor use; no inhibitors to FIX or FVIII Immune tolerance to FVIII/FIX in preclinical models**

- **Short-term safety issues: Immune response (vector, gene-engineered cells); liver toxicity (corticosteroids)**
- **Long-term safety issues: unknown?**
- **Patient-to-patient variation: “lucky draw” (risk of traumatic bleeds or thrombosis)**

Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning.

(Winston Churchill)

Dank u - thank you - धन्यवाद - 謝謝 – ½ è Õ ¿óî
falemnderit – grazie - gracias - danke

VUB *Department Gene Therapy & Regenerative Medicine*

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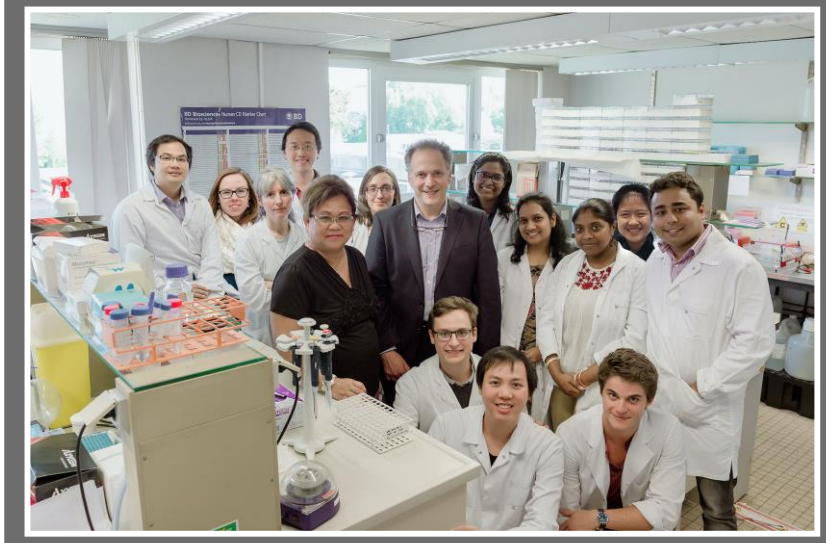
M. Loperfido
E. Samara
H. Evens

T. Jens
D. Boon
S. Landwehr
W. De Schrijver
L. Claeys
D. Manjumdar
S. Naganur
H. Pham

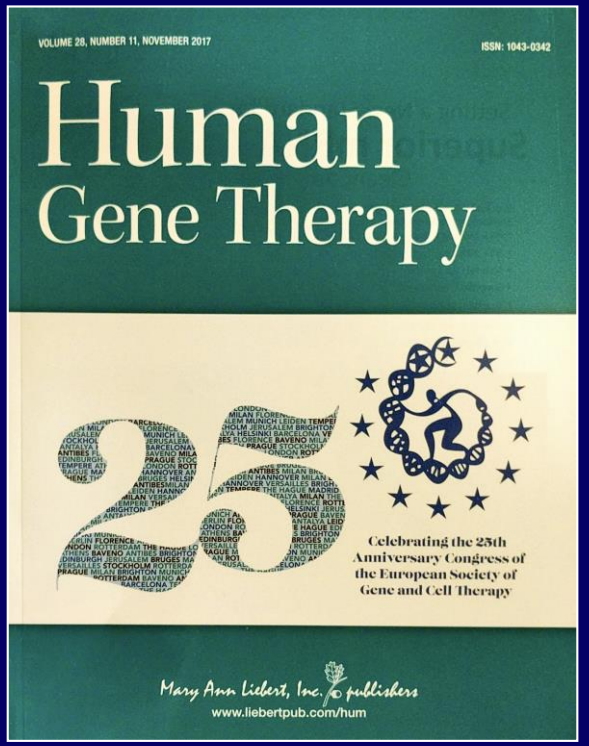


EUFP6, EUP7, EU Horizon 2020, FWO, IWT, EHA, AFM, WILLY GEPTS FONDS, KONING BOUDEWIJN STICHTING (WALTER PYLEMAN FONDS), VUB IOF GEAR, VUB "STRATEGIC RESEARCH PROJECT" GROWER STK, VUB POC IOF
Shire, Pfizer Aspire, Bayer BHAP

REVIEW ARTICLE 17 Aug 3. doi: 10.1089/hum.2017.116. [Epub ahead of print]

Hemophilia Gene Therapy: Ready for Prime Time?

Thierry VandenDriessche[1,2*] and Marinee K. Chuah[1,2*]



FIX-PADUA GENE THERAPY: CLINICAL TRIALS

BAX355

HUMAN GENE THERAPY 26:69–81 (February 2015)
Mary Ann Liebert, Inc.
DOI: 10.1089/hum.2014.106

Research Articles

Employing a Gain-of-Function Factor IX Variant R338L to Advance the Efficacy and Safety of Hemophilia B Human Gene Therapy: Preclinical Evaluation Supporting an Ongoing Adeno-Associated Virus Clinical Trial

Paul E. Monahan,[1–3] Junjiang Sun,[1] Tong Gui,[1] Genlin Hu,[1] William B. Hannah,[1] David G. Wichlan,[1,2] Zhijian Wu,[1,*] Joshua C. Grieger,[1] Chengwen Li,[1] Thipparat Suwanmanee,[1] Darrel W. Stafford,[4] Carmen J. Booth,[5] Jade J. Samulski,[6] Tal Kafri,[1,7] Scott W.J. McPhee,[6] and R. Jude Samulski[1,8]

- **FIX Padua variant gave rise to peak expression levels of 30-58%**
- **1 subject with sustained activity of ~20% with 3 years of follow-up**
- **Declining expression in other subjects**

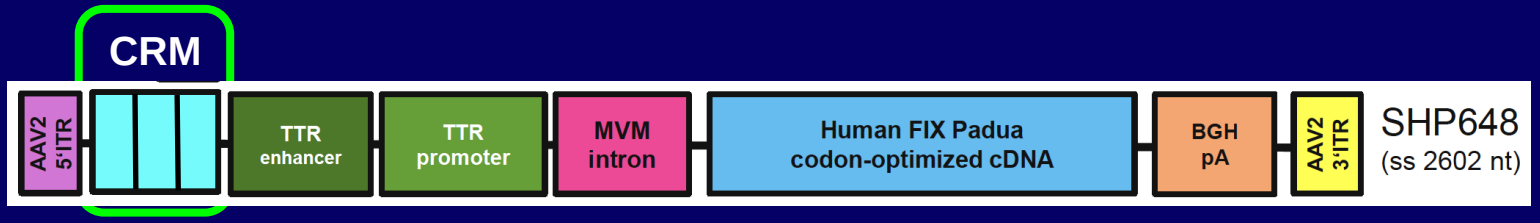
ISTH 2017

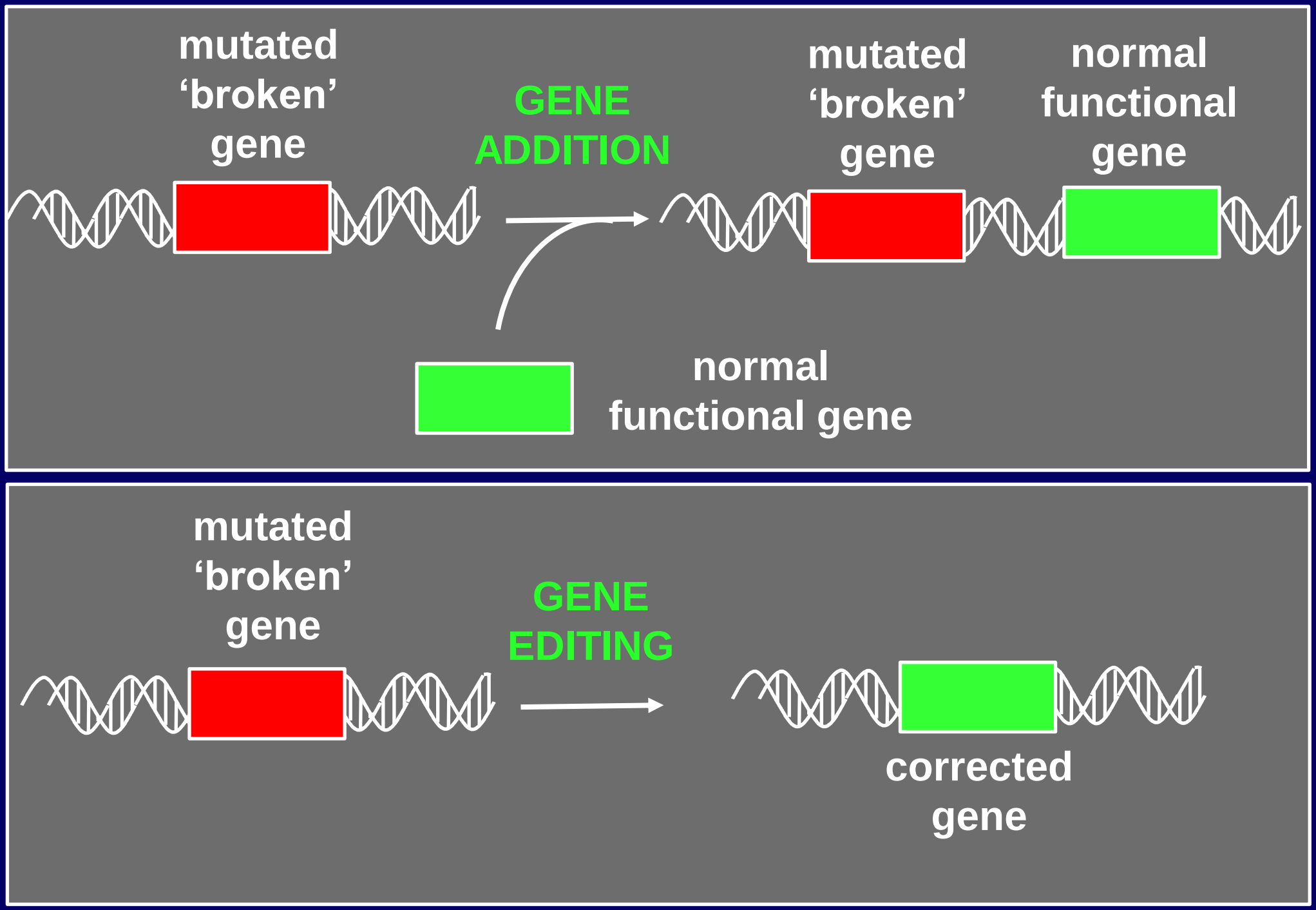
P003

Development of SHP648, Shire's high performing AAV8-based FIX gene therapy vector

Franziska Horling[1], Falko G. Falkner[1], Marinee Chuah[3], Johannes Lengler[1], Josef Mayrhofer[1], Matthias Klugmann[1], Maurus de la Rosa[1], Sogue Coulibaly[1], Werner Hoellriegl[1], Paul E. Monahan[2], Thierry VandenDriessche[3], Friedrich Scheiflinger[1], Hanspeter Rottensteiner[1]
[1] Shire, Vienna, Austria; [2] Shire, Cambridge, MA, USA; [3] VUB, Brussels, Belgium

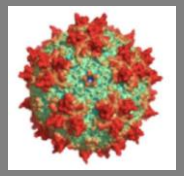
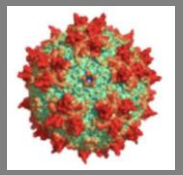
ESGCT 2017



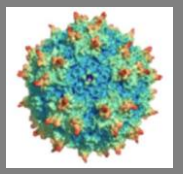
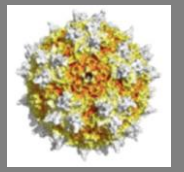

mutated
'broken'
gene
GENE
ADDITION
mutated
'broken'
gene
normal
functional
gene
normal
functional gene
mutated
'broken'
gene
GENE
EDITING
corrected
gene

CHALLENGING TO COMPARE EFFICACY/SAFETY IN DIFFERENT GENE THERAPY TRAILS

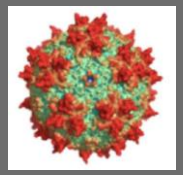
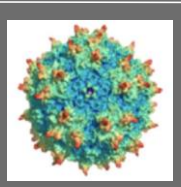
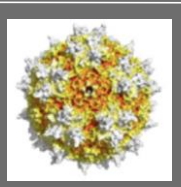
APPARENT HIGH VECTOR DOSES

BioMarin	*UniQure*
	
BMN 270	AMT-061
$6x10^{13}$ vg/kg	*$2x10^{13}$ vg/kg*

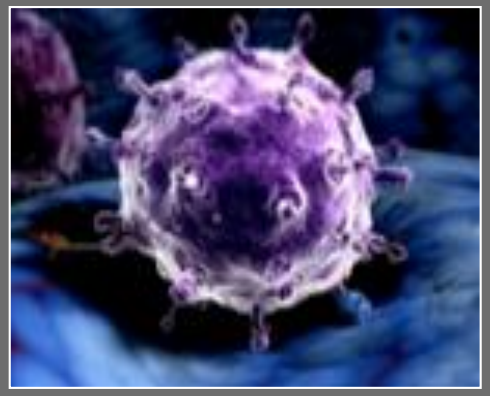
APPARENT LOW VECTOR DOSES

Freeline	*Spark*
	
AAV8-HLP-hFVIII-V3	SPK-8011
$2x10^{12}$ vg/kg	*$2x10^{12}$ vg/kg*
FLT180a	SPK-9001
$4.5x10^{11}$ vg/kg	*$5x10^{11}$ vg/kg*

GENE THERAPY CLINICAL TRIALS FOR HEMOPHILIA A

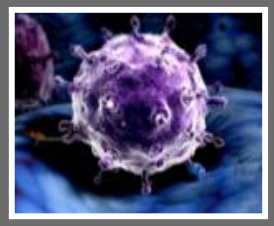
	PHASE III	PHASE I/II	PHASE I/II
company	*BioMarin*	*Freeline*	*Spark*
serotype	 AAV5 BMN 270	 AAV8 AAV8-HLP-hFVIII-V3	 Clade E Spk200 SPK-8011
dose (vg/kg) FVIII	*$6x10^{12}$ <1%* *$2x10^{13}$ 1-3%* *$6x10^{13}$ 40-50% (2 yr)*	*$6x10^{11}$ 7% (w47)* *$2x10^{12}$ 6-69% (w20)*	*$5x10^{11}$ 15%* *$1x10^{12}$* *$2x10^{12}$ 30% (w12) < 6% (*)*
	transaminitis steroids no clear dose response	*transaminitis steroids*	**transient FVIII transaminitis steroids (oral; i.v.)*

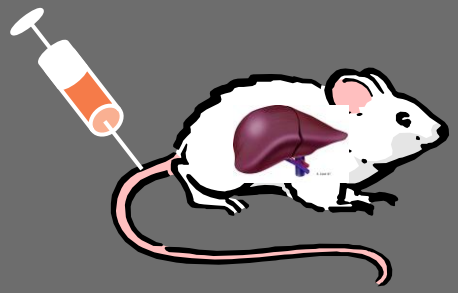
Lentiviral vector

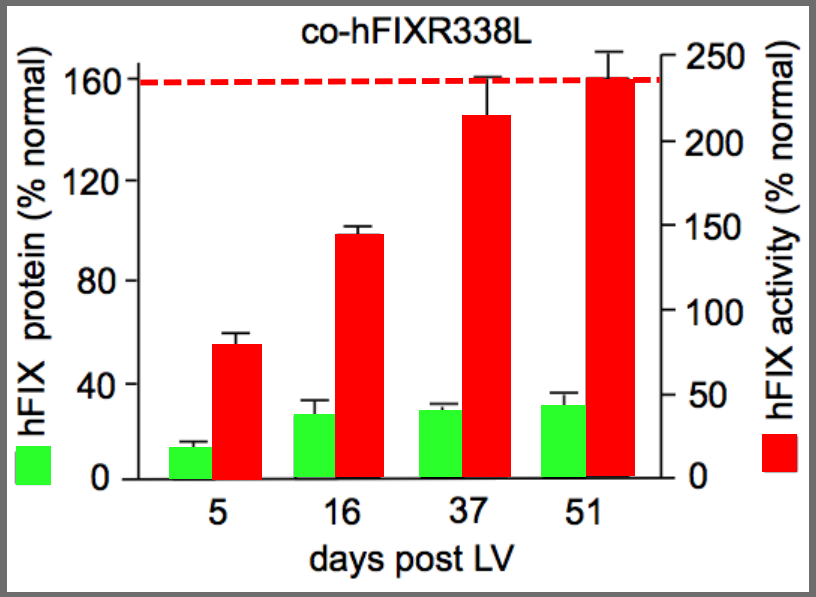


- Efficacy established in human trials
- Sustained expression
- No pre-existing immunity
- Integrated

INTEGRATING VECTOR: LENTIVIRAL-FIX PADUA







stable; 30%

>100%
pigtail macaque

THERAPEUTIC FIX EXPRESSION IN HEMOPHILIA B MICE, DOGS AND MACAQUE

Be not astonished at new ideas; for it is well known t
that a thing does not therefore cease to be true becau
not accepted by many.

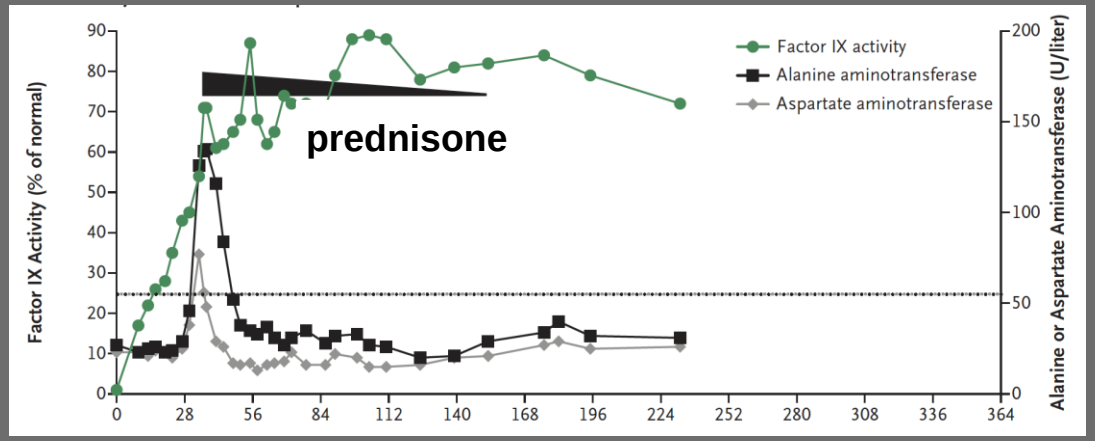
- Baruch Spinoza

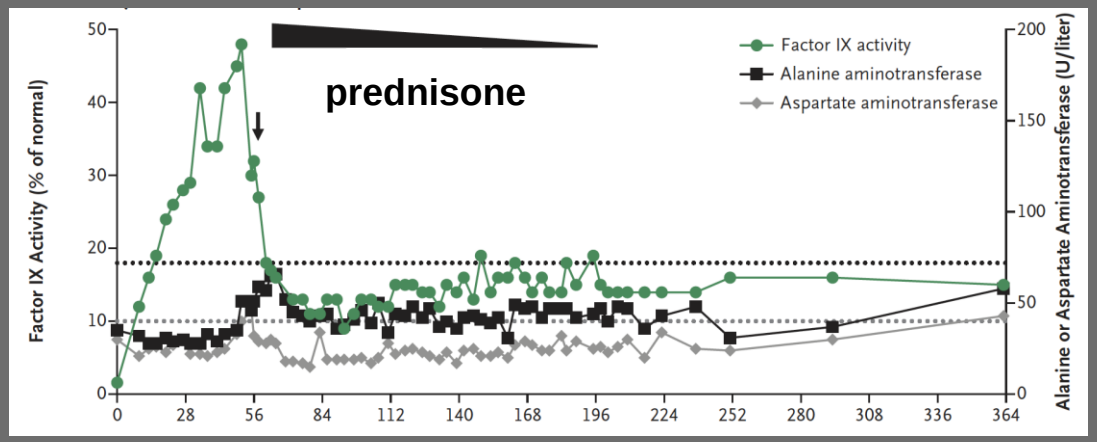
Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

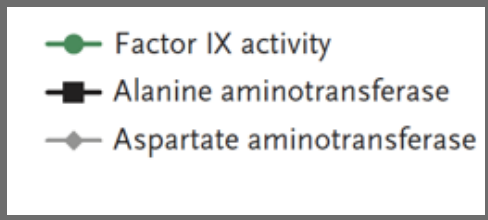
December 7, 2017
N Engl J Med 2017; 377:2215-2227
DOI: 10.1056/NEJMoa1708538

Lindsey A. George, M.D., Spencer K. Sullivan, M.D., Adam Giermasz, M.D., Ph.D., John E.J. Rasko, M.B., B.S., Ph.D., Benjamin J. Samelson-Jones, M.D., Ph.D., Jonathan Ducore, M.D., M.P.H., Adam Cuker, M.D., Lisa M. Sullivan, M.D., Suvankar Majumdar, M.D., Jerome Teitel, M.D., Catherine E. McGuinn, M.D., Margaret V. Ragni, M.D., M.P.H., et al.

single i.v. AAV-FIX vector injection 5x10^{11} vg/kg







GENE THERAPY FOR HEMOPHILIA A: BOTTLENECKS IN FVIII PRODUCTION

secretion & stabilization

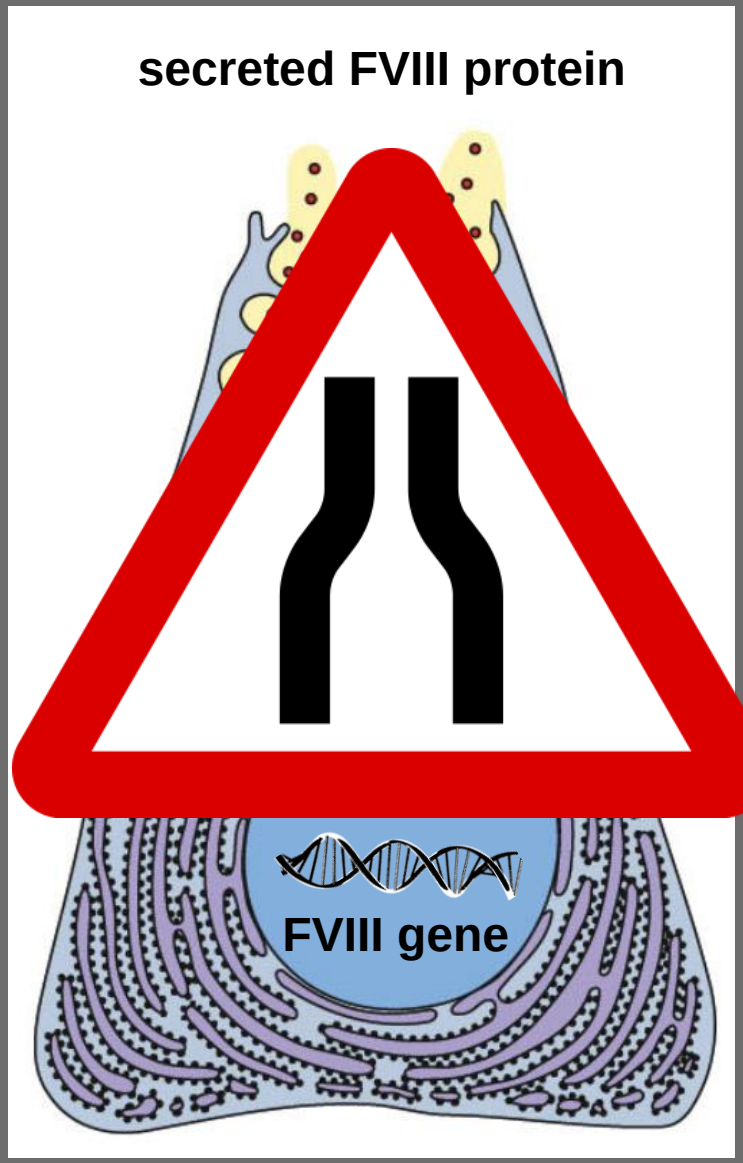
FVIII transport ER to Golgi

FVIII mannose binding to LMAN1

FVIII translation

FVIII binding to BiP ER retention

FVIII transcription



vWF binding

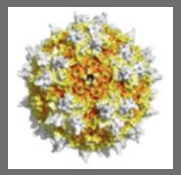
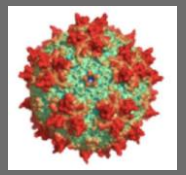
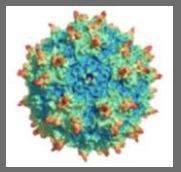
include glycosylation sites B-domain (N6 226aa, V3)

reduce BiP binding (F309S)

FVIII codon-optimization

***de novo* promoter design delete B-domain**

GENE THERAPY CLINICAL TRIALS FOR HEMOPHILIA B

	PHASE III	PHASE III	PHASE I/II
company	*Spark Pfizer*	*UniQure*	*Freeline*
serotype	 Clade E Spk200 SPK-9001	 AAV5 AMT-061	 AAV8 FLT180a
dose (vg/kg) FIX	*$5x10^{11}$ 33% (w52)*	*$2x10^{13}$ 31% (w6)*	*$4.5x10^{11}$ 48-66% (w12)*
	transaminitis steroids	*transaminitis steroids*	*transaminitis steroids*

LENTIVIRAL FIX-PADUA

blood

Nov 29, 120(23) 2012

Hyper-functional coagulation factor IX improves the efficacy of gene therapy in hemophilic mice

Alessio Cantore, Nisha Nair, Patrizia Della Valle, Mario Di Matteo, Janka Màtrai, Francesca Sanvito, Chiara Brombin, Clelia Di Serio, Armando D'Angelo, Marinee Chuah, Luigi Naldini and Thierry VandenDriessche

First validation of FIX Padua in gene therapy (preclinical)

RESEARCH ARTICLE

GENE THERAPY

Liver-directed lentiviral gene therapy in a dog model of hemophilia B

Alessio Cantore,[1,2*] Marco Ranzani,[1,2*†] Cynthia C. Bartholomae,[3] Monica Volpin,[1,2] Patrizia Della Valle,[4] Francesca Sanvito,[5] Lucia Sergi Sergi,[1] Pierangela Gallina,[1] Fabrizio Benedicenti,[1] Dwight Bellinger,[6] Robin Raymer,[6] Elizabeth Merricks,[6] Francesca Bellintani,[7] Samia Martin,[8] Claudio Doglioni,[5] Armando D'Angelo,[4] Thierry VandenDriessche,[9,10] Marinee K. Chuah,[9,10] Manfred Schmidt,[3] Timothy Nichols,[6‡] Eugenio Montini,[1‡] Luigi Naldini[1,2‡§]

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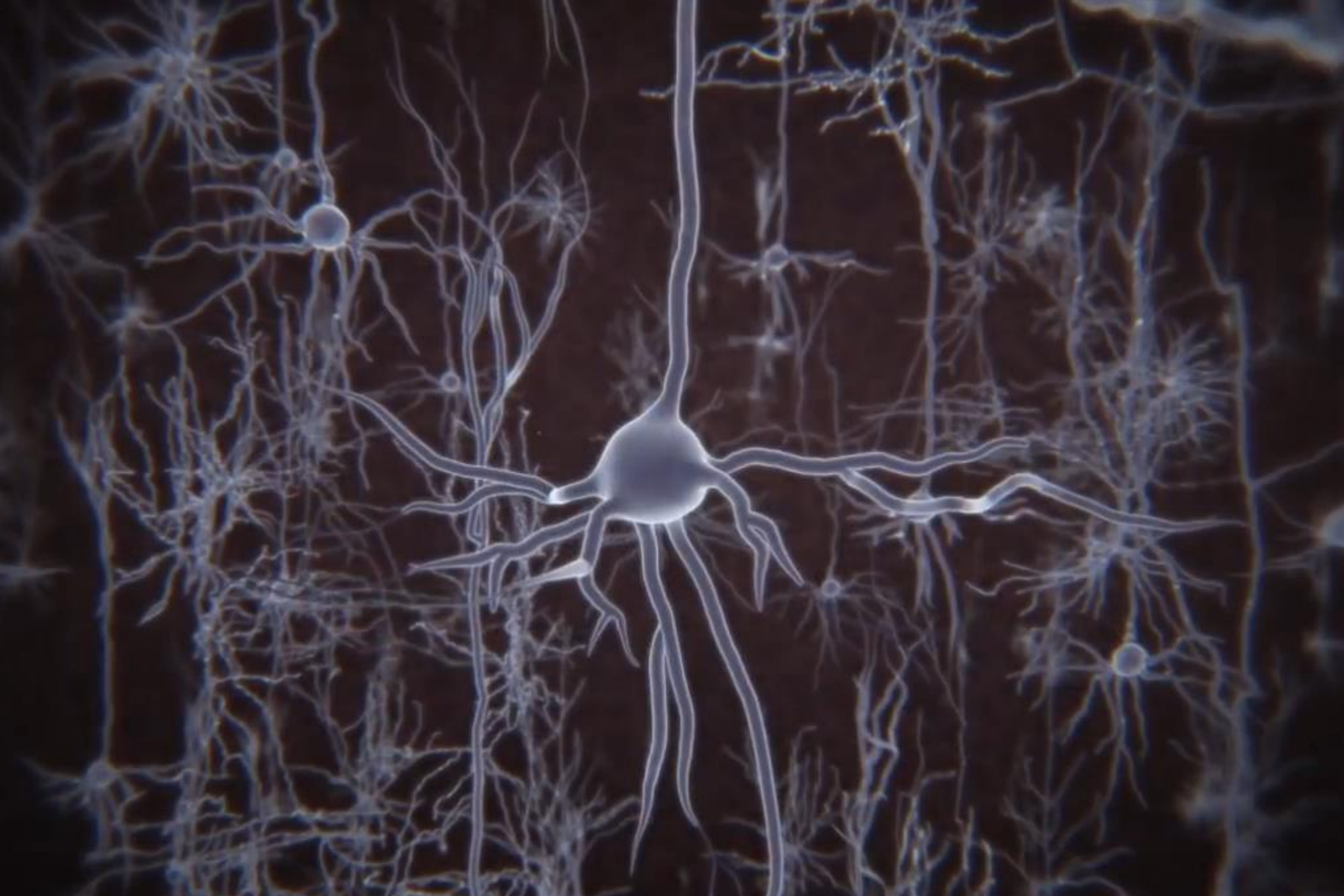
RANDOM GENOMIC INTEGRATION
NO CONTROL - SAFETY RISK



CHALLENGE GENE-SPECIFIC EDITING

FINDING THE NEEDLE IN THE HAYSTACK …
WITHOUT ANY OFF-TARGET EFFECTS !