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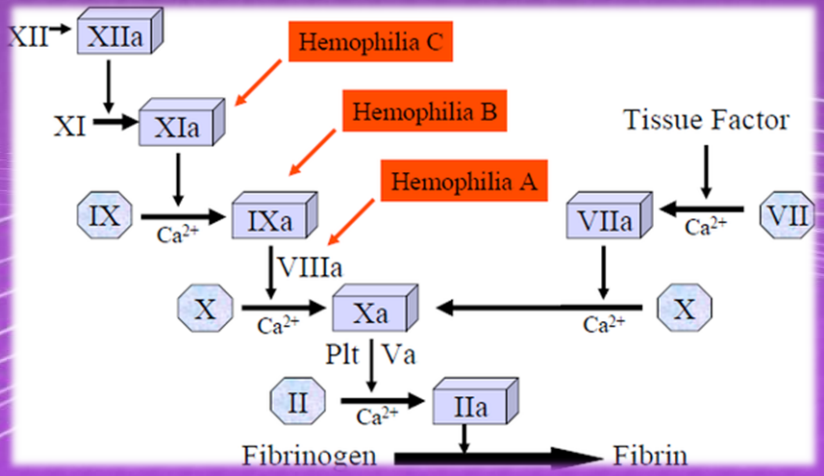
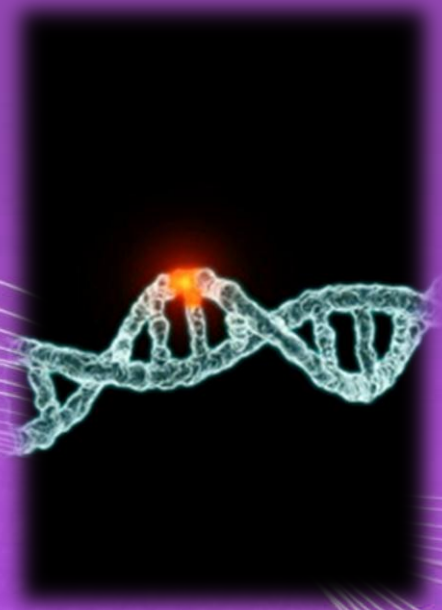
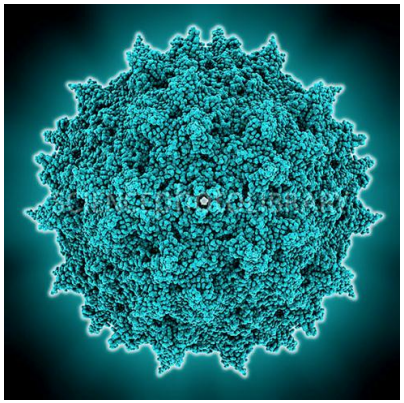




Terapia génica de la hemofilia



Jordi Barquinero

XII Jornadas farmacéuticas sobre el tratamiento de las coagulopatías congénitas

Hospital Universitario La Paz, 1 - 12 - 2017

Vall d'Hebron Hospital · Generalitat de Catalunya Departament de Salut · Institut Català de la Salut · UAB Universitat Autònoma de Barcelona · Generalitat de Catalunya Departament d'Economia i Coneixement · Institució CERCA Centres de Recerca de Catalunya · Instituto de Salud Carlos III

Enfermedades pioneras en la historia de la TG

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- Disease targets

Single gene defect	Gene(s involved)	Tissues
Severe combined immunodeficiency	Adenosine deaminase	Lymphoid tissue
α-AT deficiency	α-Antitrypsin	Lungs,liver(cirrhosis)
Cystic fibrosis	CFTR	Lungs,pancreas
Hemophilia A & B	Factor VIII & IX	Blood clotting
Gaucher disease	Acid β-glucosidase,glucocerebrosidase	Macrophages,liver,spleen,lungs

La hemofilia es una buena candidata para la TG

Enfermedad monogénica, gen recesivo, fisiopatología conocida

FVIII y FIX se secretan, cualquier tejido diana puede ser válido

Expresión transgén no requiere excesiva regulación

Niveles de corrección modestos (1-5%) son terapéuticos

Existen buenos modelos animales

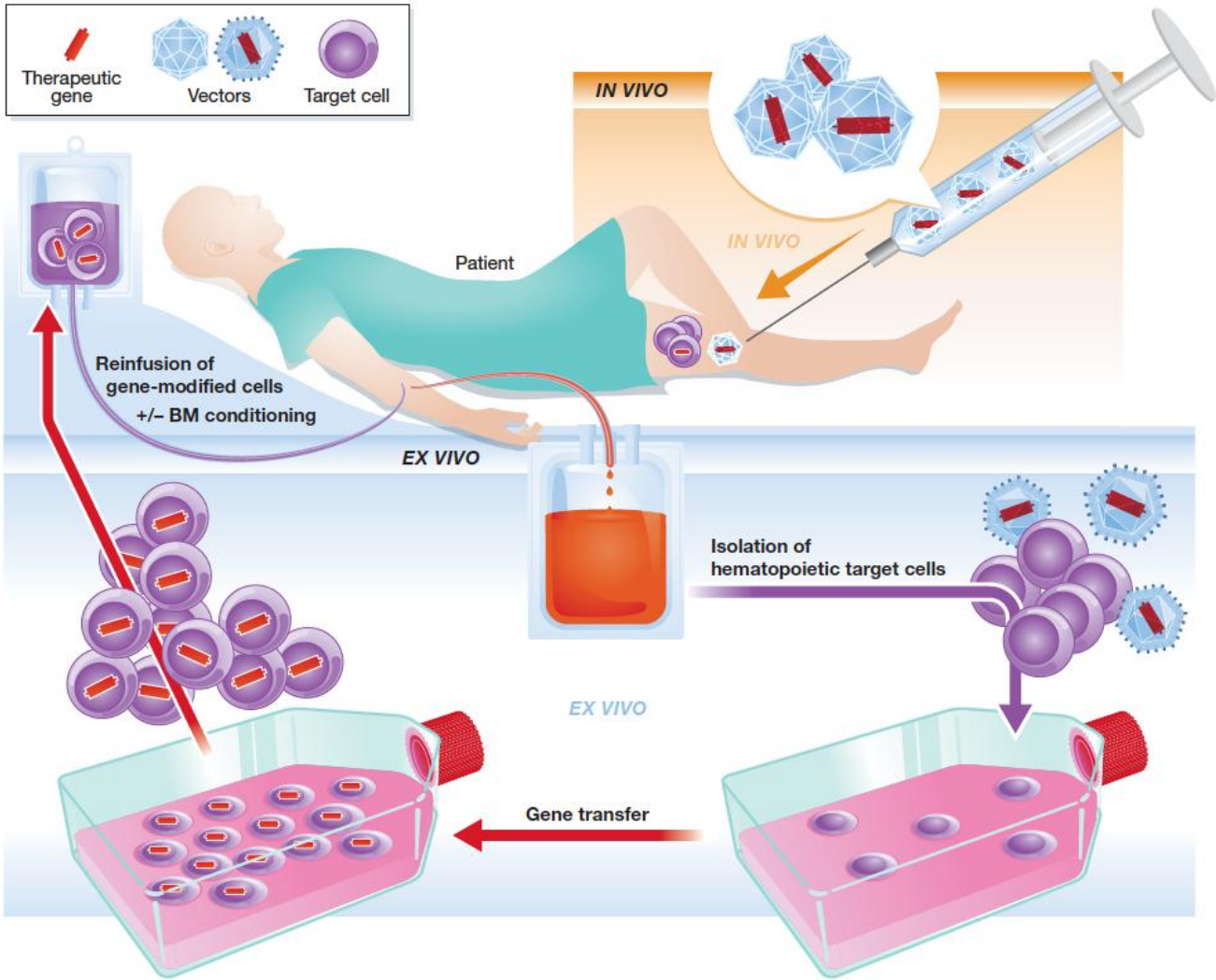
Tamaño del cDNA (FVIII)

No hay ventaja selectiva de las células corregidas

Requiere una terapia *in vivo* (con vectores no integrativos)

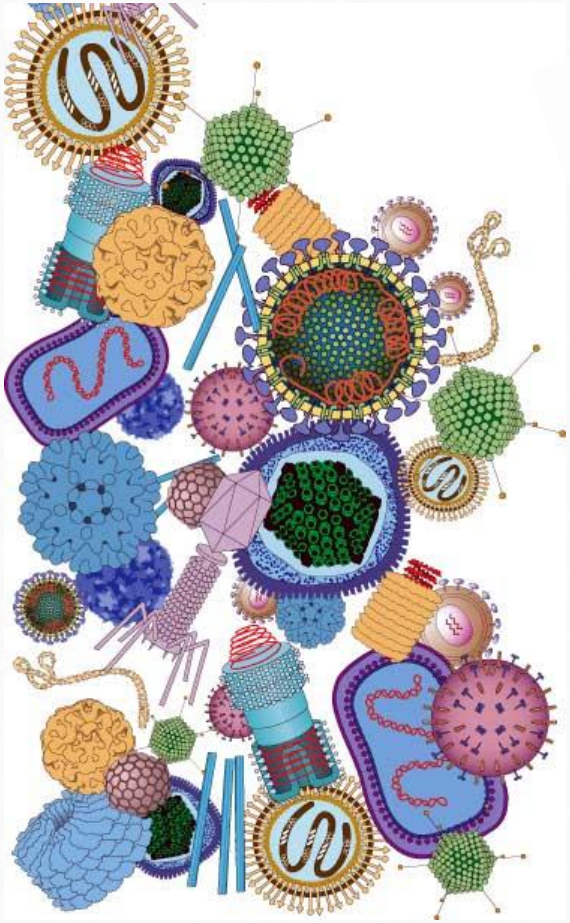
Posibles riesgos de los vectores (oncogenicidad, inmunogenicidad)

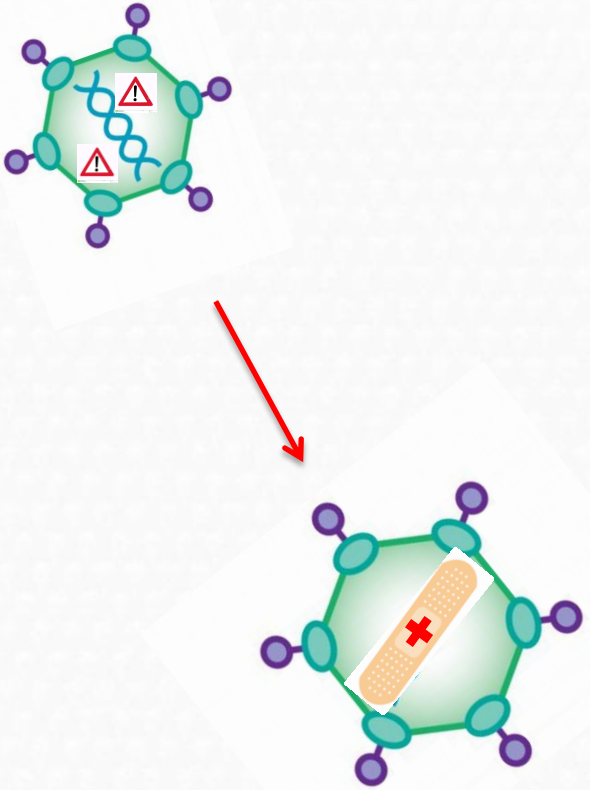
Terapia génica *in vivo vs ex vivo*

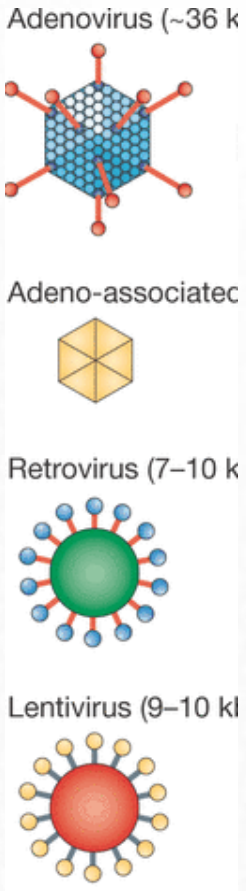


Kaufmann KB *et al*. *EMBO Mol Med* (2013)

Virus y vectores





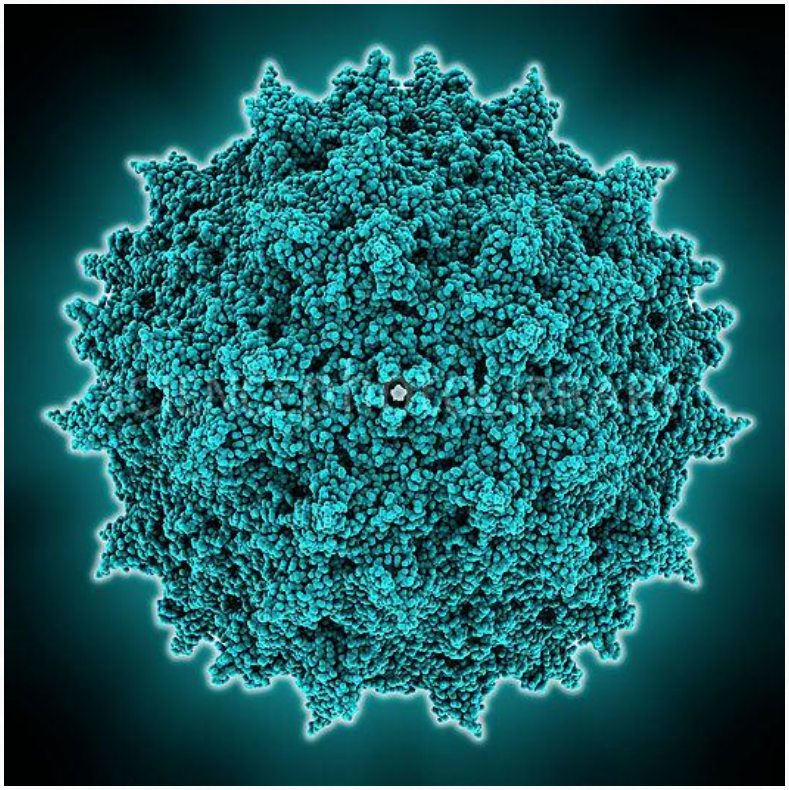


Virus

Virus

Vectores

Vectores Adenoasociados (AAV): óptimos para la TG de la hemofilia



Virus DNA, no integrativos

Parvovirus (virus defectivos)

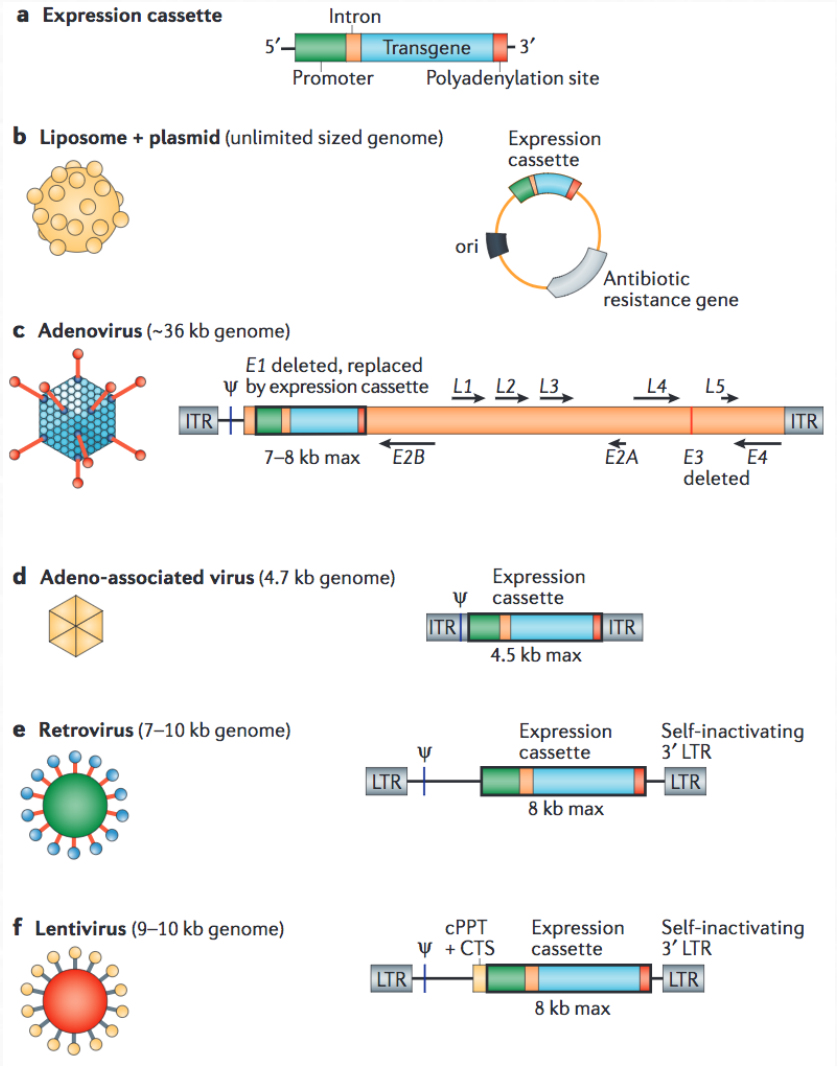
No producen enfermedades

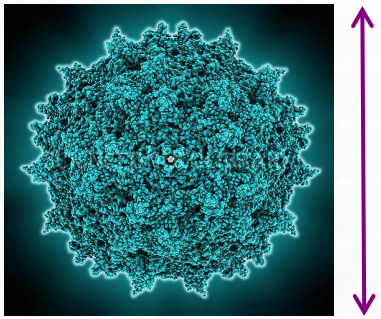
Escasa inmunogenicidad

Tropismo hepático

Adecuados para la TG *in vivo*

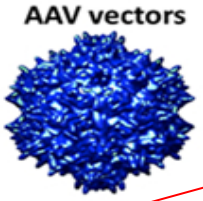
El tamaño de los vectores limita su capacidad de cargo





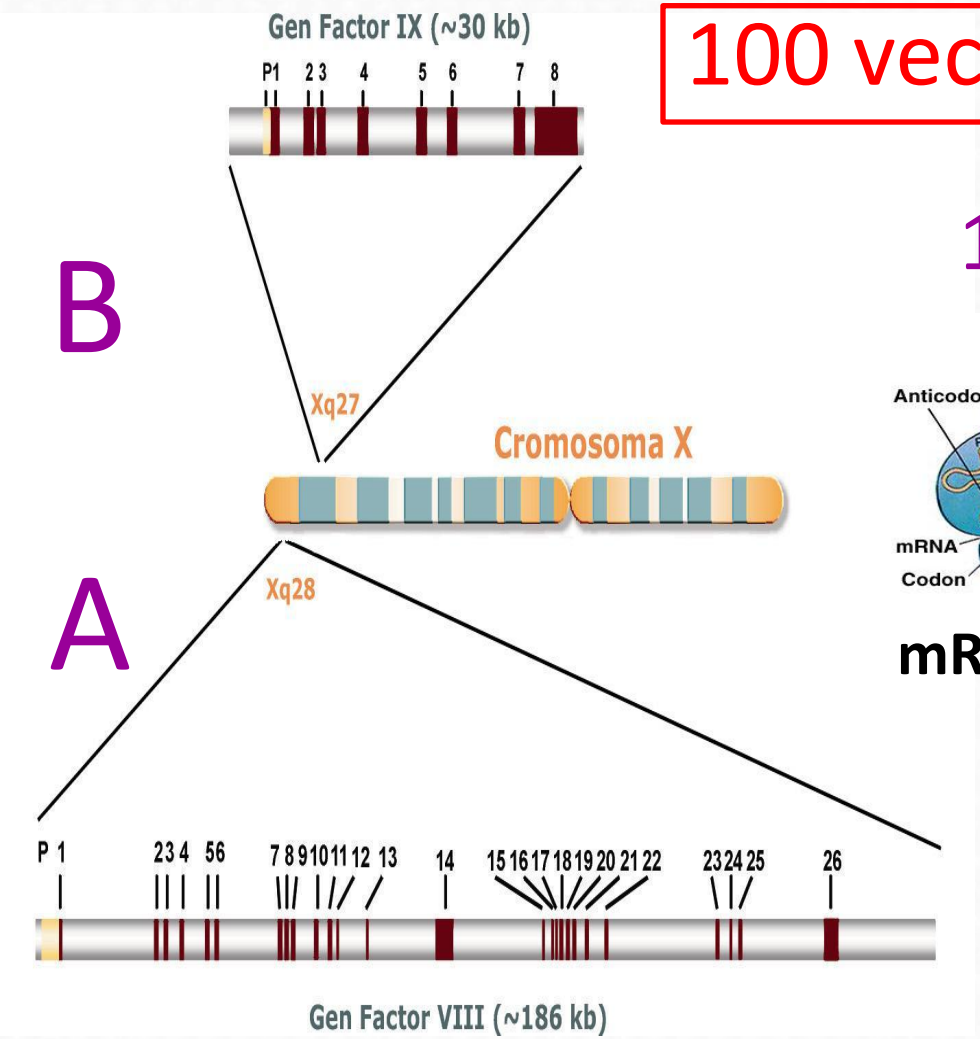
AAVs
45-60 nm
5 kb

www.discoverymedicine.org



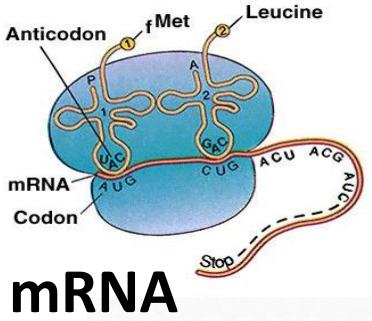
cDNA F8: 9 kb

Características F VIII y F IX



100 veces más moléculas de FIX

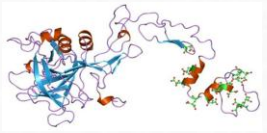
1,4 Kb 56 Kda 5 µg/ml



mRNA

tΔ 18-24 h

proteína **[conc. pl.]**



9 Kb 265 Kda 150 ng/ml

tΔ 8-12 h

Elementos en terapia génica

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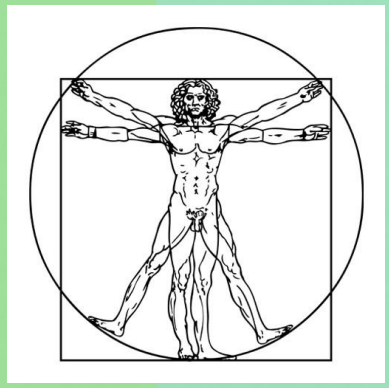
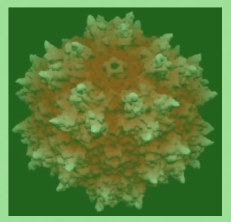
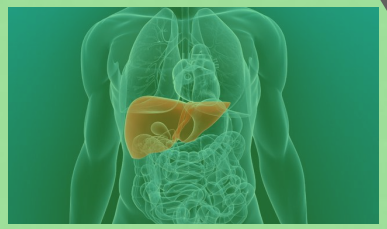
Gen o secuencia terapéutica

enfermedad



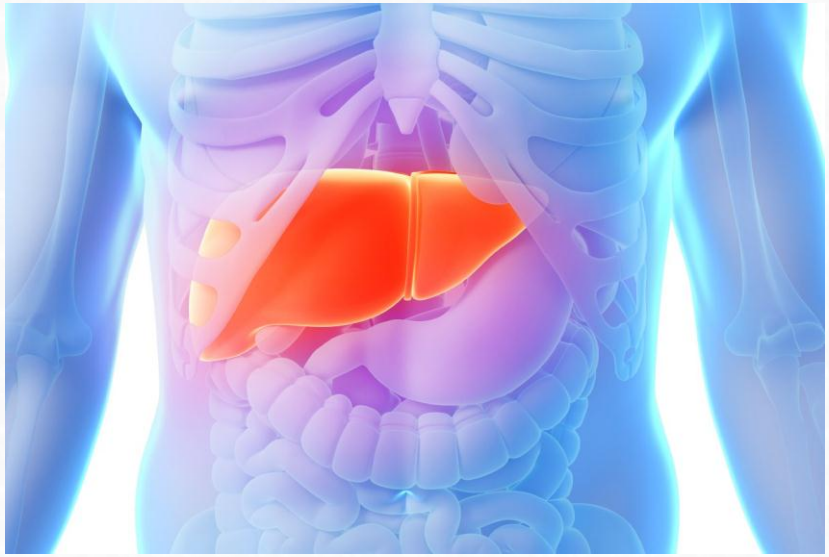


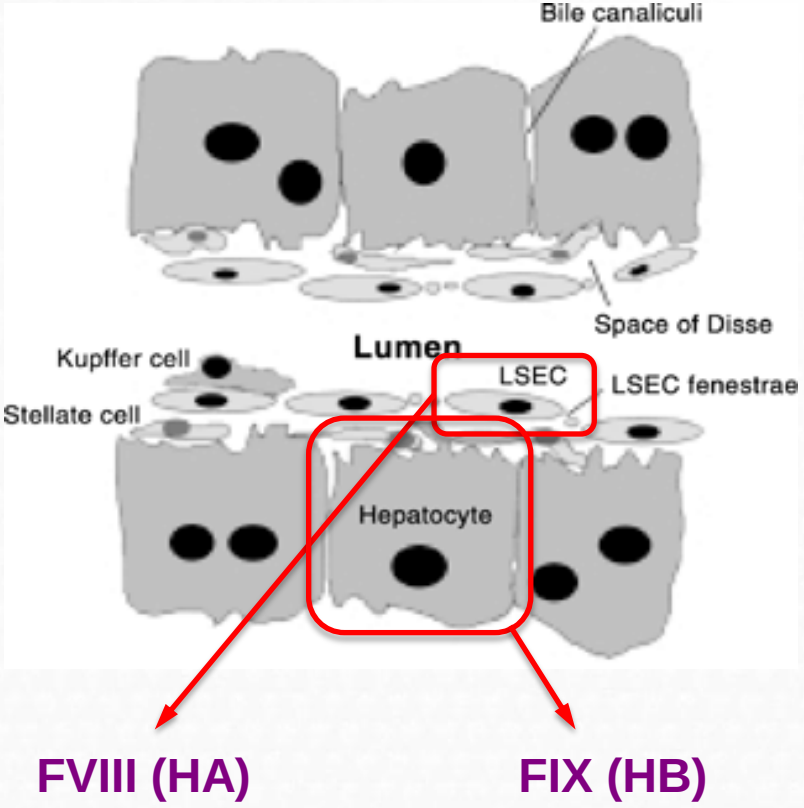


Vector

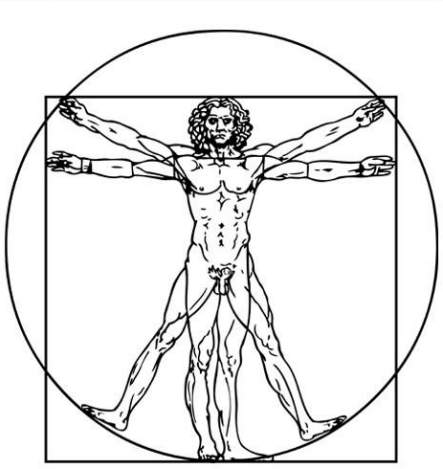
Tejido diana

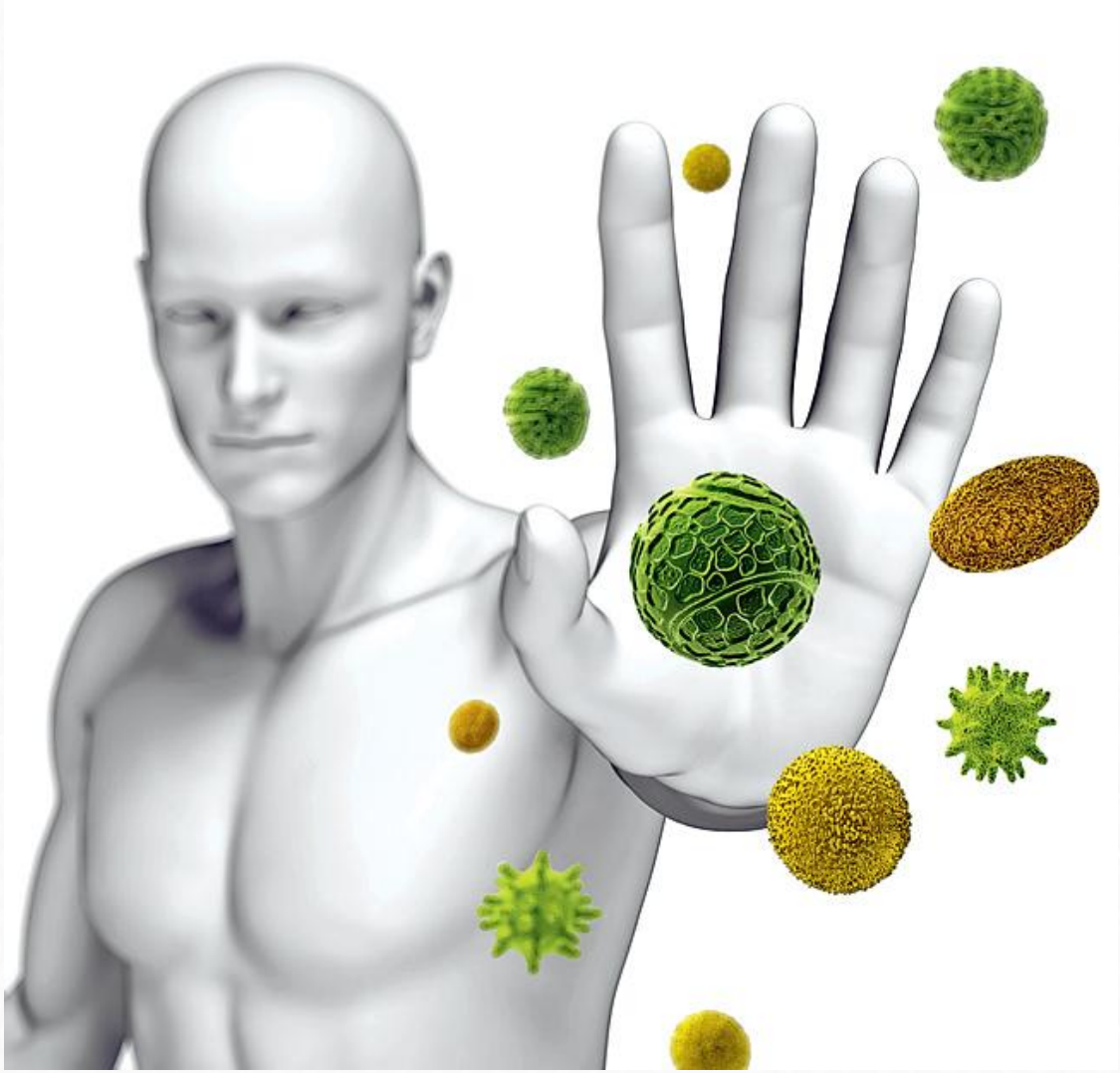
En la hemofilia el tejido relevante és el hepático





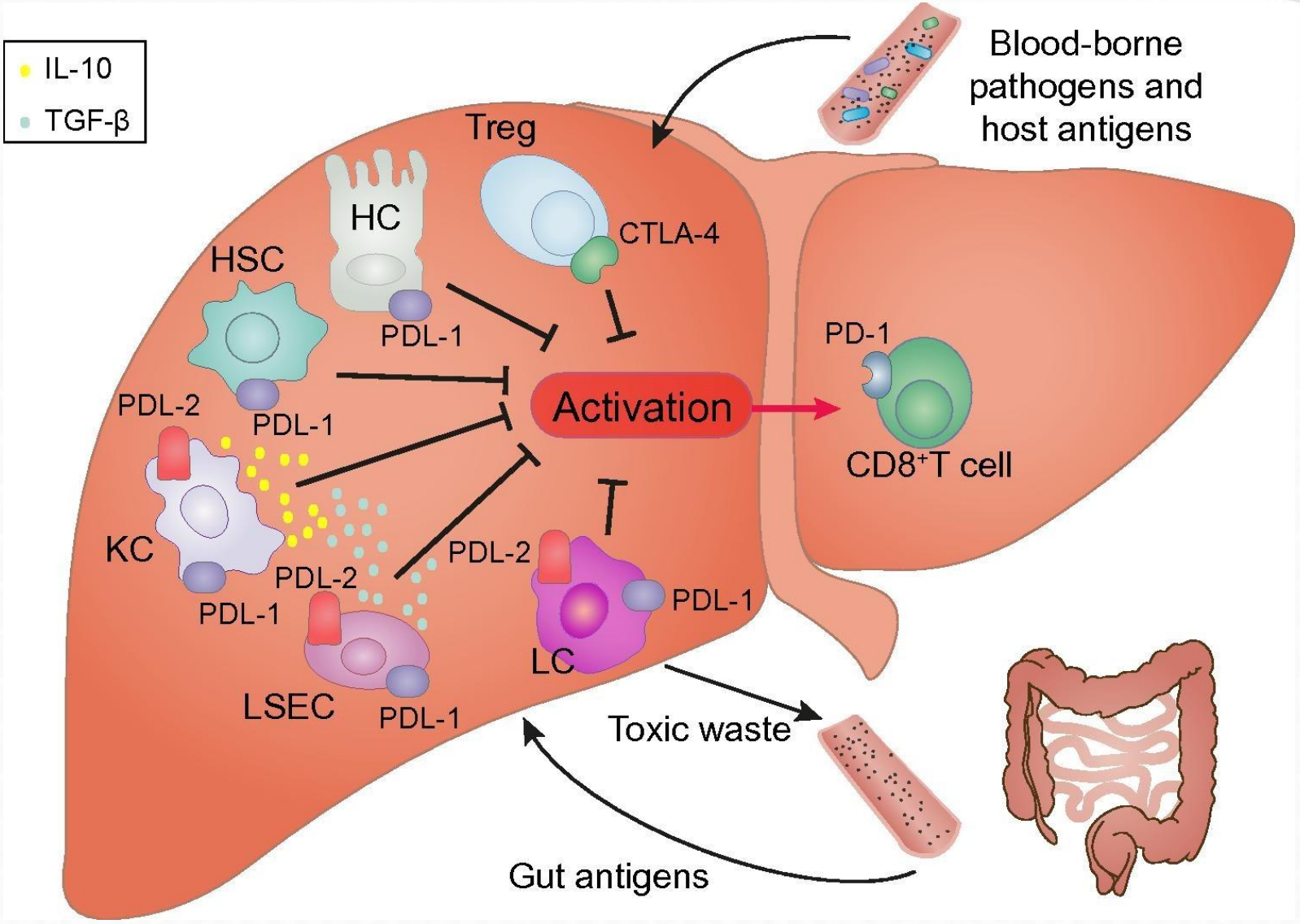
La barrera inmunitaria





http://www.mid-day.com/articles/health-special-be-the-immunity-superhero/15523190

El hígado es un órgano tolerogénico



La barrera immunitaria frente a AAVs

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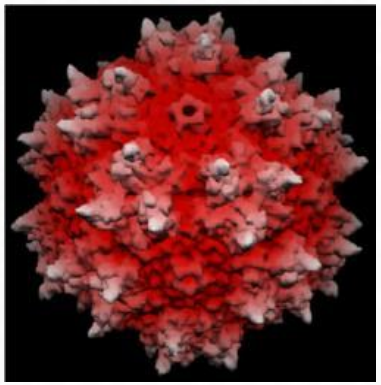
PRE

30-50% de la población tiene AcN frente a AAVs



POST

Tras una inyección i.v. el 100% los desarrollan

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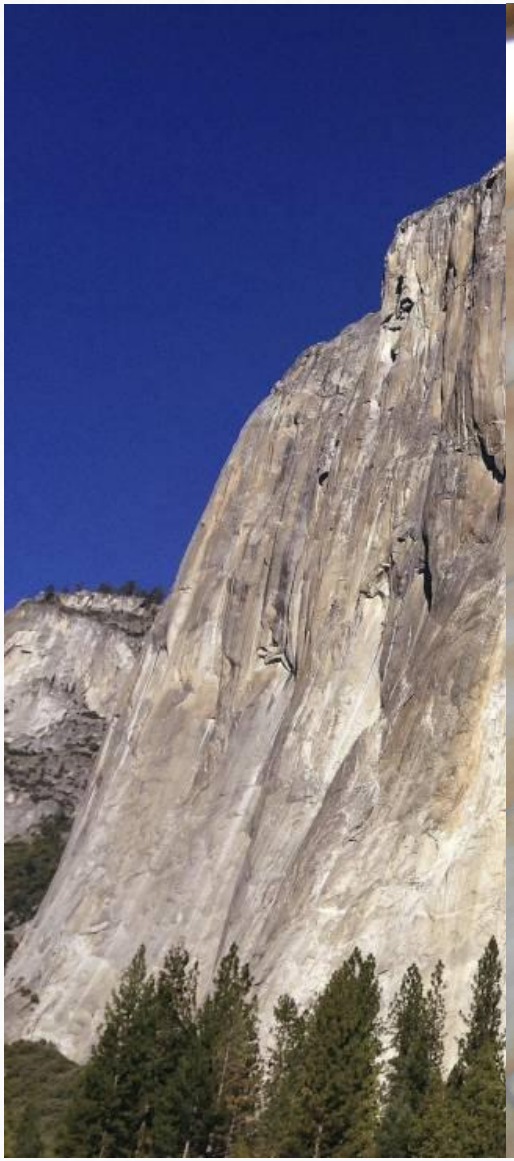
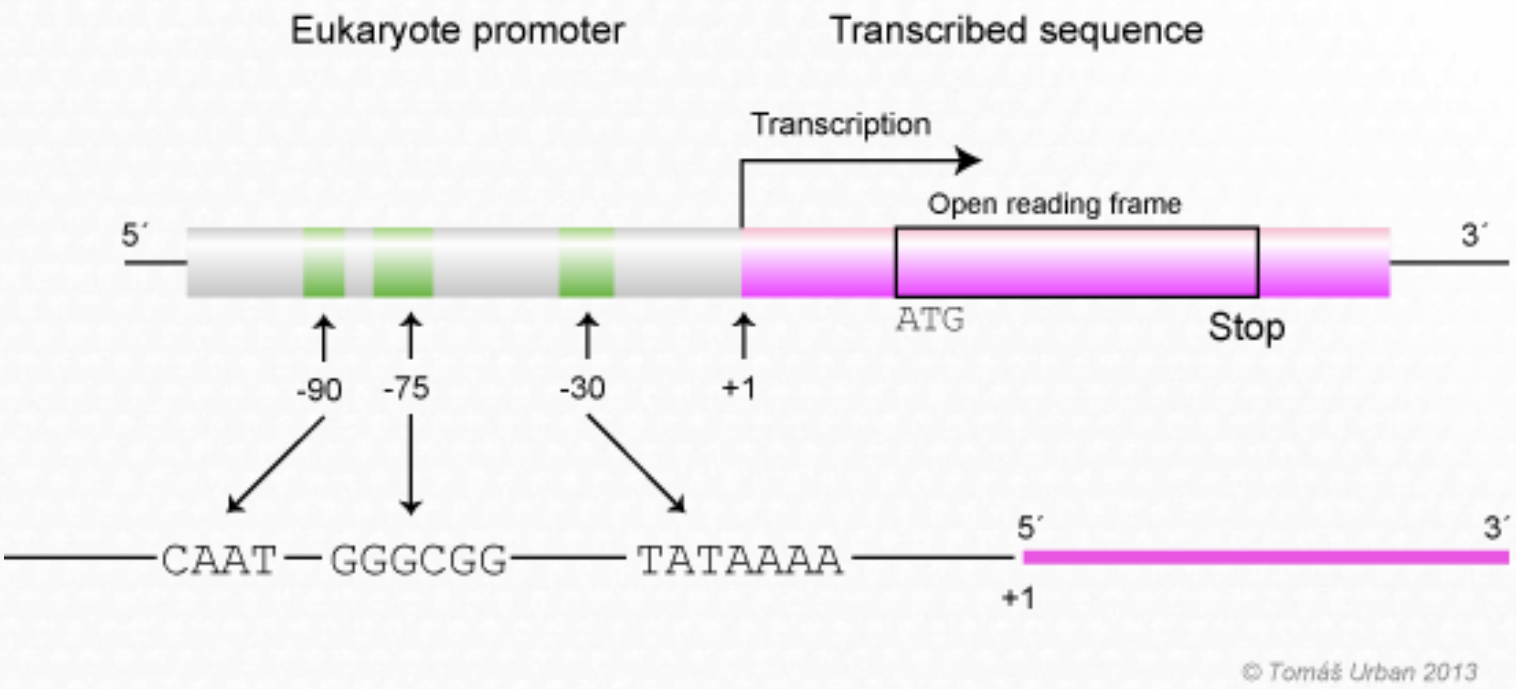


Estrategias para mejorar la eficacia de la TG en hemofilia

Promotores



constitutivos vs específicos de tejido

quiméricos

Promotores específicos de hígado

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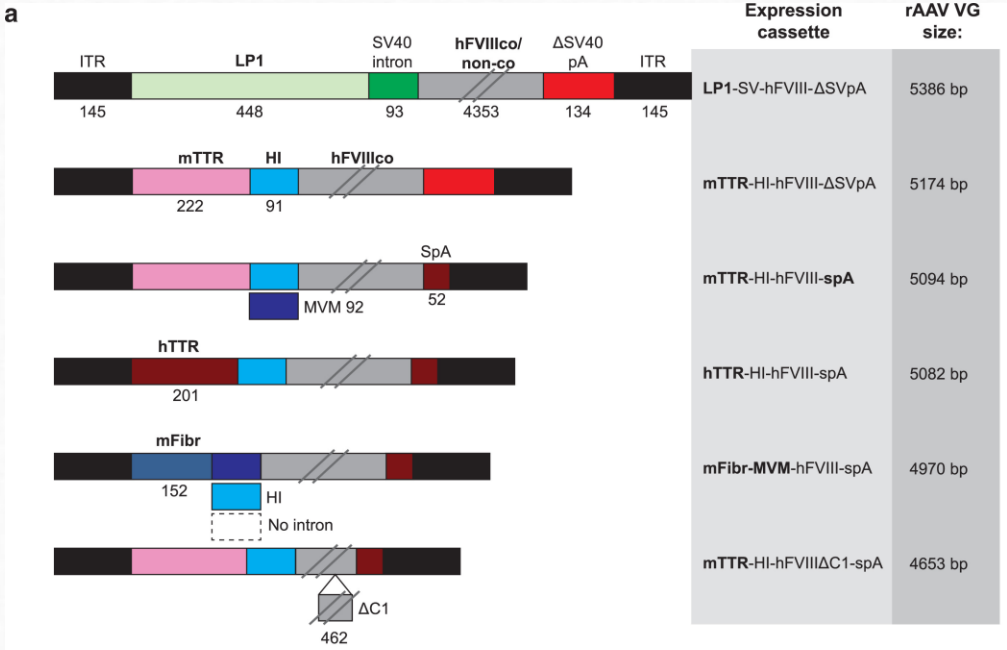

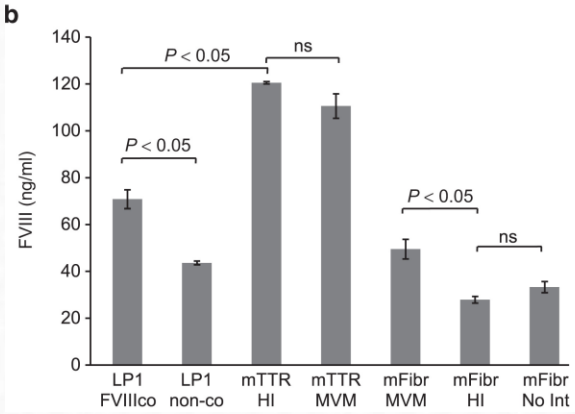

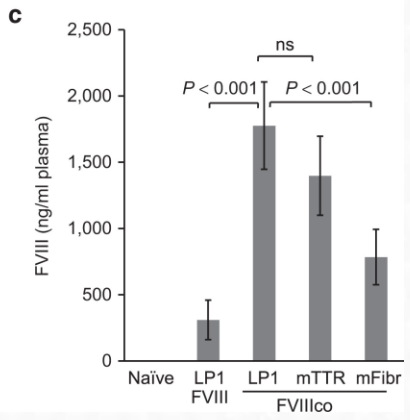

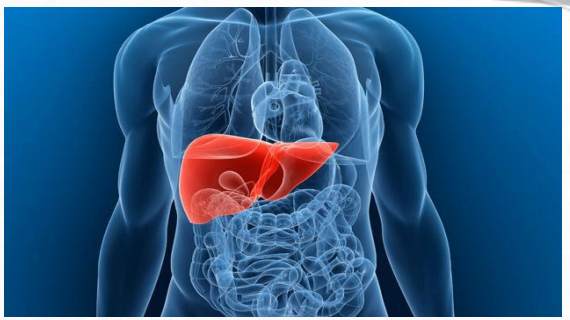

Alb: Albúmina

TTR: Transtiretina

AAT: α1-Antitripsina

Quiméricos

LP-1 HLP ET

Optimización de los transgenes

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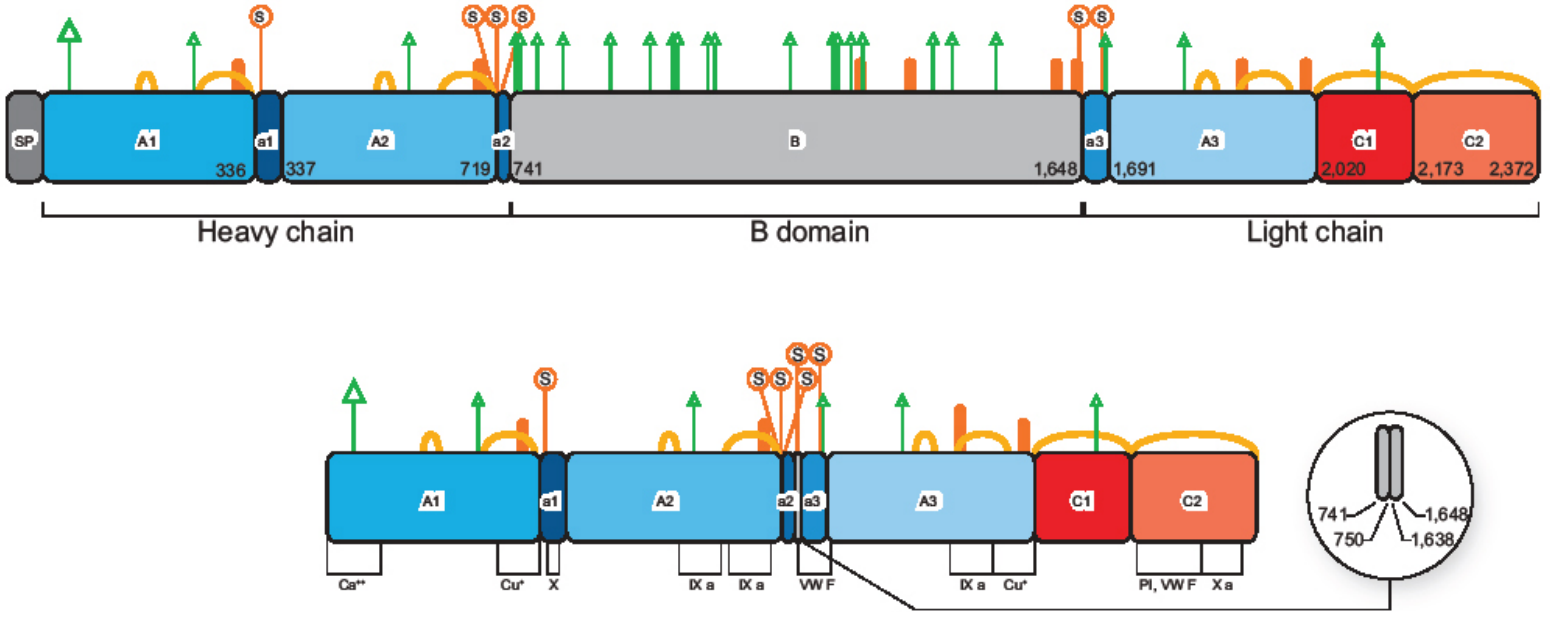
F VIII



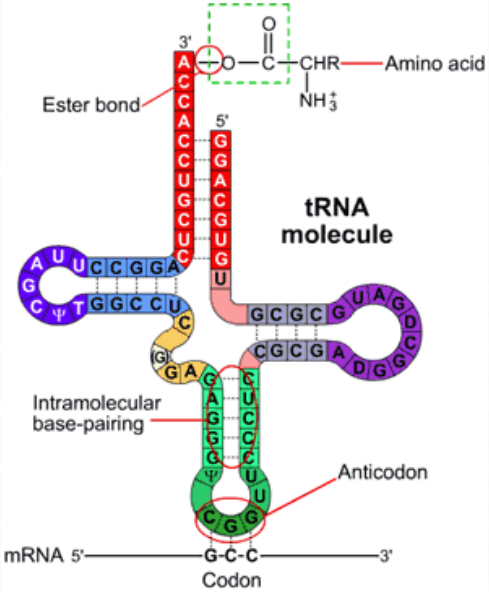
B domain deleted (BDD) FVIII

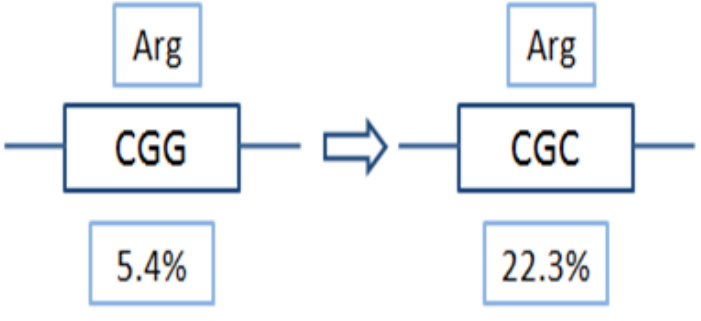
Meulien P *et al*, Prot Eng 1988

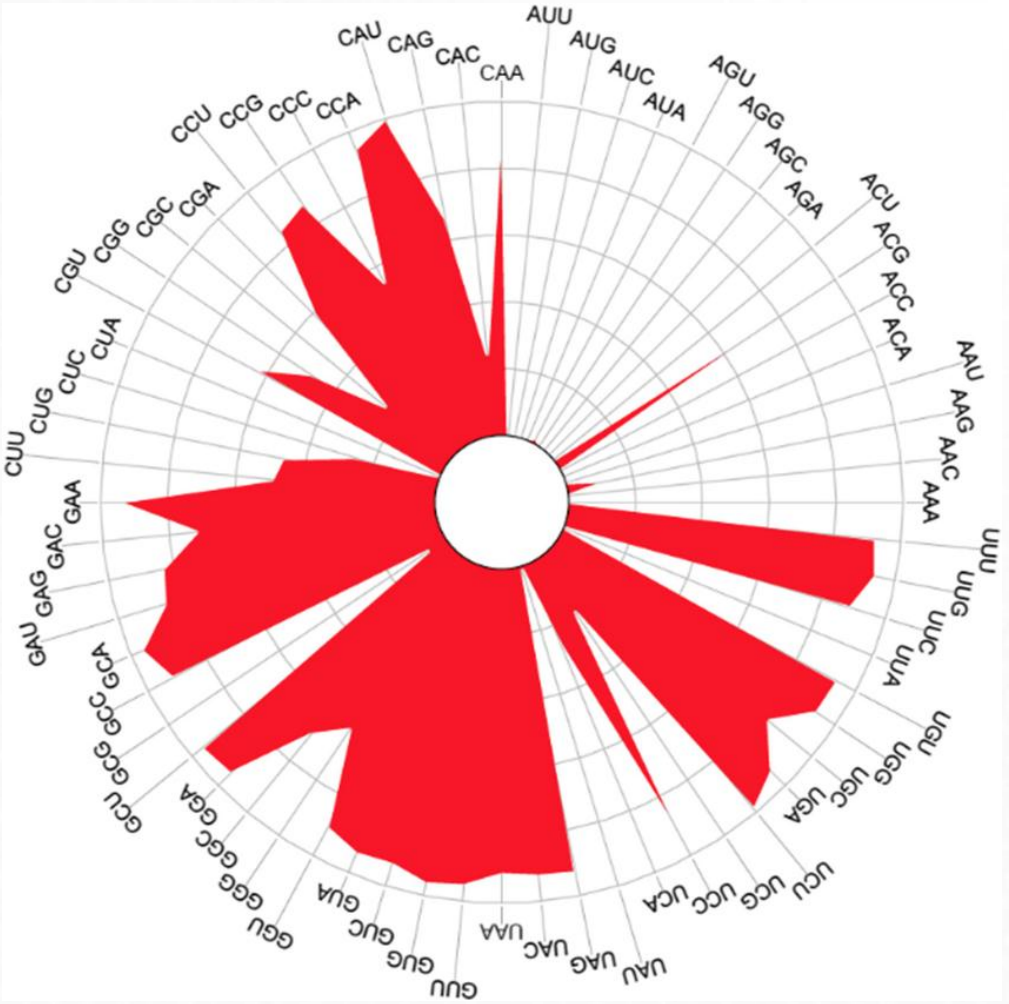
Optimización de las secuencias codificantes

Vall d'Hebron
Institut de Recerca

Optimización de codones







Variantes hiperactivas

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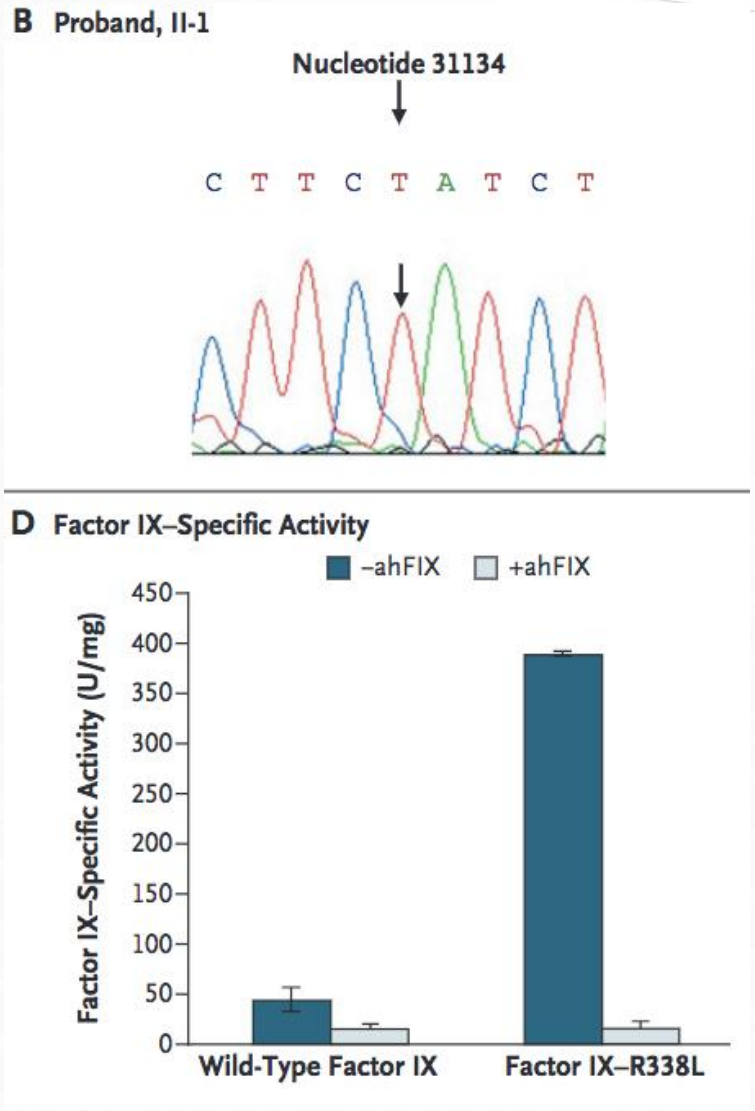
F IX

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

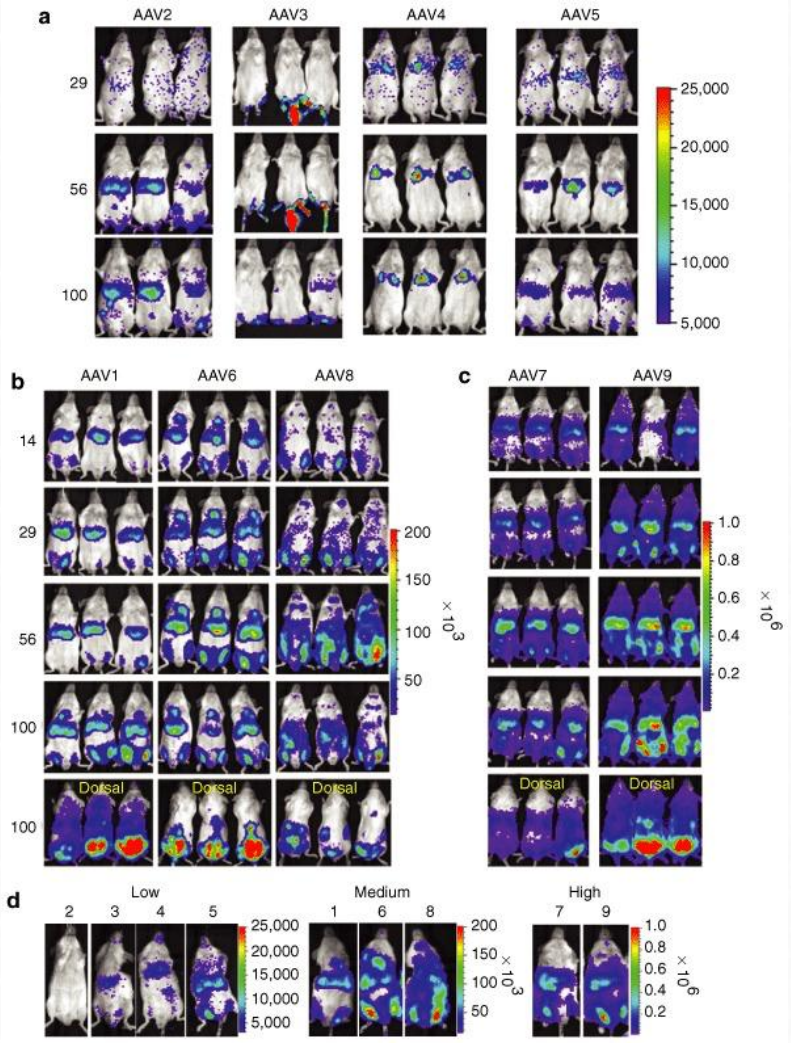
X-Linked Thrombophilia with a Mutant Factor IX (Factor IX Padua)

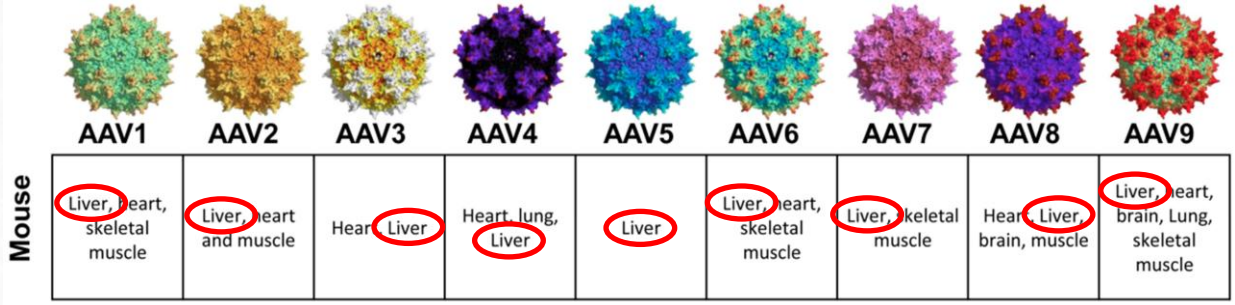
Paolo Simioni, M.D., Ph.D., Daniela Tormene, M.D., Ph.D., Giulio Tognin, M.D., Sabrina Gavasso, Ph.D., Cristiana Bulato, Ph.D., Nicholas P. Iacobelli, B.A., Jonathan D. Finn, Ph.D., Luca Spiezia, M.D., Ph.D., Claudia Radu, Ph.D., and Valder R. Arruda, M.D., Ph.D.

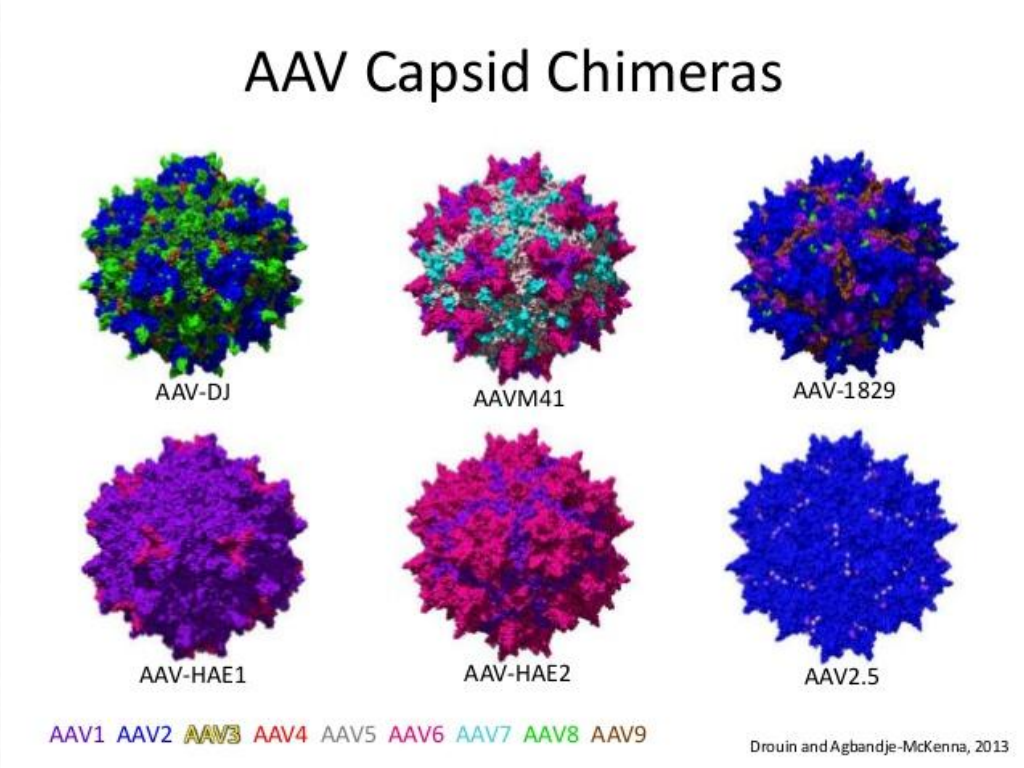


Vectores AAV y tropismo

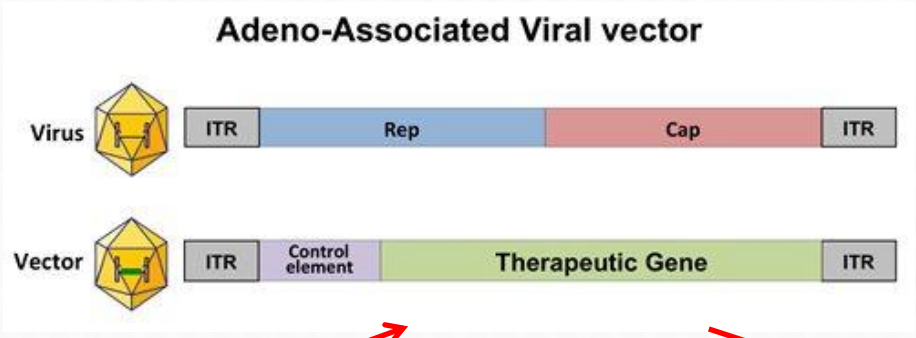
Vall d'Hebron
Institut de Recerca

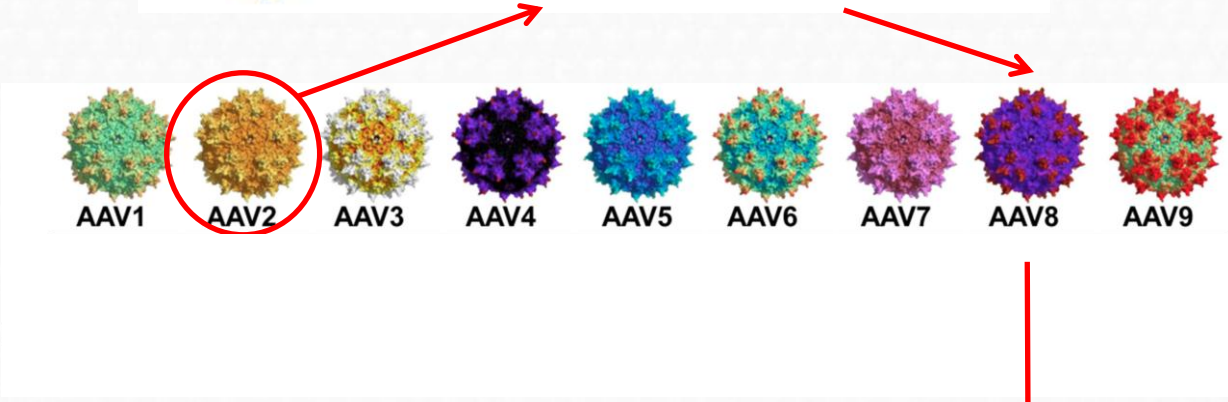






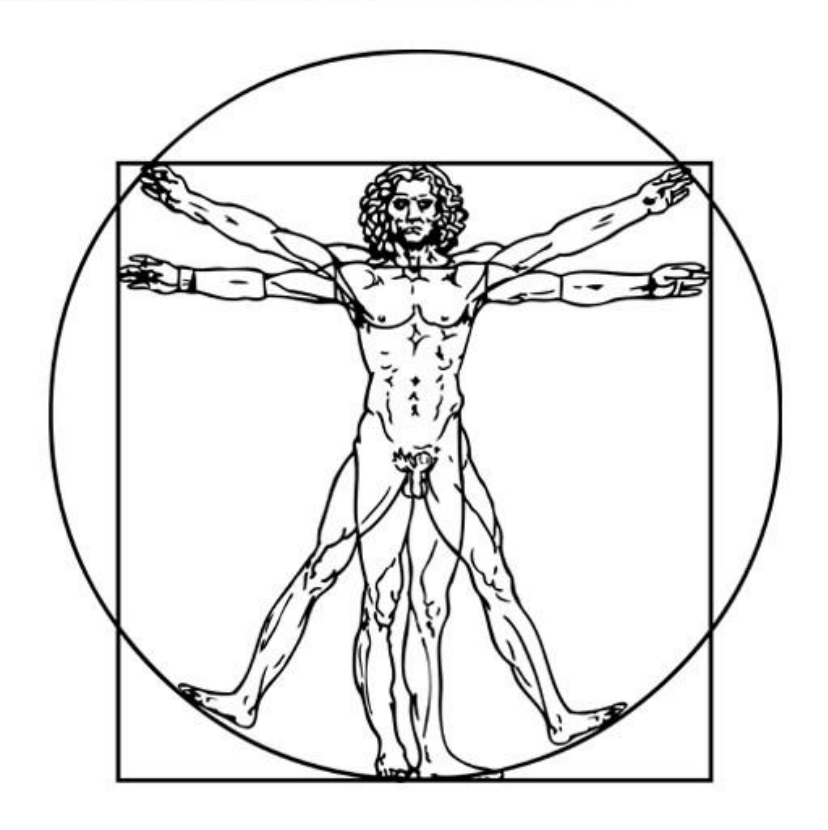
Pseudotipado de vectores





AAV2/8

Eficiencia de transducción: ratón vs humano



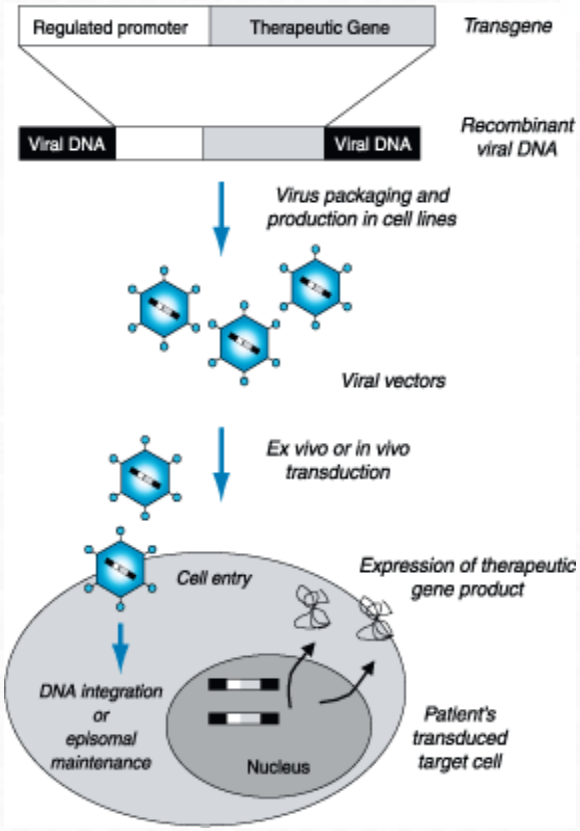
80-90

% hepatocitos transducidos

< 5

Ensayos clínicos en hemofilia

1er ensayo clínico de TG HB, Shanghai, China, 1993

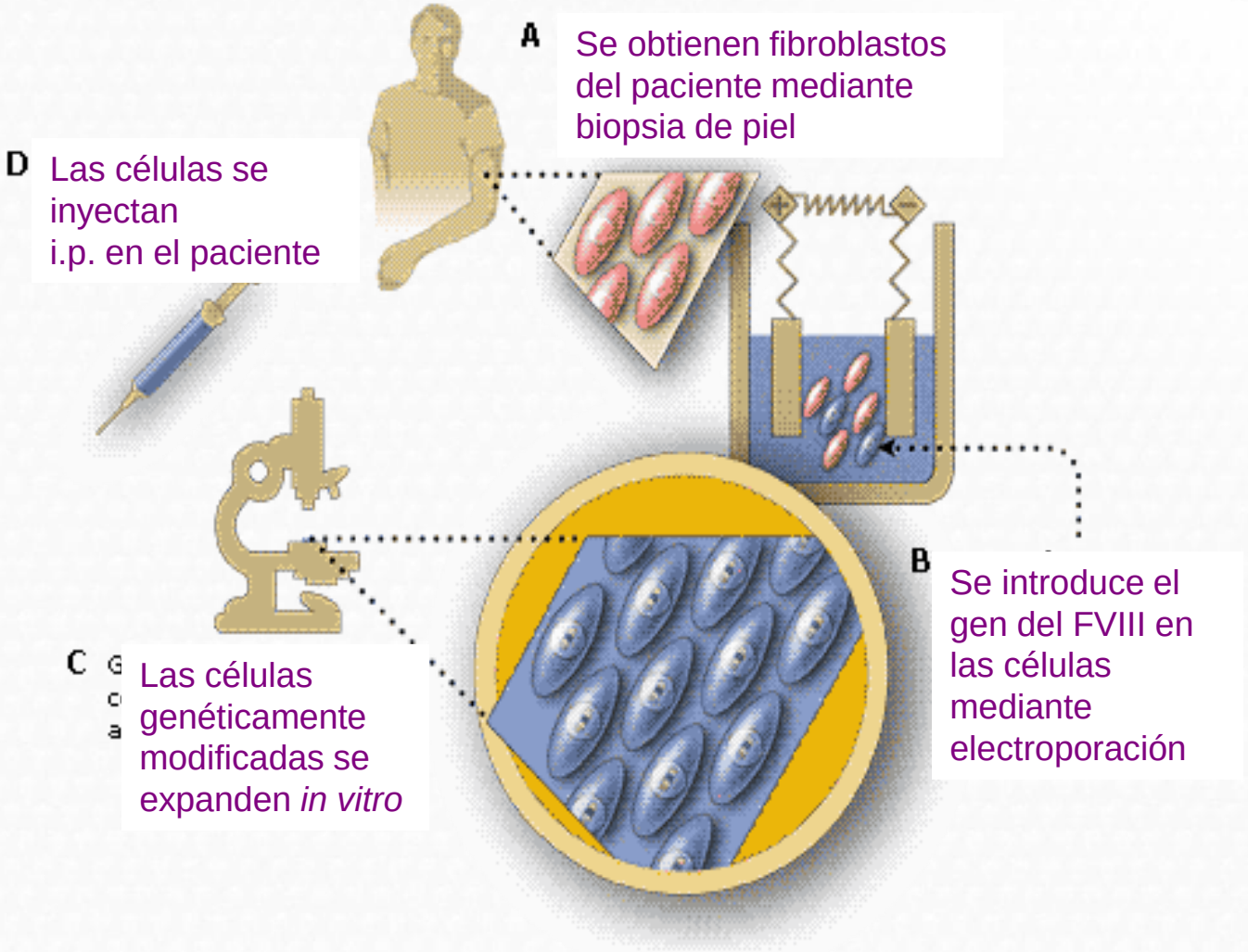


2 hermanos con HB moderada

Retrovirus, promotor CMV

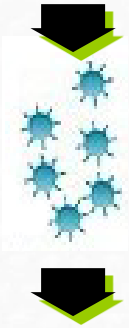
Fibroblastos piel autólogos

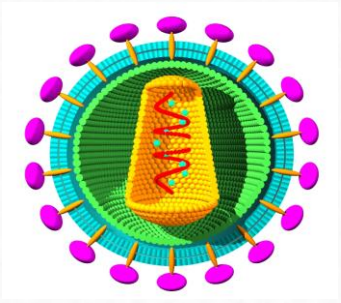
Inyección i.p. de fibroblastos transfectados con pFVIII



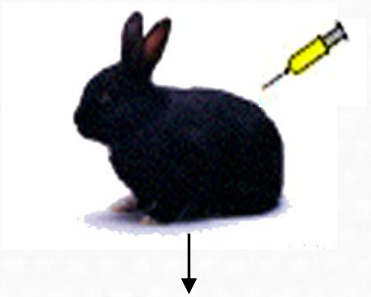
Ensayo Chiron: Inyección i.v. de retrovirus - FVIII

Vector Retroviral





Inyección
i.v.



nivel FVIII
15-20%

Ensayo F. I/II (1999-2002)

U. North Carolina (Chapel Hill)

13 pacientes HA grave

inyección i.v. hasta $8{,}8 \times 10^8$/Kg

actividad >1% en 6 pacientes (max 6%)

↓ requerimientos FVIII

Powell JS *et al*. Blood 2003

Ensayo Avigen (*Children's Hospital* Filadelfia), 1999

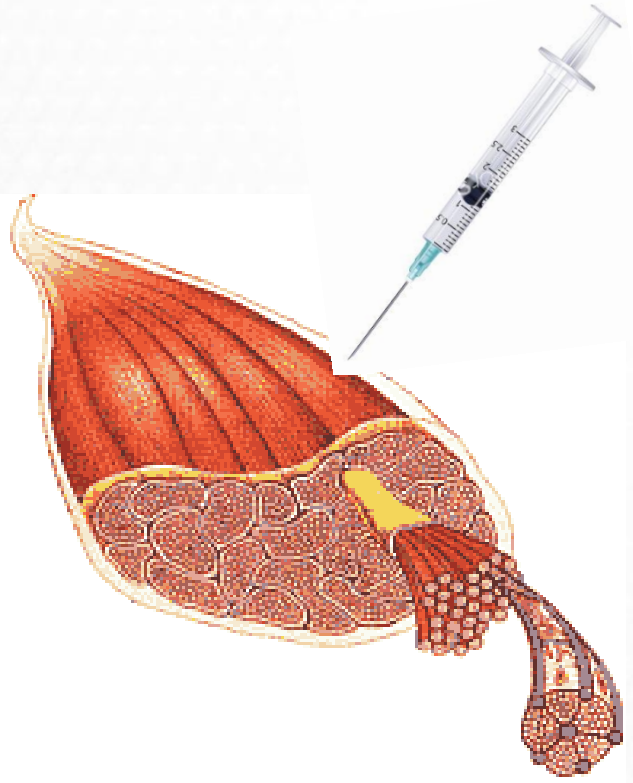
Había funcionado en perros

8 pacientes con HB grave

inyección i.m. (10-72 dosis)

actividad > 1% transitoria

↓ requerimientos FIX



Inyección intraarteria hepática de AAV-FIX

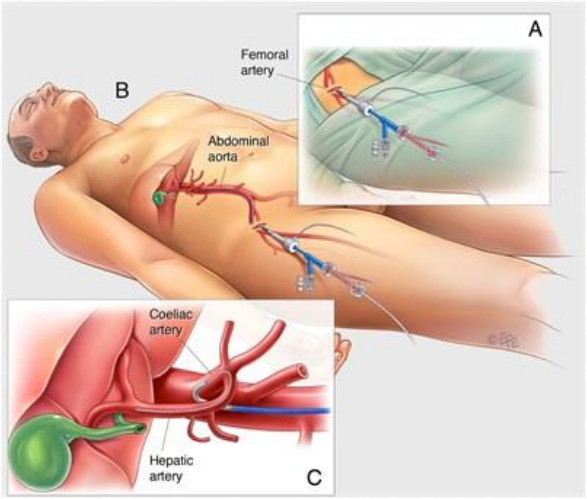
Vall d'Hebron Institut de Recerca

LETTERS

nature medicine

Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response

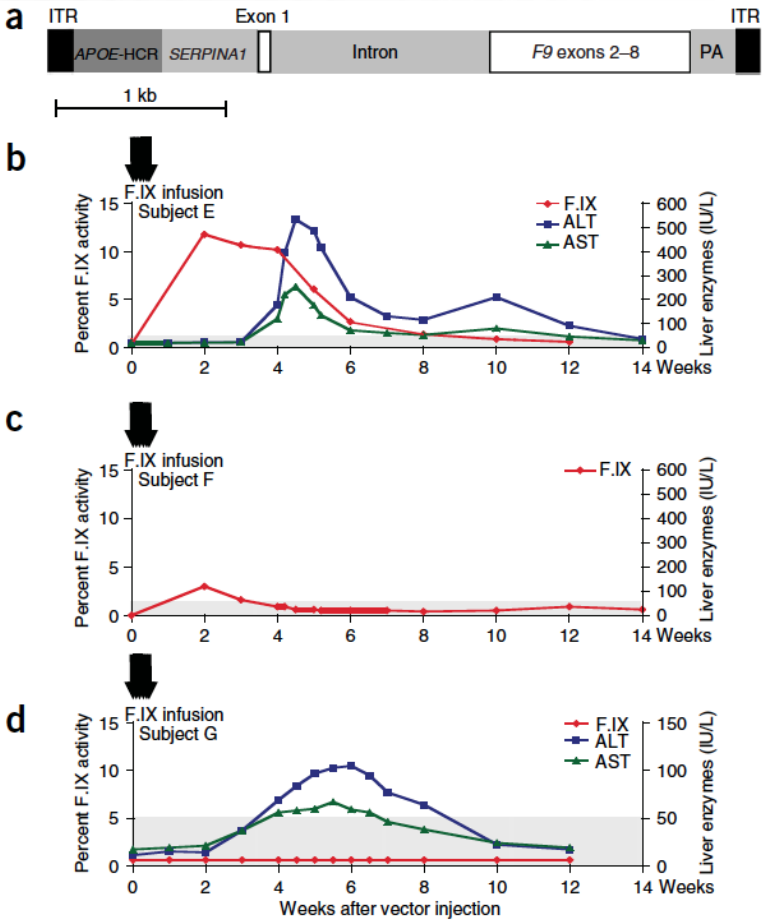
Catherine S Manno[1,2,15], Glenn F Pierce[3,15], Valder R Arruda[1,2,15], Bertil Glader[4,15], Margaret Ragni[5], John J E Rasko[6], Margareth C Ozelo[7], Keith Hoots[8], Philip Blatt[9], Barbara Konkle[10], Michael Dake[4], Robin Kaye[1,2], Mahmood Razavi[4], Albert Zajko[10], James Zehnder[4], Pradip K Rustagi[11], Hiroyuki Nakai[4], Amy Chew[1,3], Debra Leonard[2,12], J Fraser Wright[3], Ruth R Lessard[3], Jürg M Sommer[3], Michael Tigges[3], Denise Sabatino[1], Alvin Luk[3], Haiyan Jiang[3], Federico Mingozzi[1], Linda Couto[3], Hildegund C Ertl[1,13], Katherine A High[1,2,14] & Mark A Kay[4]



Mark Kay, U. Stanford
Nat Med 2006; 12: 342

dosis: 8 x 10^{10} – 2 x 10^{12} gc / Kg

1 paciente: niveles FIX 12%



Primer ensayo clínico exitoso en HB

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Institut de Recerca

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S., Cecilia Rosales, Ph.D., Jenny McIntosh, Ph.D., David C. Linch, M.B., B.Chir., Pratima Chowdary, M.B., B.S., Anne Riddell, B.Sc., Arnulfo Jaquilmac Pie, B.S.N., Chris Harrington, B.S.N., James O'Beirne, M.B., B.S., M.D., Keith Smith, M.Sc., John Pasi, M.D., Bertil Glader, M.D., Ph.D., Pradip Rustagi, M.D., Catherine Y.C. Ng, M.S., Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D., John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D., Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D., Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

N Engl J Med 2011; 365: 2357

Resultados (6 pacientes)

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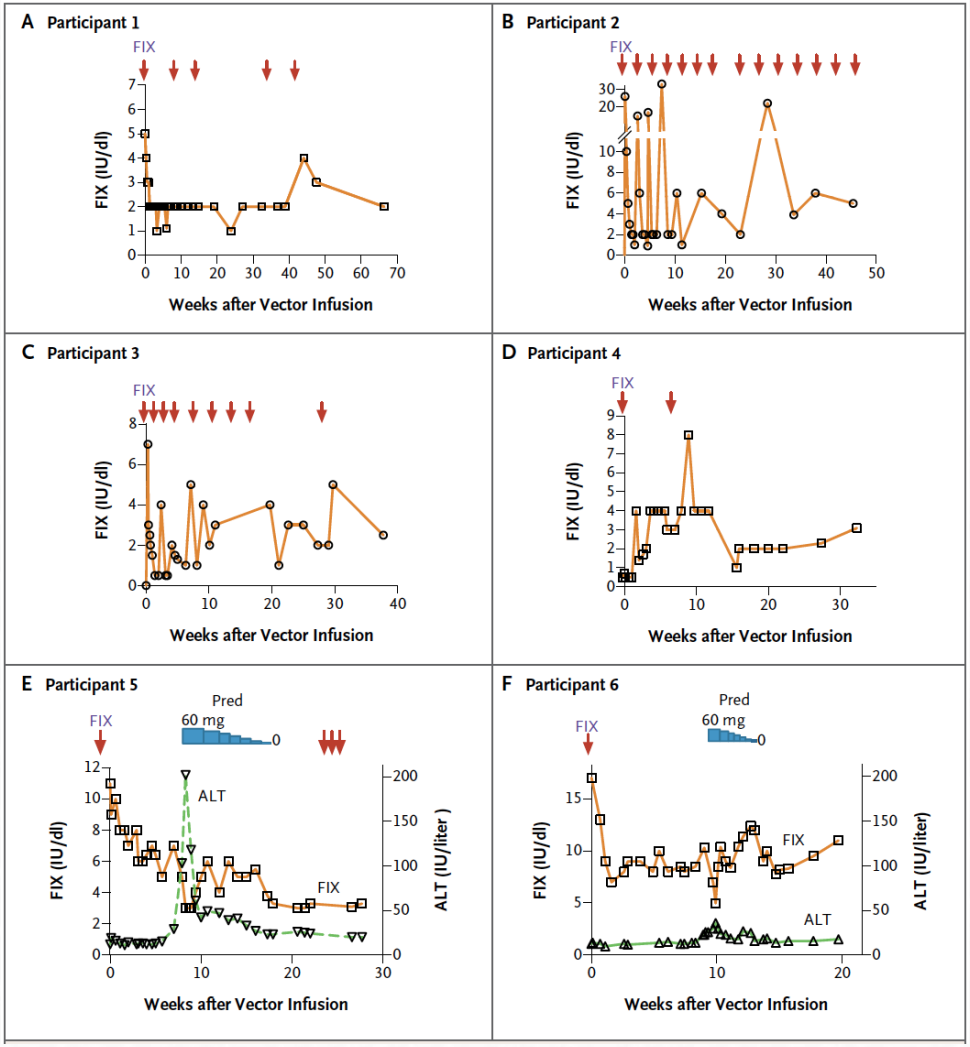
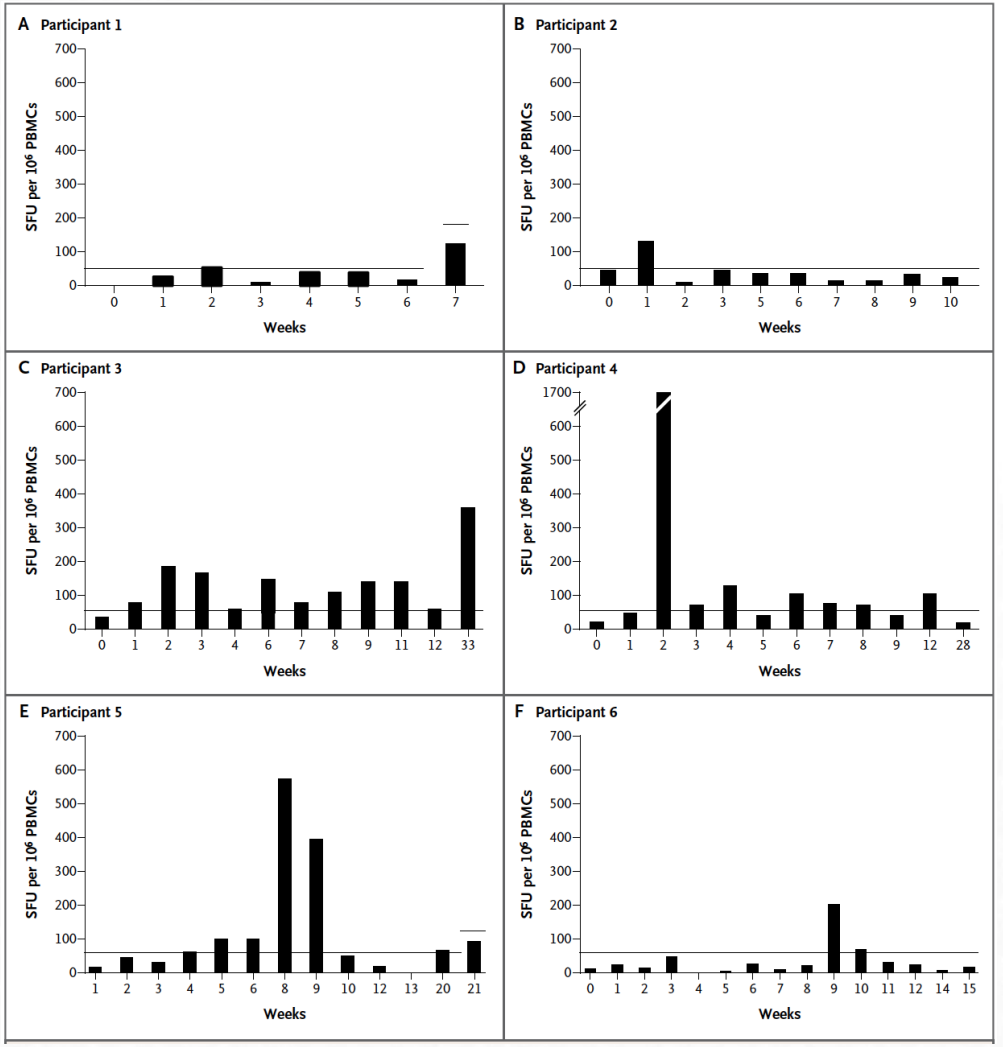
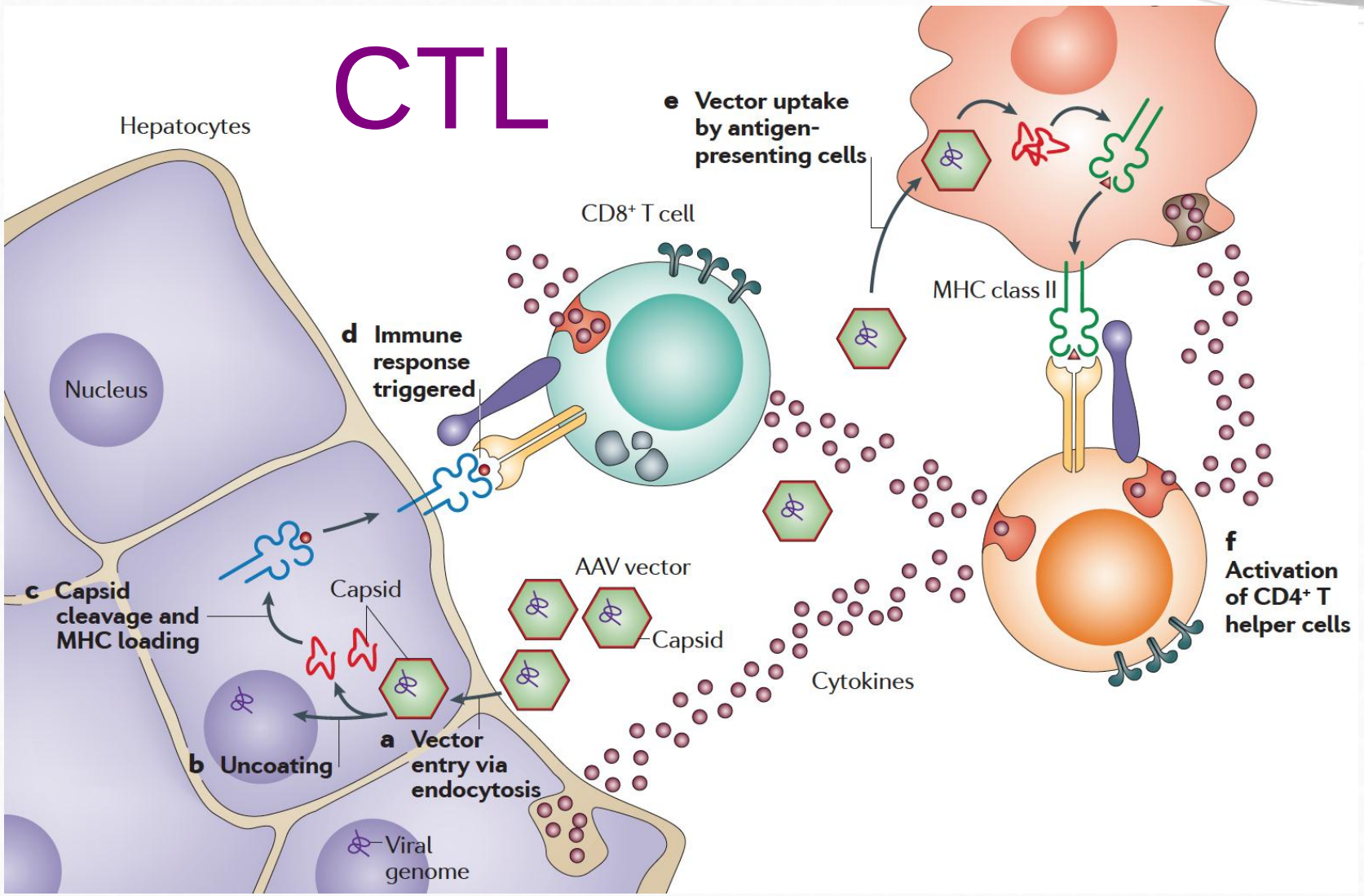




Respuesta inmune celular frente al vector



Riesgos: genotoxicidad (oncogenicidad)

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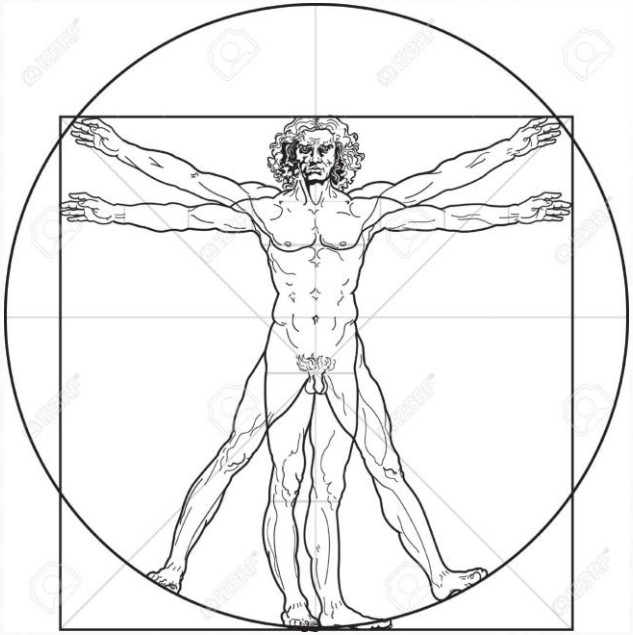
Probabilidad integración: 10^{-3}

Tumorigenicidad hepática

AAVs integrados en céls tumorales

Sólo en periodo neonatal

Sólo con dosis altas de vector



En >100 ensayos clínicos con AAVs no se han observado casos de oncogenicidad

El “mercado” de la TG en hemofilia

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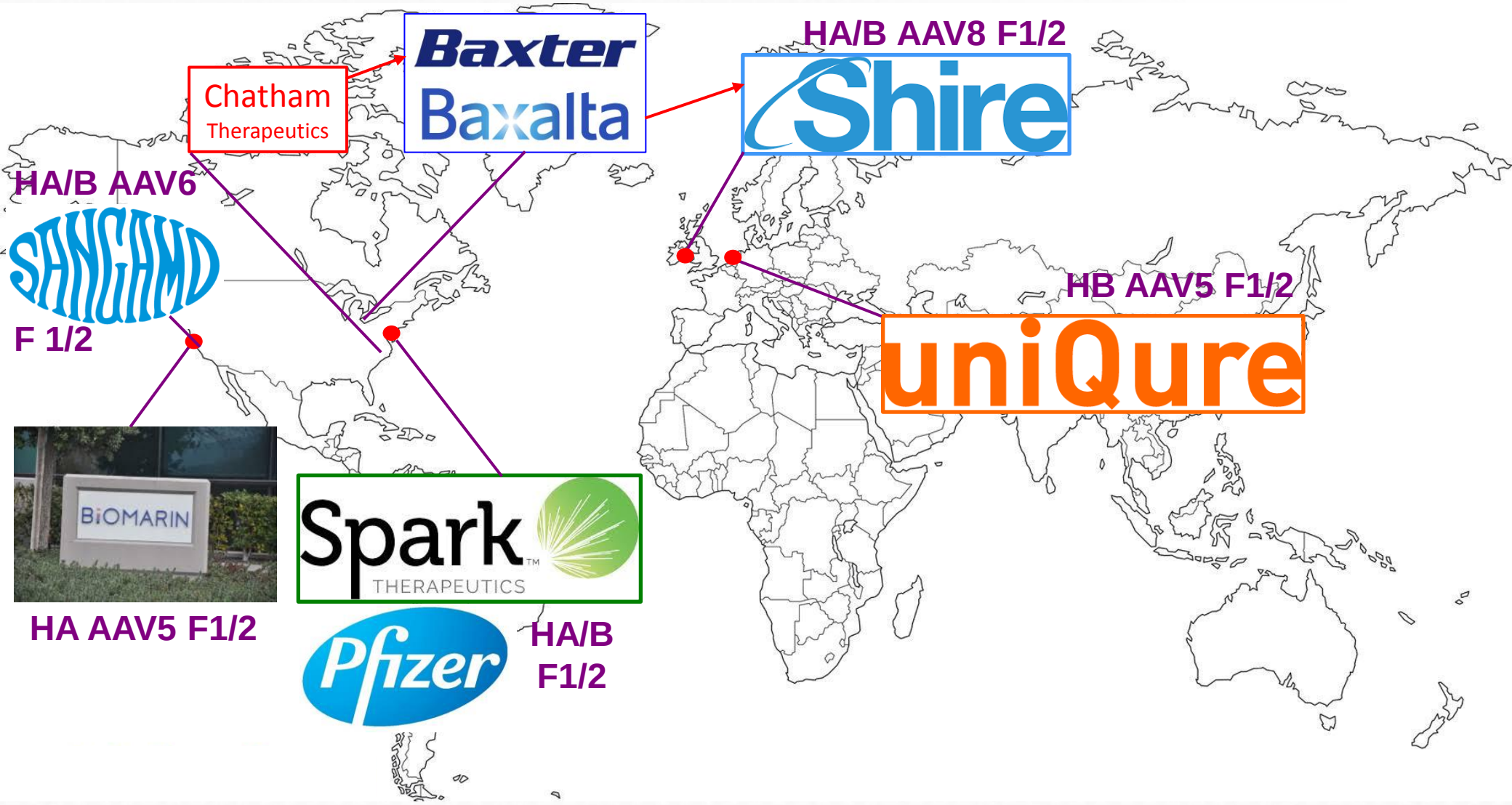
Institut de Recerca



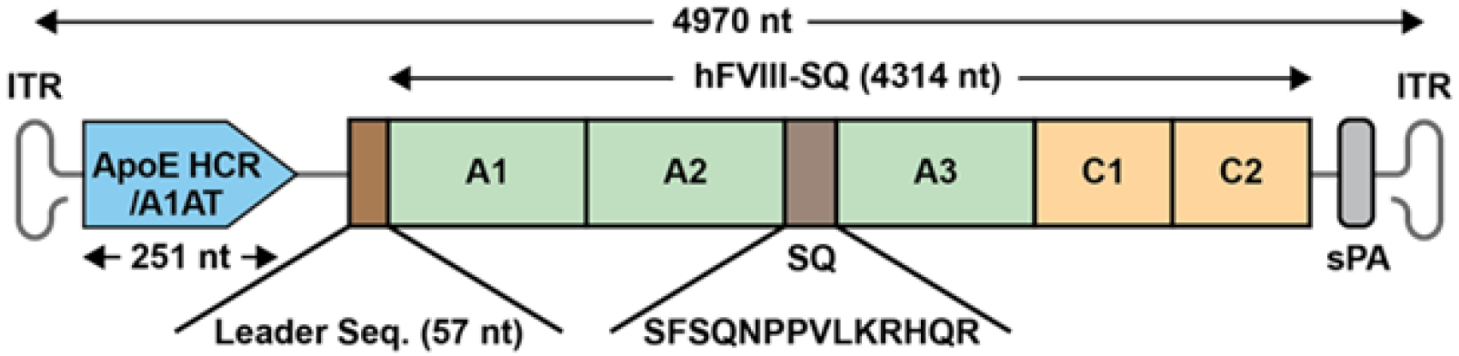
AAV5-FVIII (BMN 270) gene transfer in HA

Vall d'Hebron Institut de Recerca

BIOMARIN R&D Day 2017

BMN 270: AAV5-hFVIII-SQ

In vivo transfer of a functional FVIII gene into hepatocytes



- Recombinant, replication-incompetent AAV5 vector
- Hybrid liver-specific promoter
- Codon-optimized human B domain-deleted (BDD) coagulation factor VIII with 14 amino acid "SQ" linker sequence

AAV5-FVIII (BMN 270) gene transfer in HA

Vall d'Hebron Institut de Recerca

BIOMARIN R&D Day 2017

BMN 270: Proof of Concept with First-in-Human Study

Phase 1/2 Study Design

- Subjects enrolled sequentially into one of up to four cohorts based on FVIII activity at 3 weeks:
  1. 6E12 vg/kg given as a single IV dose
  2. 2E13 vg/kg
  3. 6E13 vg/kg
  4. 4E13 vg/kg
- Dose escalation occurred in cohorts 1-3 if the resulting FVIII activity at the Week 3 visit was < 5 IU/dL and gated by safety assessment
- Endpoints
  - Safety of a single IV administration of a recombinant AAV5-human FVIII vector
  - Change from baseline FVIII activity level
  - Annualized FVIII replacement therapy infusion rate
  - Annualized bleed rate
  - Change in health-related quality of life

AAV5-FVIII (BMN 270) gene transfer in HA

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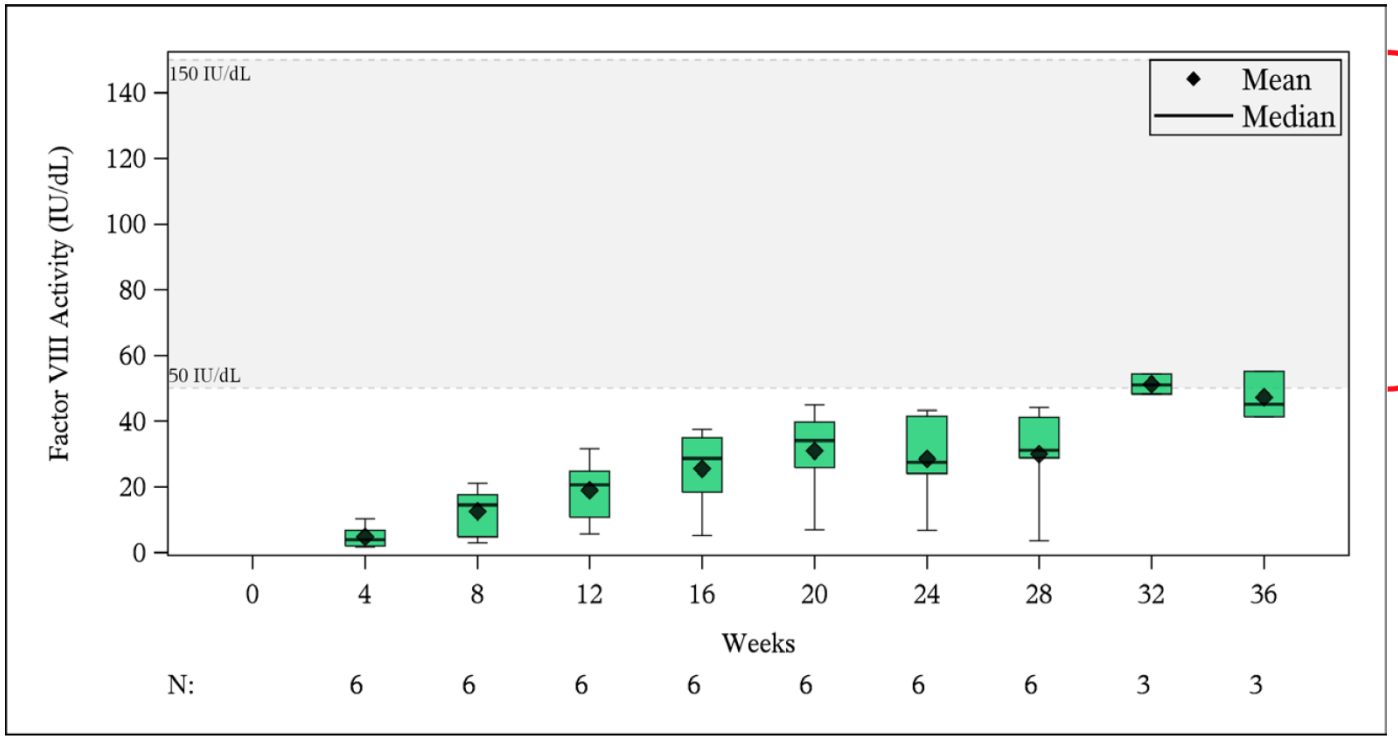
BIOMARIN R&D Day 2017

BMN 270 4E13 vg/kg Update: Approaching/In Normal Range

Baseline FVIII activity for all subjects at study start was ≤1% of normal level

Results from 4E13 vg/kg cohort
(September 14, 2017 datacut)



AAV5-FVIII (BMN 270) gene transfer in HA

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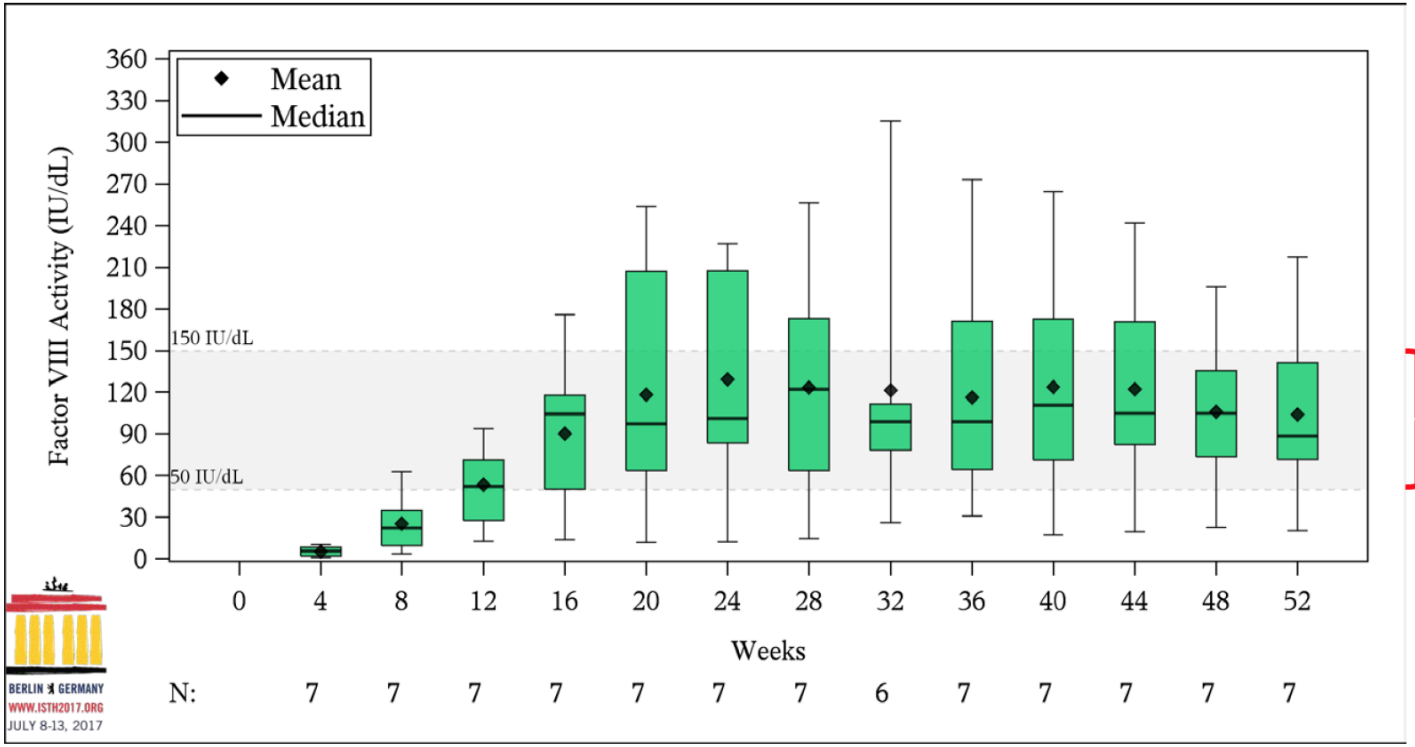
BIOMARIN R&D Day 2017

BMN 270: FVIII Levels Sustainably Normalized After 52 Weeks

Baseline FVIII activity for all subjects at study start was ≤1% of normal level

Results from 6E13 vg/kg cohort

(May 31, 2017 datacut)



Normal FVIII Range: 50-150 IU/dL

BERLIN GERMANY
WWW.ISTH2017.ORG
JULY 8-13, 2017

SPARK (SPK-9001) gene transfer in HB

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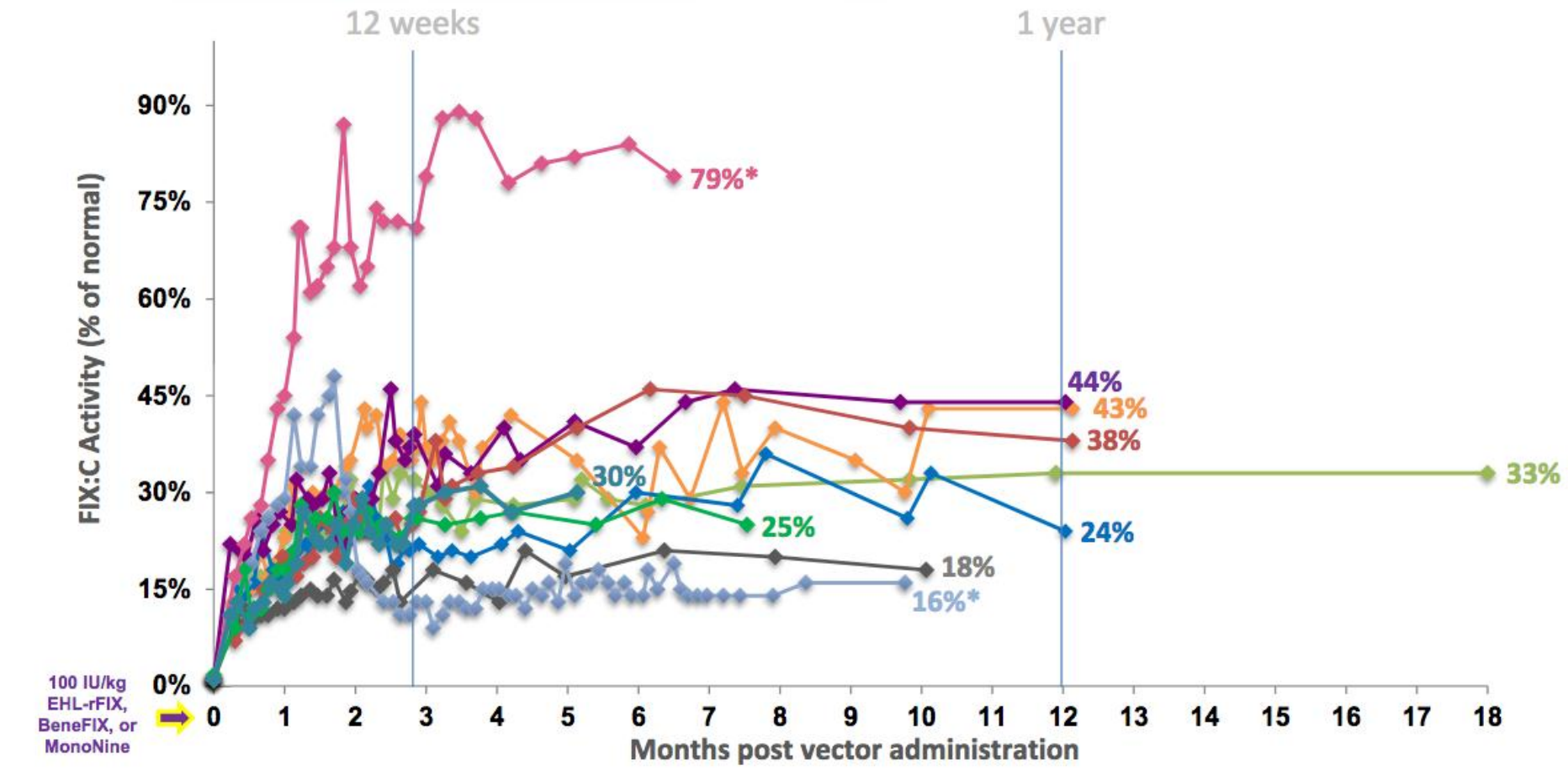
Preliminary *SPK-9001* Phase 1/2 data in hemophilia B: Consistent and sustained FIX activity within target range in initial 10 participants



¿CUANTOS PACIENTES OPTARAN POR LA TG?

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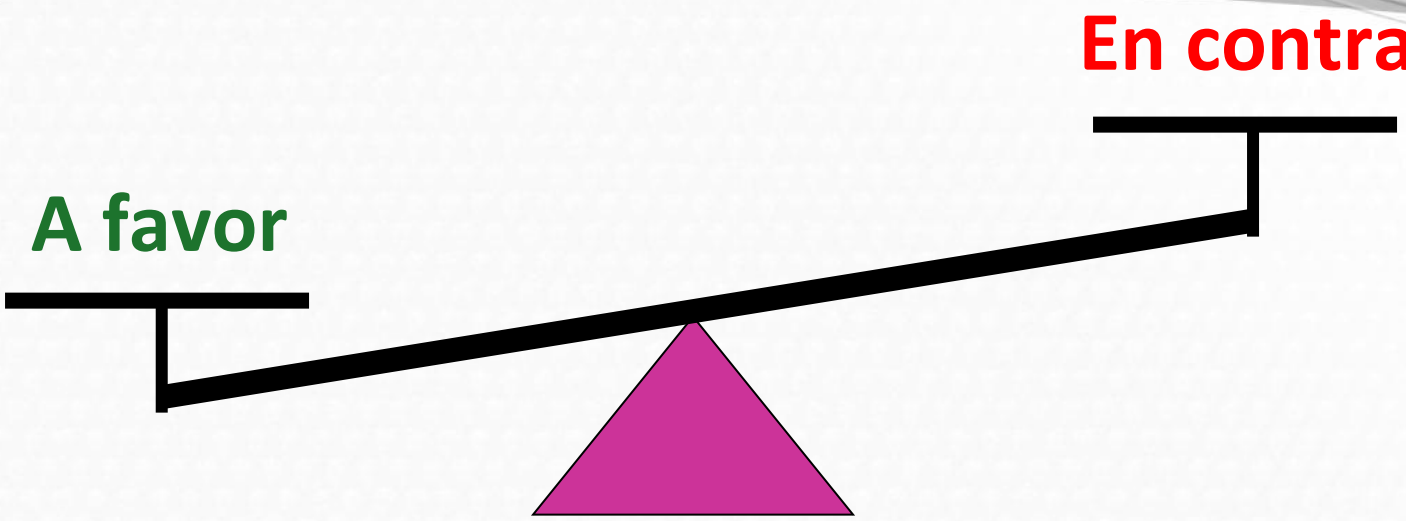
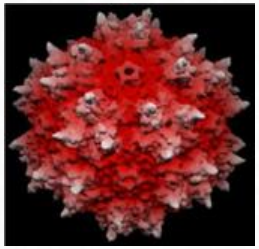


Un único tratamiento
< coste económico



Inmunización (AcN)
Pérdida de expresión
¿Riesgos a largo plazo?

Bioseguridad alta
No veta futuras opc.

Admin. periódicas
> coste económico

Modelos de éxito en TG

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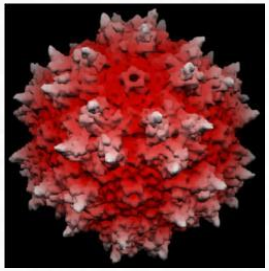
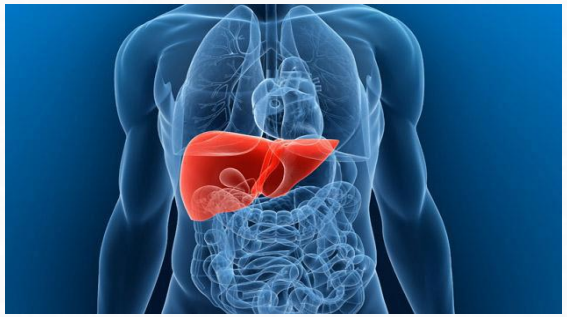
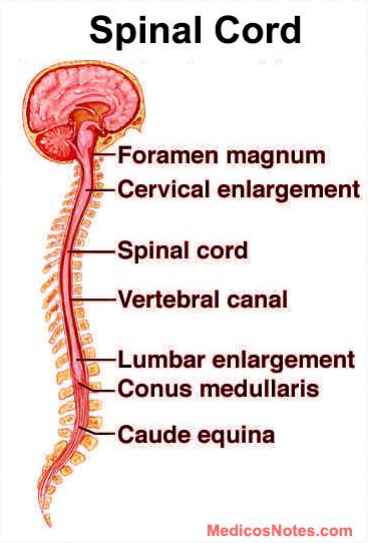
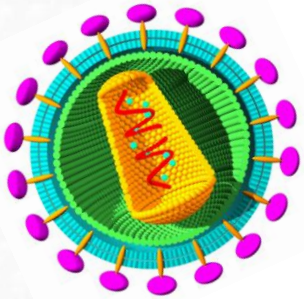


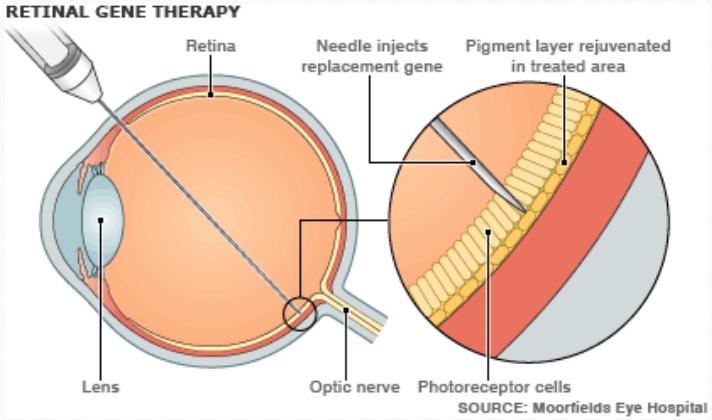
AAVs

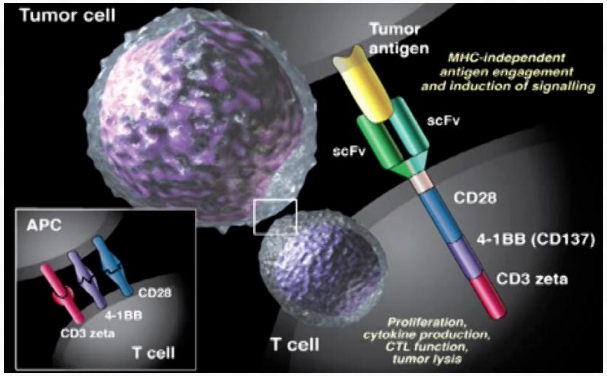




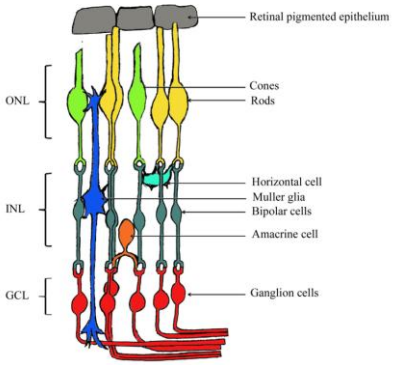


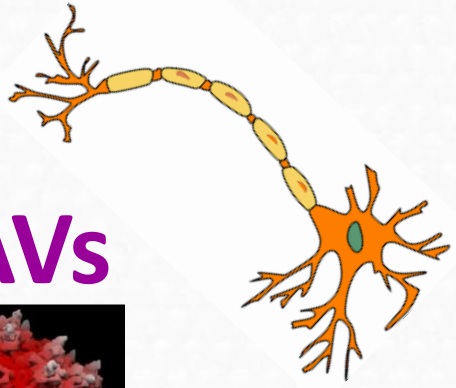
RVs



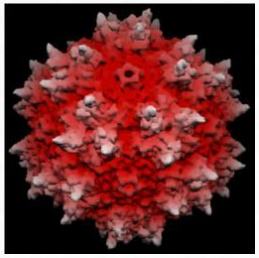


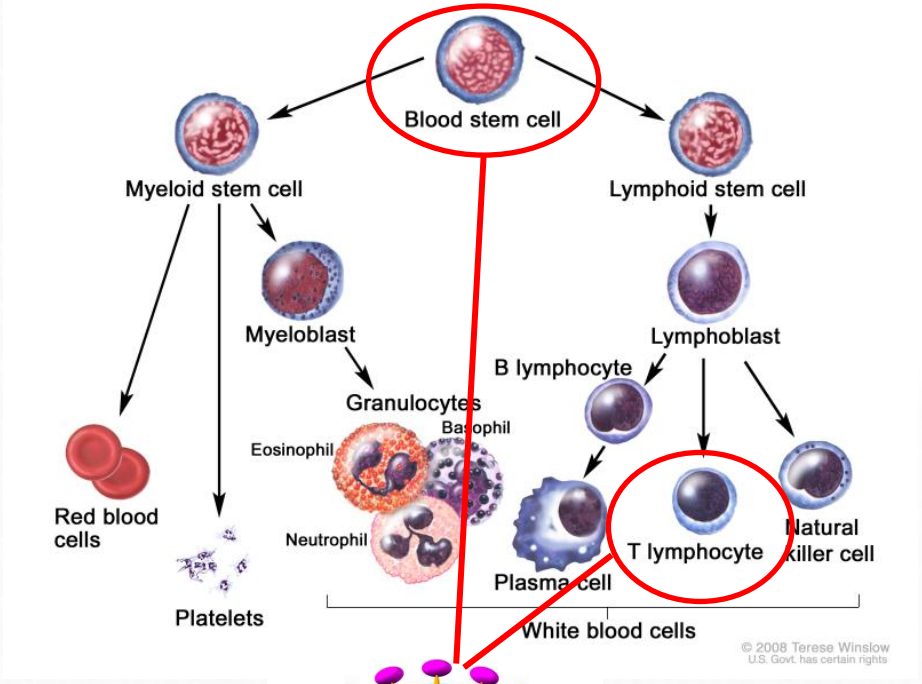
Modelos de éxito en TG

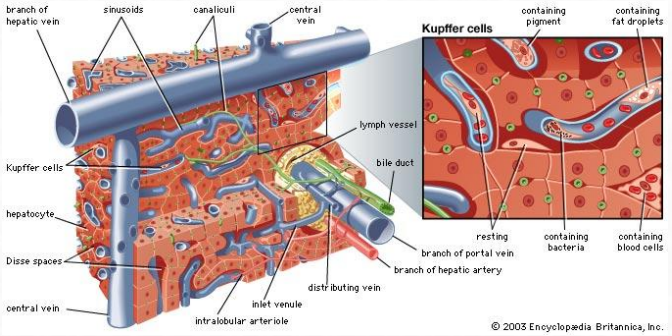


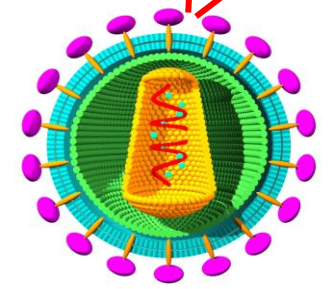


AAVs









RVs

Tejidos postmitóticos

Tejidos proliferativos

el futuro

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Edición génica

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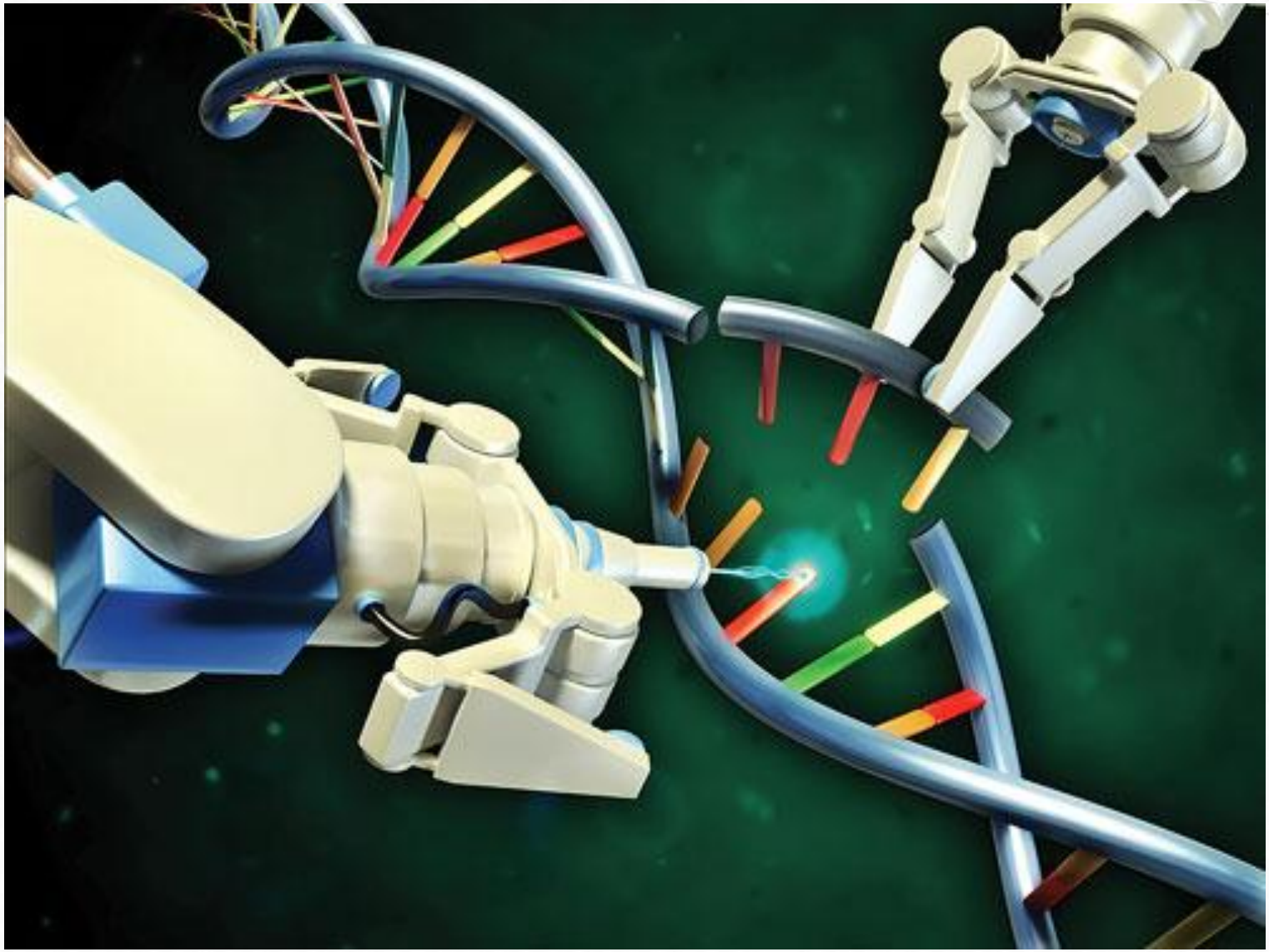
Institut de Recerca



Edición génica

Vall d'Hebron
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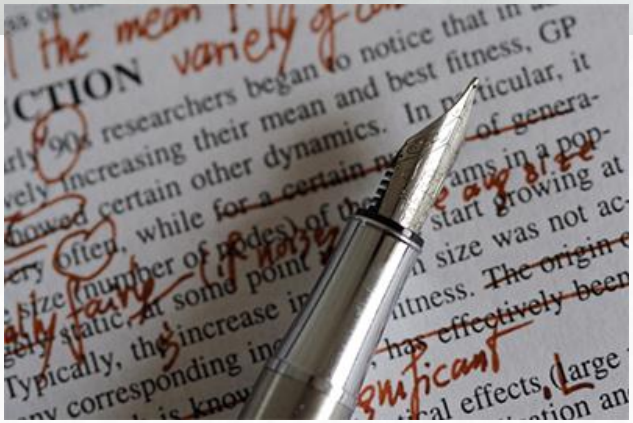

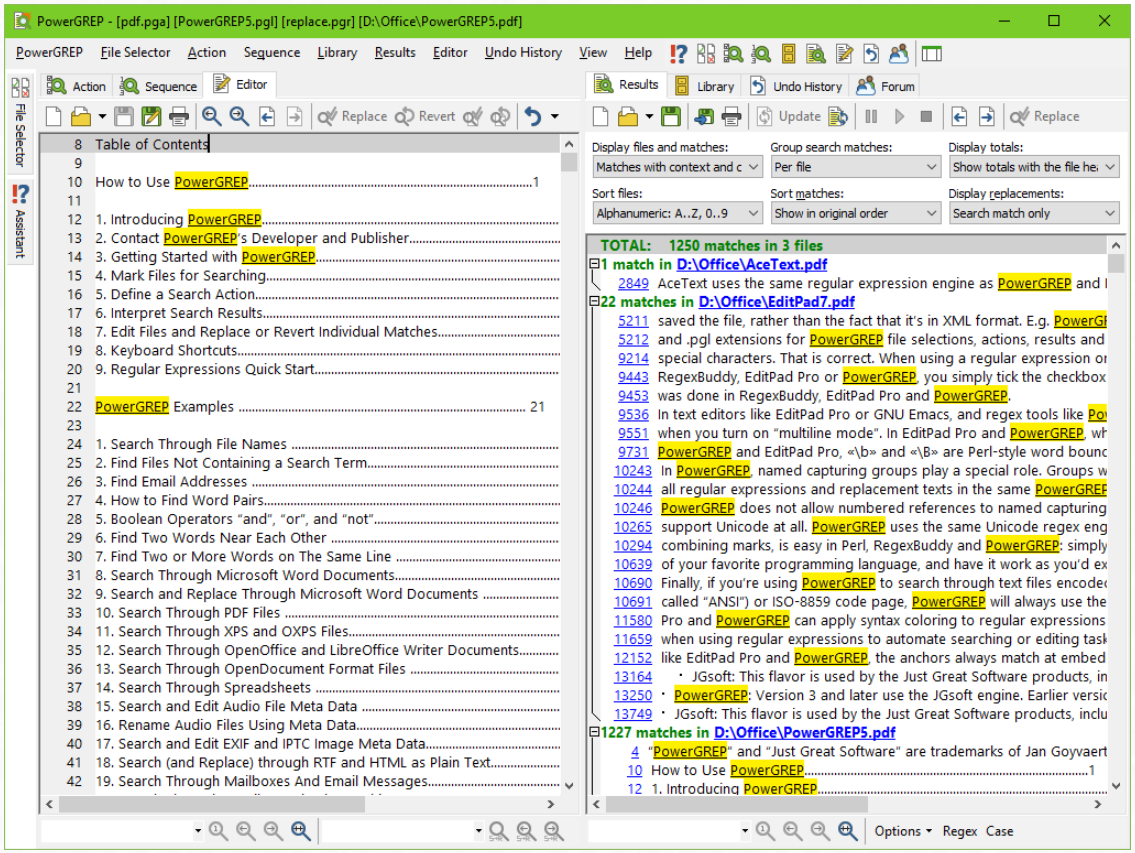

Herramientas para la edición génica

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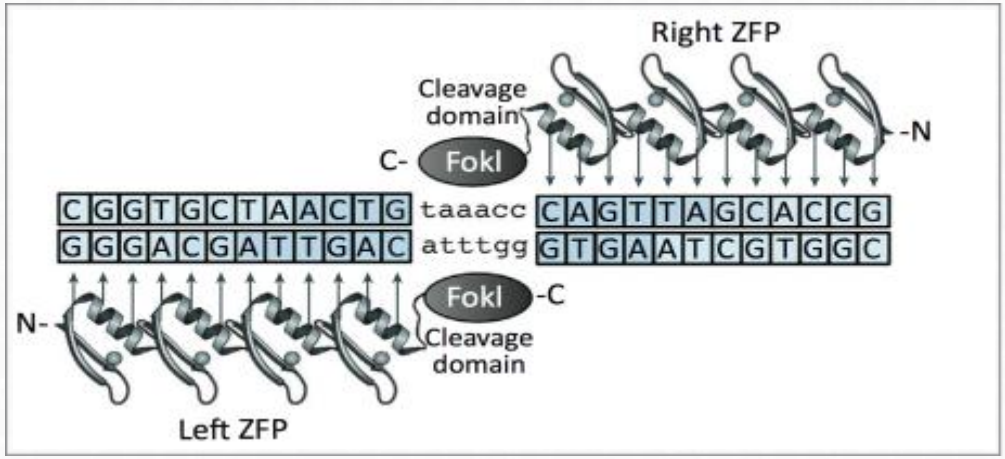
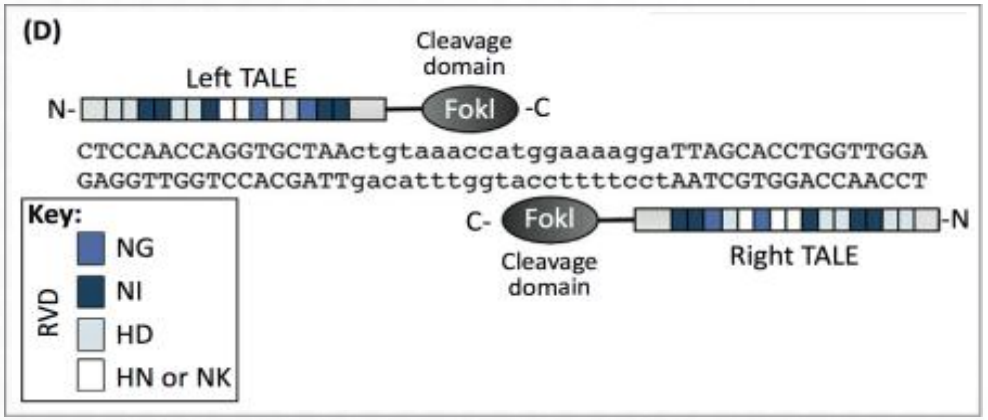
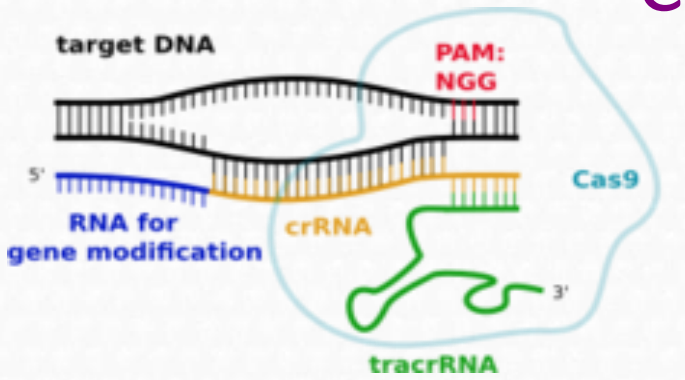
Nucleasas en dedos de zinc

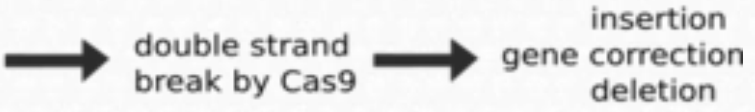


TALEN



CRISPR/Cas9

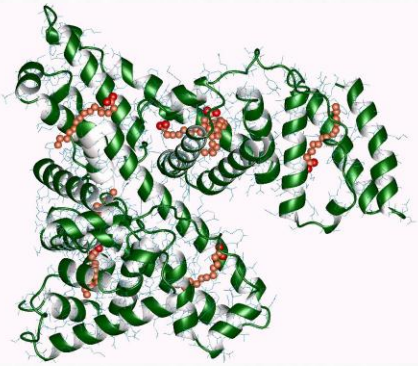




Vall d'Hebron
Barcelona Campus Hospitalari

Modificado de X. Anguela

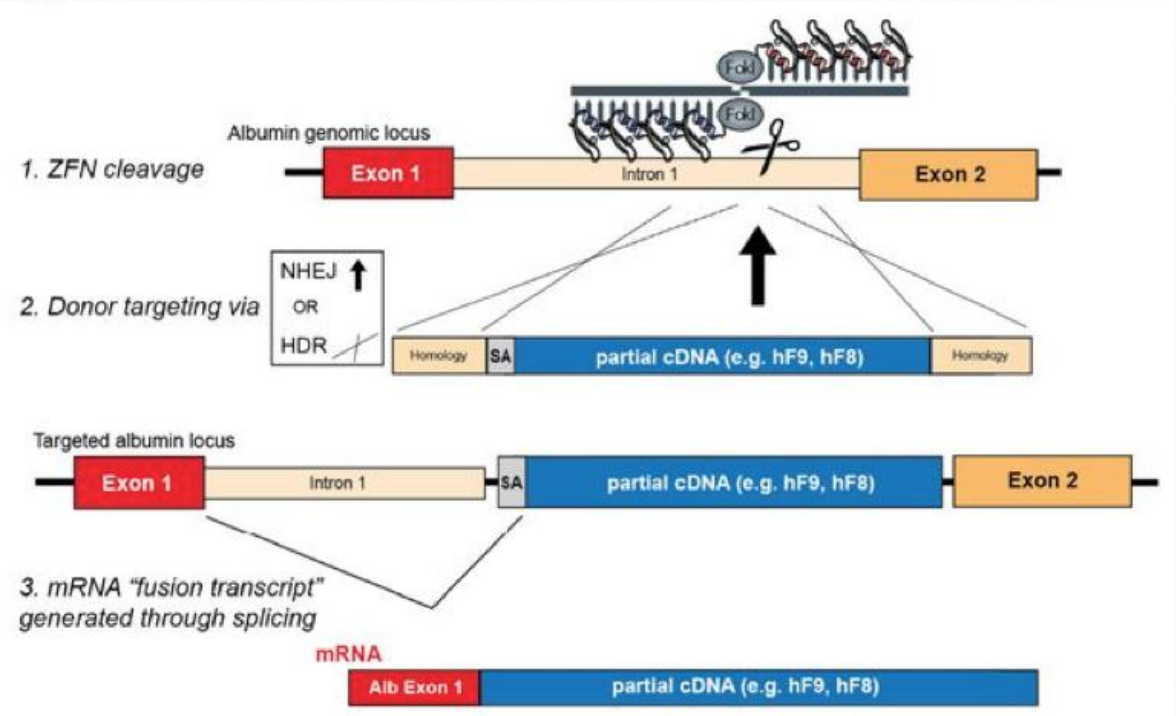
Utilización del locus de la albúmina (I)



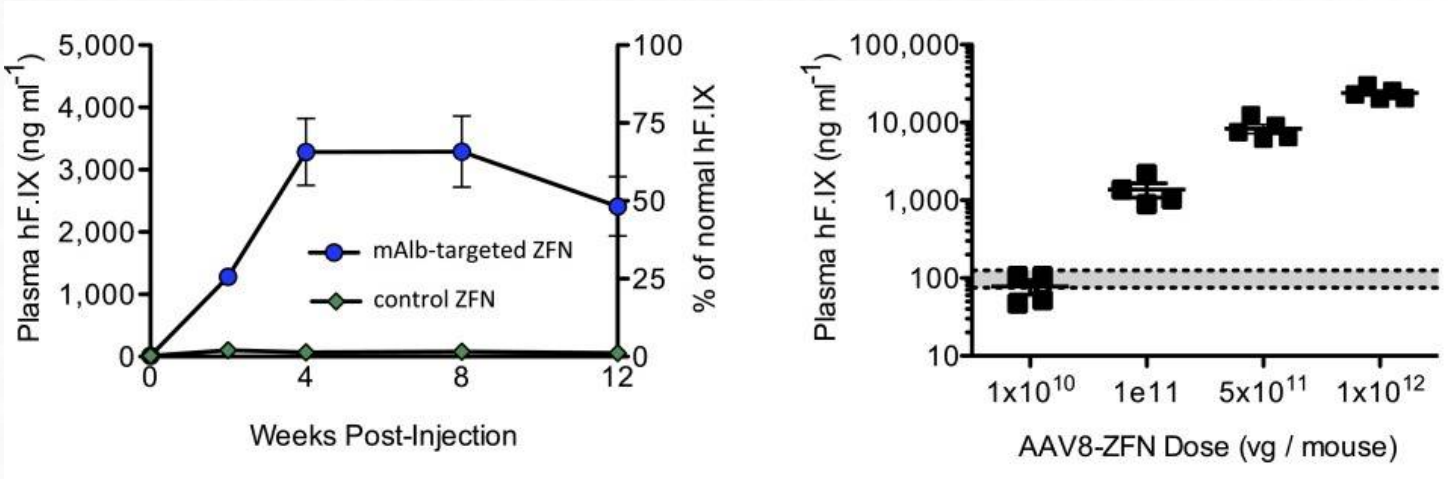
albúmina

Proteína plasmática más abundante

[ ]: 35-50 g/L: se sintetizan 15 g/día



Utilización del locus de la albúmina (II)



GENE THERAPY

In vivo genome editing of the albumin locus as a platform for protein replacement therapy

Rajiv Sharma,[1,*] Xavier M. Anguela,[1,2,*] Yannick Doyon,[3,*] Thomas Wechsler,[3] Russell C. DeKelver,[3] Scott Sproul,[3] David E. Paschon,[3] Jeffrey C. Miller,[3] Robert J. Davidson,[1] David Shivak,[3] Shangzhen Zhou,[1] Julianne Rieders,[1] Philip D. Gregory,[3] Michael C. Holmes,[3] Edward J. Rebar,[3] and Katherine A. High[1,2]

[1]Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Howard Hughes Medical Institute, Philadelphia, PA; and [3]Sangamo BioSciences, Richmond, CA

Blood 2015; 126: 1777

Cambio de paradigma

TG convencional

vectores muy eficientes

expresión transgén a largo plazo

TG mediante edición

vectores muy eficientes

expresión transitoria de los elementos de edición

“hit & run”

Resumen

1) La TG mediante AAVs dirigidos a hígado parece segura y está funcionando razonablemente en ensayos clínicos en HA y HB.

2) La respuesta inmune celular sólo se produce con dosis altas de vector pero puede controlarse con inmunosupresión convencional. La respuesta humoral no se evita, pero no perjudica a la terapia actual (aunque podría vetar futuras opciones). ¿Pacientes con AcN?

3) Quedan dudas sobre la expresión de los transgenes a largo plazo.

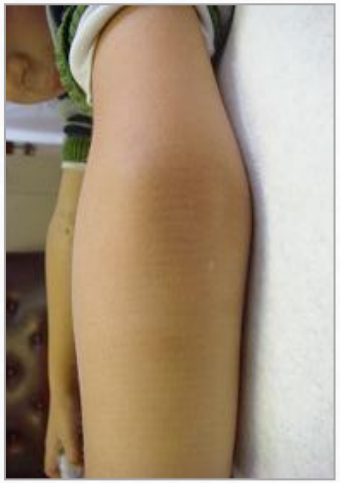
4) Coste económico, quien podrá y querrá tratarse con TG.

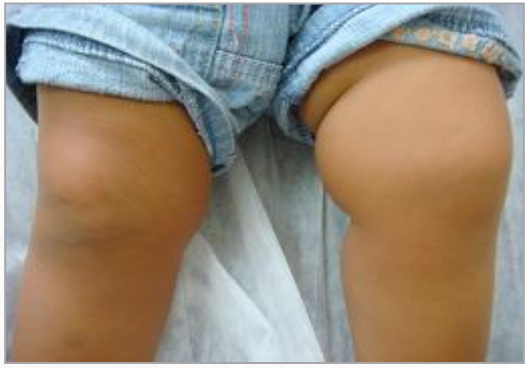
5) En el futuro habrá vectores más seguros y eficientes. TG convencional vs TG con edición génica.

Clínica: hemorragias

Grave (<1% de actividad): 60%

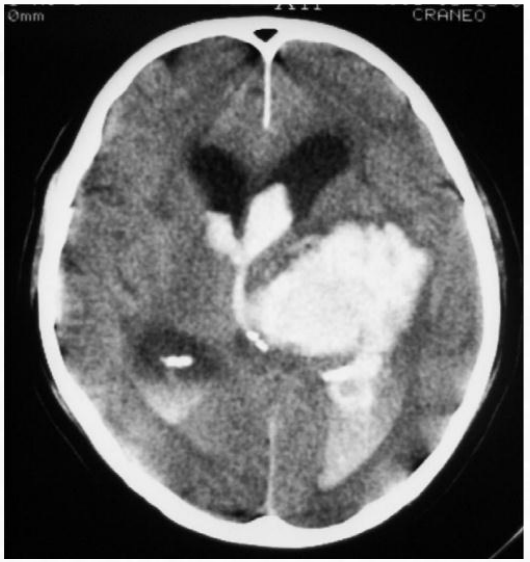
hemorragias espontáneas
traumatismos mínimos





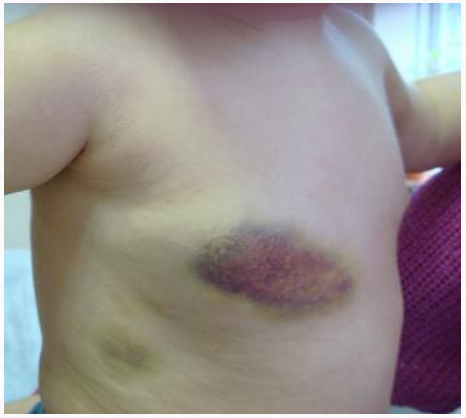
Moderada (1-5% de actividad): 15%

traumatismos leves o moderados

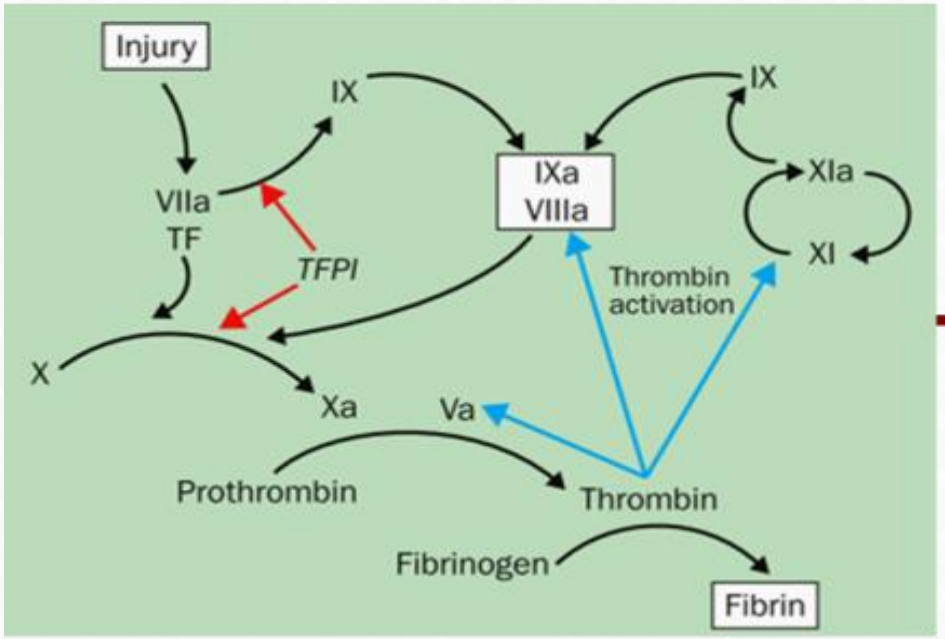


Leve (6-30% de actividad): 25%

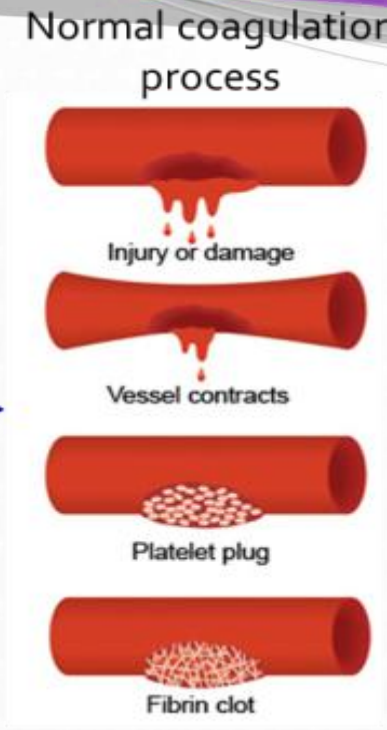
asintomática
estudio familiar
estudio de la hemostasia
sangrado post quirúrgico



Tratamiento



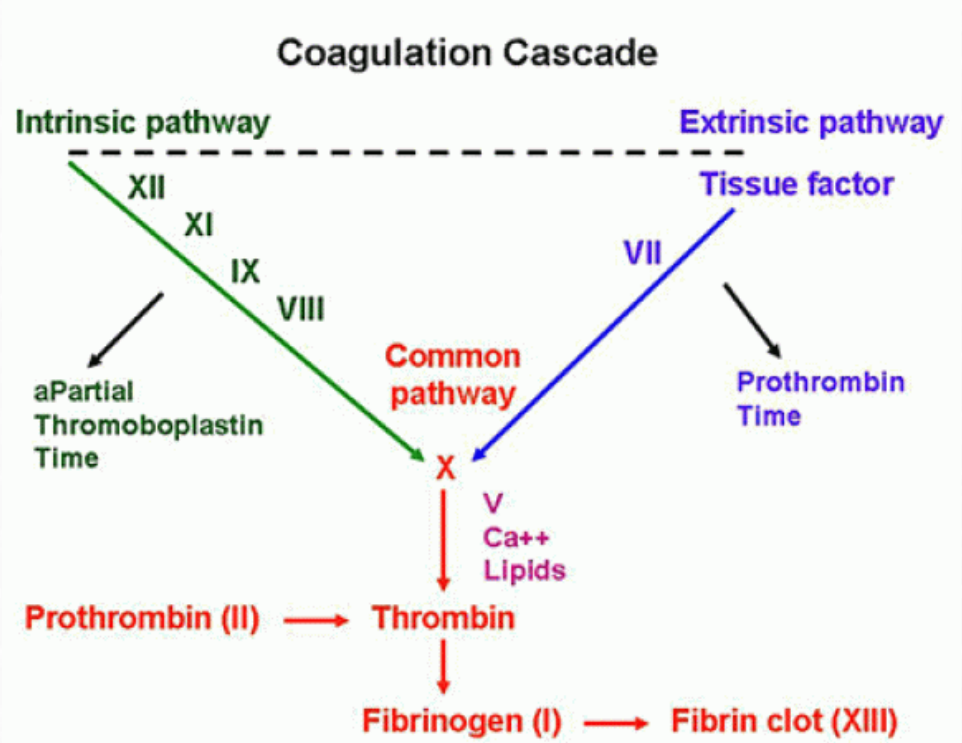
Schematic model of coagulation in vivo



“”haima” = blood, “philia” = love

Incidence: 1/6000 males (HA), 1/25.000 (HB)

200-300.000 people affected in the world

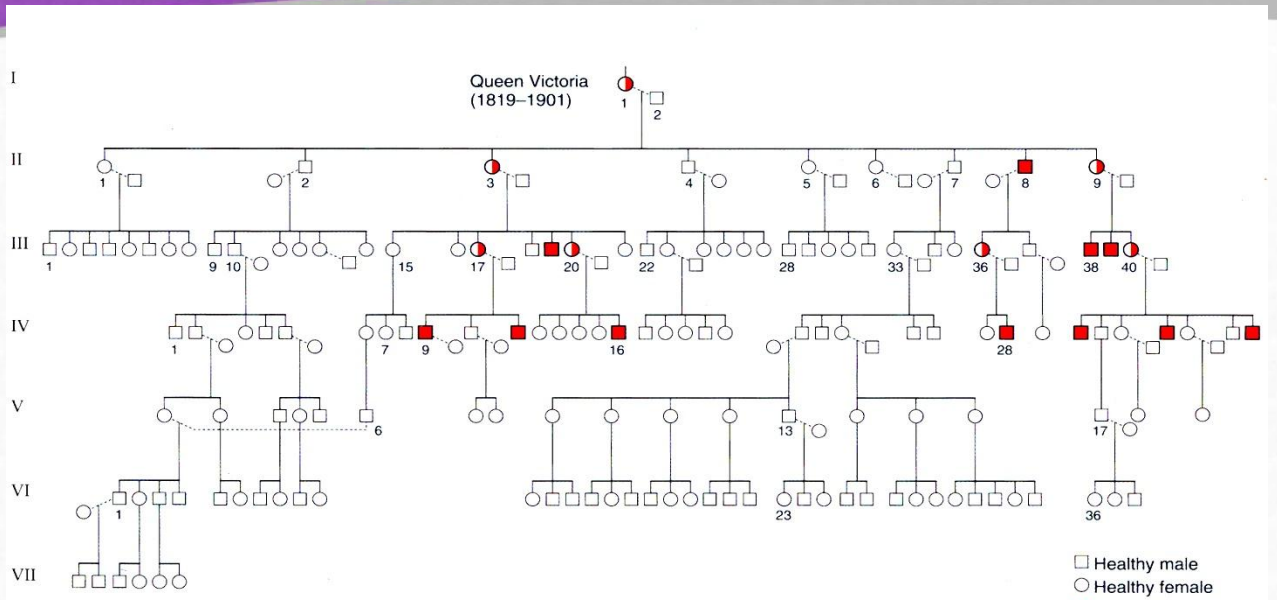


Deficiencia de FVII (HA) o FIX (HB)

1/5000 – 1/10.000 hombres

Ligada al cromosoma X

Hemophilia A, a "Royal" disease



I: 1 Queen Victoria (1819-1901)
2 Prince Albert

II: 1 Victoria, non-carrier, mother of Kaiser Wilhelm II
2 Edward VII, healthy
3 Alice, carrier, m. Ludvig IV of Hesse
4 Alfred I of Coburg, healthy
5 Helene, non-carrier
6 Louise, non-carrier
7 Arthur of Connaught, healthy
8 Leopold of Albany, hemophiliac, d. cerebral hemorrhage, aged 31
9 Beatrice, carrier

III: 1-8 Healthy children of Victoria and Frederick III (III:1, Kaiser Wilhelm II)
9 King George V
10
11
12
13 Queen Maud of Norway
14
15 Victoria, non-carrier
16 non-carrier
17 Irene, carrier, m. Henry of Prussia
18 Ernest, healthy son
19 Frederick, hemophiliac, d. cerebral hemorrhage aged three
20 Alexandra, carrier, m. Nicholas II, Tsar of Russia
21 Mary, d. at the age of four
22-27 Neither hemophiliac nor carrier
28-32 -"-
33 Crown Princess Margareta, m. Gustav VI Adolf
34 Arthur, healthy
35 Patricia Ramsay
36 Alice, Princess of Teck, carrier
37 Charles-Edward of Saxe-Coburg, healthy (father of Princess Sibylla)
38 Leopold, hemophiliac
39 Maurice, -"-
40 Victoria Eugenie, carrier, m. Alfonse XIII of Spain

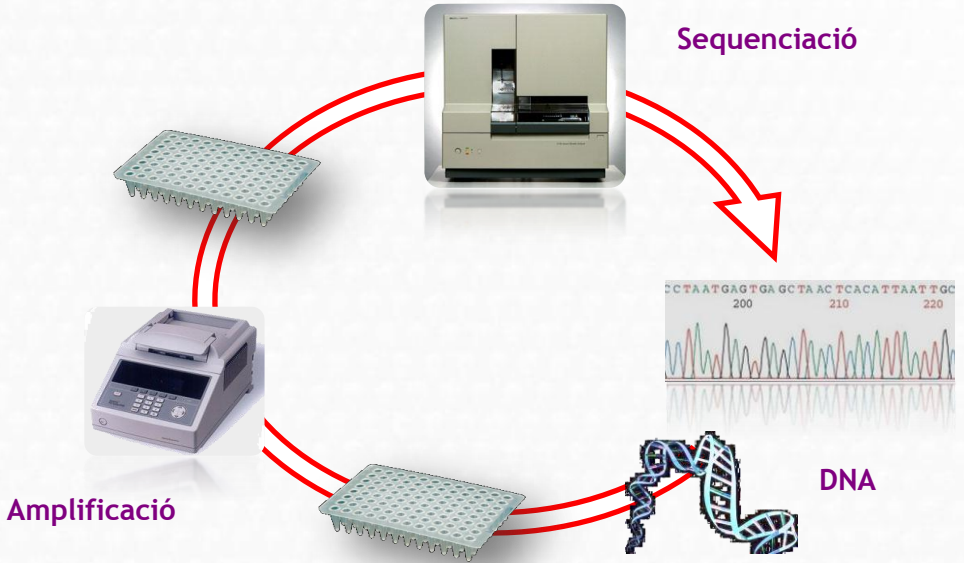
IV: 1 Duke of Windsor, healthy
2 George VI
3 Mary
4 Henry, Duke of Gloucester
5 George, Duke of Kent
6 Princess Alice, non-carrier, m. Andrew of Greece
7 Queen Louise of Sweden
8 Lord George Mountbatten
9 Waldemar, hemophiliac, d. 56 years of age, no progeny
10 Sigismund, non-hemophiliac
11 Henry, hemophiliac, d. at the age of four
12 Olga } Alexandra Feodorovna's daughters
13 Tatiana } carriers?
14 Marie } murdered 1918
15 Anastasia, b. 1901, carrier?
16 Alexis, b. 1904, d. 1918, hemophiliac
17-21 Neither hemophiliac nor carrier
22 Prince Gustav Adolf, m. Princess Sibylla
23 Sigvard
24 Queen Ingrid of Denmark
25 Prince Bertil
26 Carl Johan
27 Name not known
28 Rupert, hemophiliac, d. cerebral hemorrhage at the age of 28
29 Princess Sibylla
30 Alfonso, hemophiliac, d. at the age of 31
31 Juan, healthy
32 Carrier?
33 Hemophiliac?
34 Carrier
35 Healthy male
36 Gonzalo, hemophiliac, d. at the age of 20

V: 1 Queen Elizabeth II
2 Princess Margaret
3 Edward, Duke of Kent
4 Alexandra
5 Michael
6 Prince Philip, Duke of Edinburgh
7 Non-carrier
8 -"-
9 Princess Margaretha
10 Princess Birgitta
11 Princess Desirée
12 Princess Christina
13 King Carl XVI Gustav (m. to Queen Silvia)
14 Queen Margarethe
15 Princess Benedikte
16 Queen Anna-Maria
17 King Juan Carlos of Spain (m. to Queen Sophia)
18 Name not known
19 Name not known

VI: 1 Prince Charles (m. to Princess Diana)
2 Princess Anne
3 Prince Andrew
4 Prince Edward
5 David
6 Lady Sarah
7 George
8 Helen
9 James
10 Marina
11-13 Princess Margaretha's children
14-16 Princess Birgitta's children
17-10 Princess Desirée's children
20-22 Princess Christina's children
23 Crown Princess Victoria
24 Prince Carl-Philip
25 Princess Madeleine
26 Crown Prince Frederik
27 Prince Joachim
28-30 Princess Benedikte's children
31-35 Queen Anna-Maria's children
36-38 King Juan Carlos's children

VII: 1-2 Prince Charles' children
3-4 Princes Anne's children
5-6 Prince Andrew's children

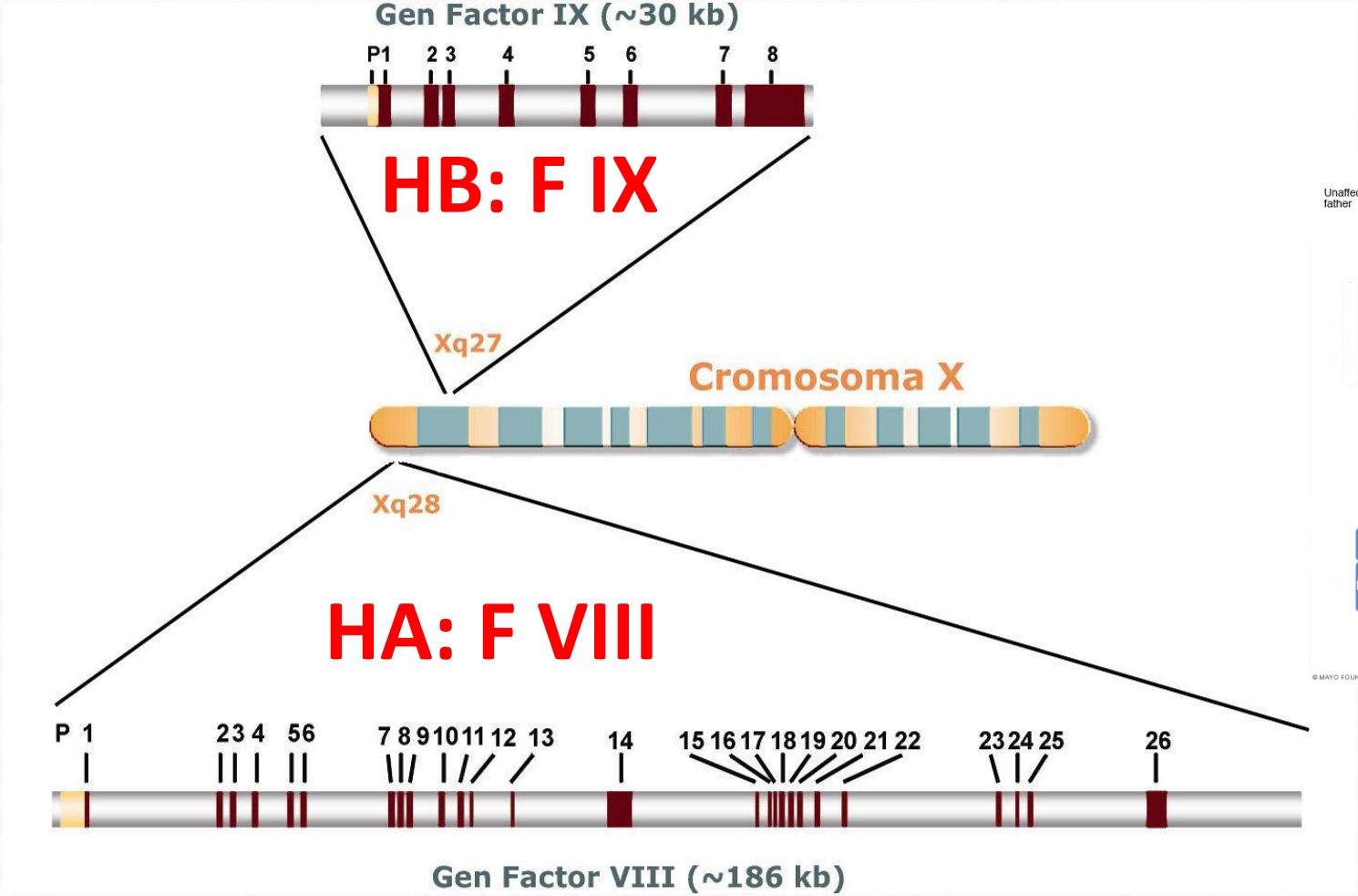
Identificación de la mutación

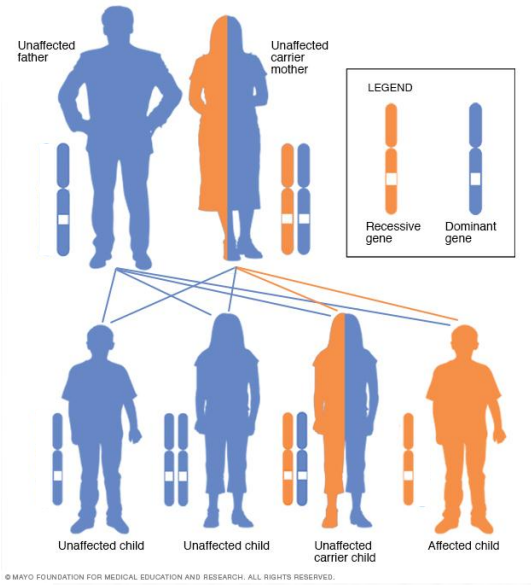


Gen F8

Gen F9

Hemofilia A o B?





HEMO-iPS

Vall d'Hebron
Institut de Recerca

2011

Use of patient-specific induced pluripotent stem cells (iPSCs) to improve diagnosis and treatment of hemophilia A

HEMO-iPS

Vall d'Hebron
Institut de Recerca


PARIS
A. Dubart & A. Weber (INSERM)
Hepatocyte differentiation

TORINO
P. Schinco. Hemophilia Unit

NOVARA
A. Follenzi. Endothelial differentiation, preclinical gene therapy




BARCELONA
F. Vidal & R. Parra (BST).
Molecular diagnosis of hemophilia
Hemophilia Unit
J. Barquinero, LL. Martorell (VHIR).
Preclinical gene therapy
A. Raya (IBEC).
Control of stem cell potency

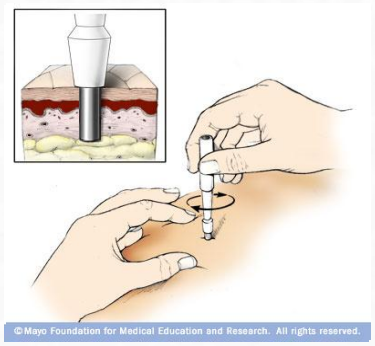
E-Rare

Selección y reclutamiento de pacientes

www.hemobase.com

Unidad de Hemofília / BST / VHIR

Biòpsia de pell



7 pacientes (HA) reclutados

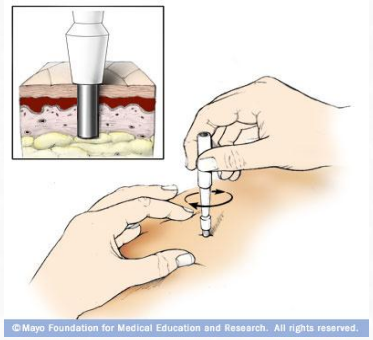
Mutation type	***Nonsense***	***Splicing***
# of patients	3	4

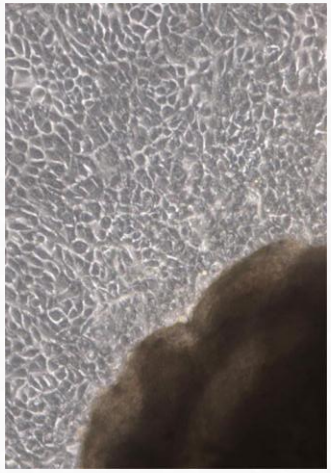
Se decide incluir también el paciente con HB y su madre

HEMO-iPS: generación de iPSCs

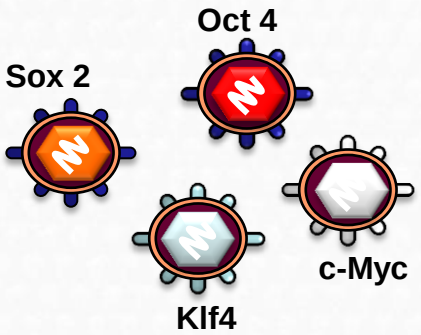
Vall d'Hebron
Institut de Recerca

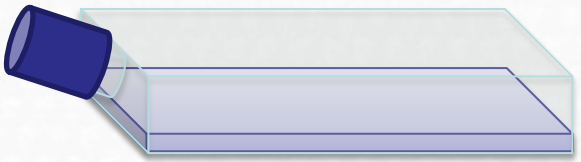
Biopsia de piel



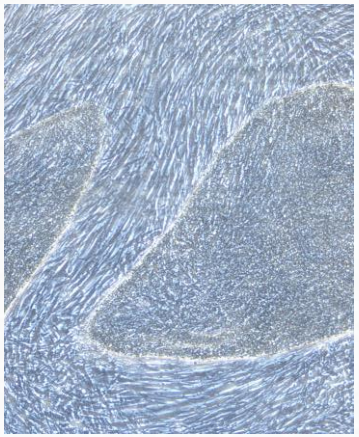


Queratinocitos
y fibroblastos





Cultivos primarios



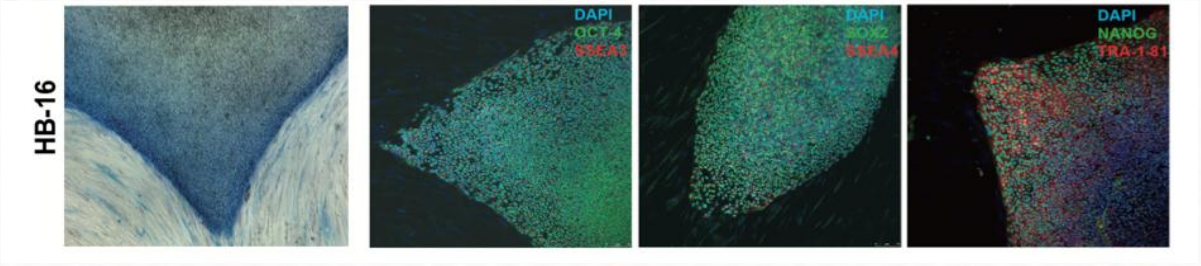
Colonias de iPSCs

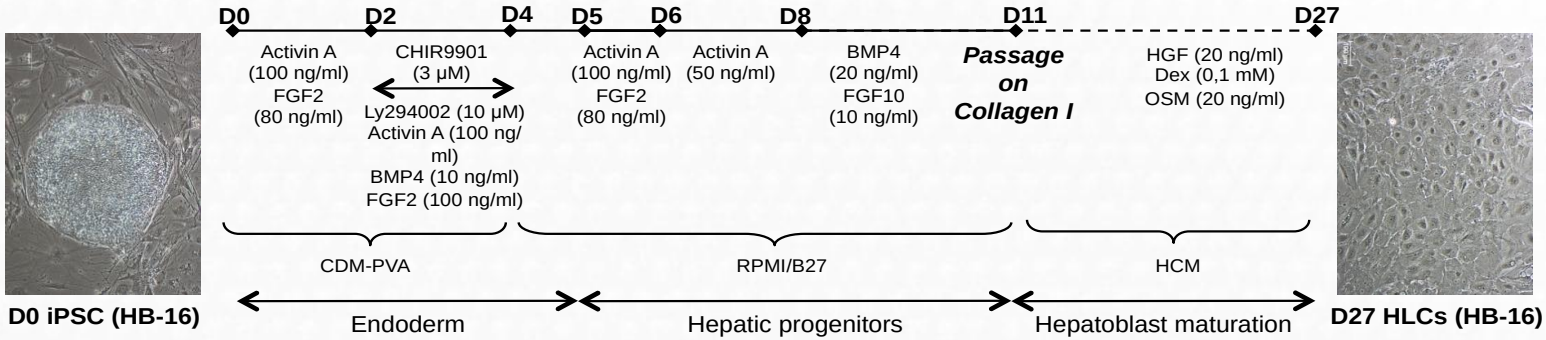
A. Raya
(CMRB)

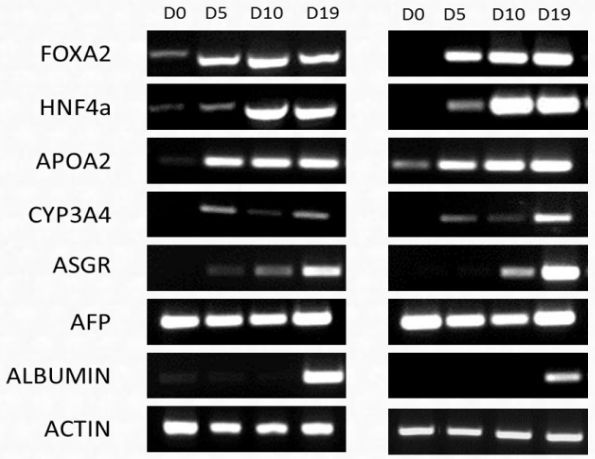
E-Rare

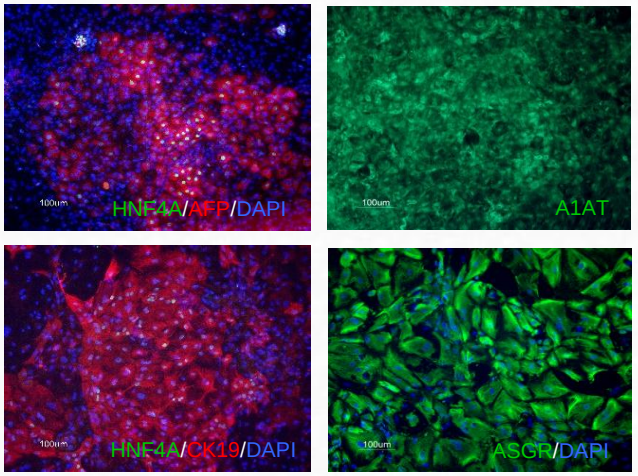
Protocolo de diferenciación a hepatocitos (HLCs)

Vall d'Hebron
Institut de Recerca





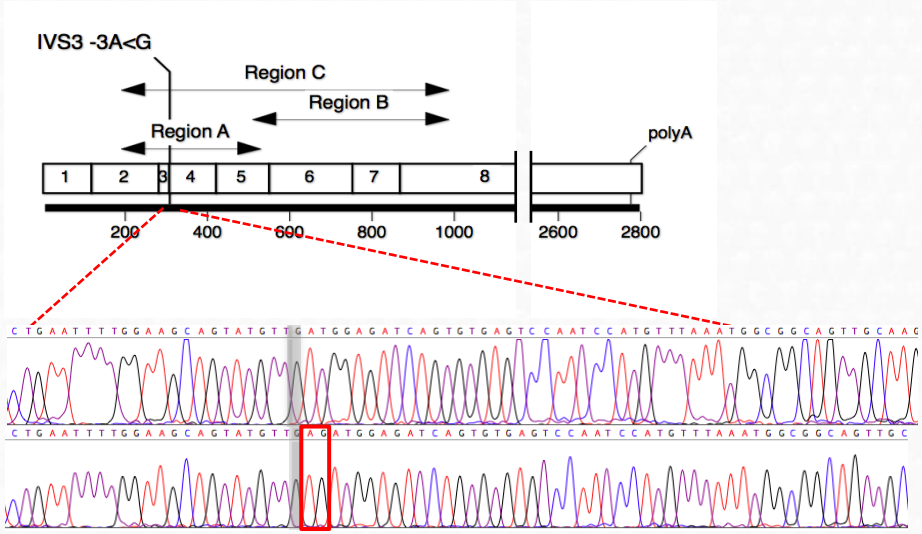




Hannan *et al*. Nat Protoc 2013; 8: 430

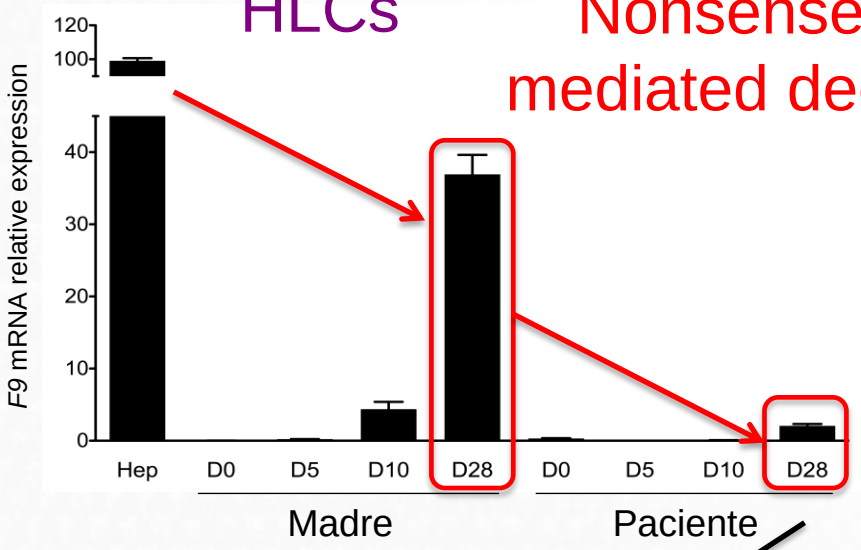
HEMO-iPSC: RESULTADOS

mutación de *splicing*

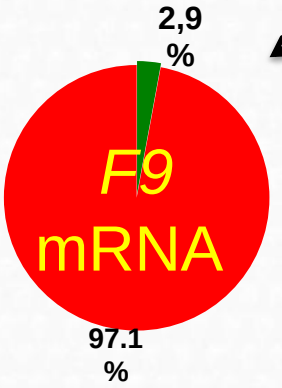


HLCs

Nonsense-mediated decay



NGS



wt

mutado

Martorell L *et al*. 2017

Vall d'Hebron
Institut de Recerca

Journal of Thrombosis and Haemostasis, **15**: 2188–2197 DOI: 10.1111/jth.13808

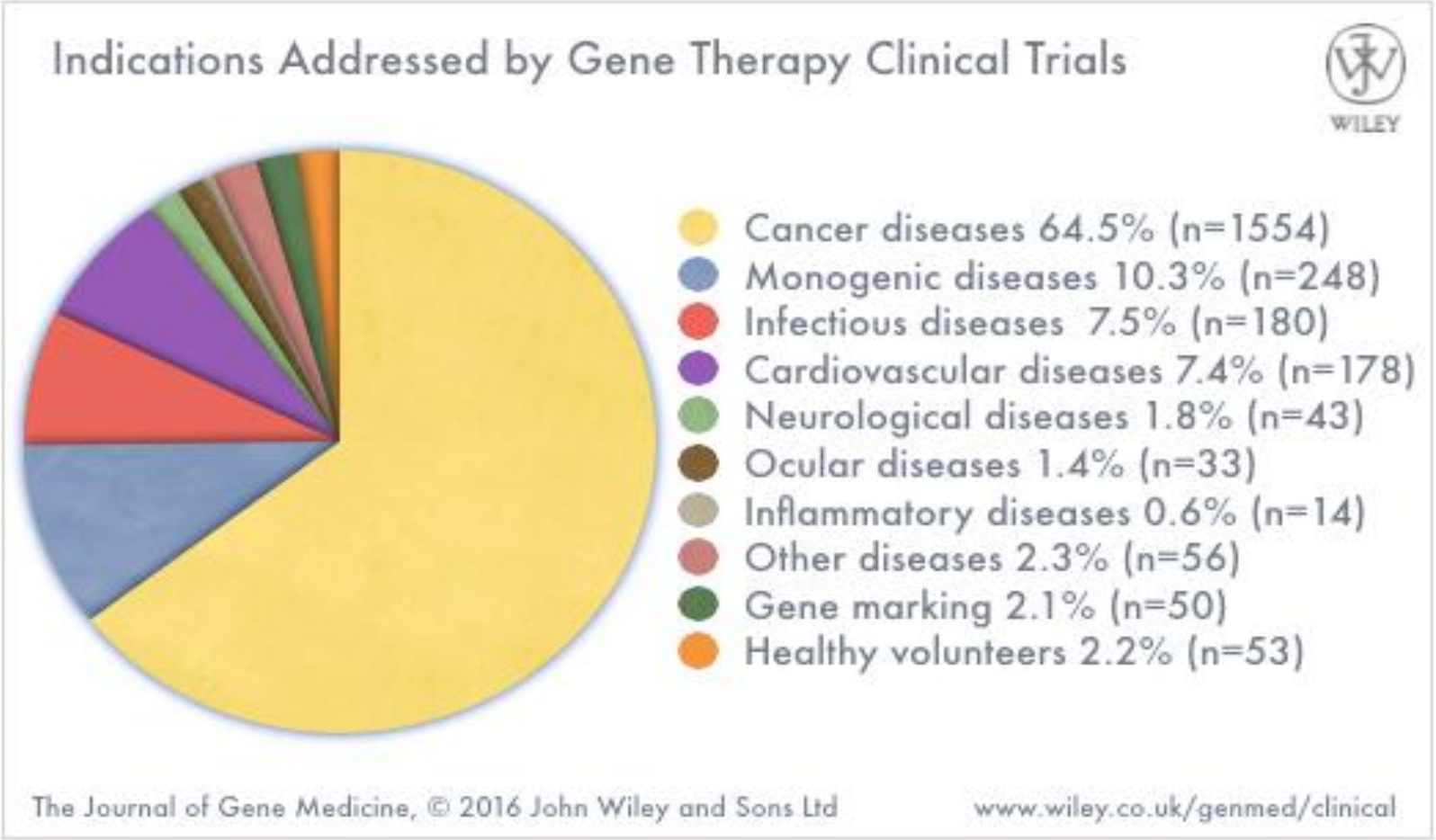
ORIGINAL ARTICLE

Advanced cell-based modeling of the royal disease: characterization of the mutated *F9* mRNA

L. MARTORELL,*†‡ E. LUCE,§¶** J.L. VAZQUEZ,†† Y. RICHAUD-PATIN,††
S. JIMENEZ-DELGADO,†† I. CORRALES,† N. BORRAS,† S. CASACUBERTA-SERRA,* A. WEBER,§¶**
R. PARRA,‡‡‡ C. ALTISENT,‡‡ A. FOLLENZI,§§ A. DUBART-KUPPERSCHMITT,§¶**
A. RAYA,††¶¶*** F. VIDAL†‡††† and J. BARQUINERO*

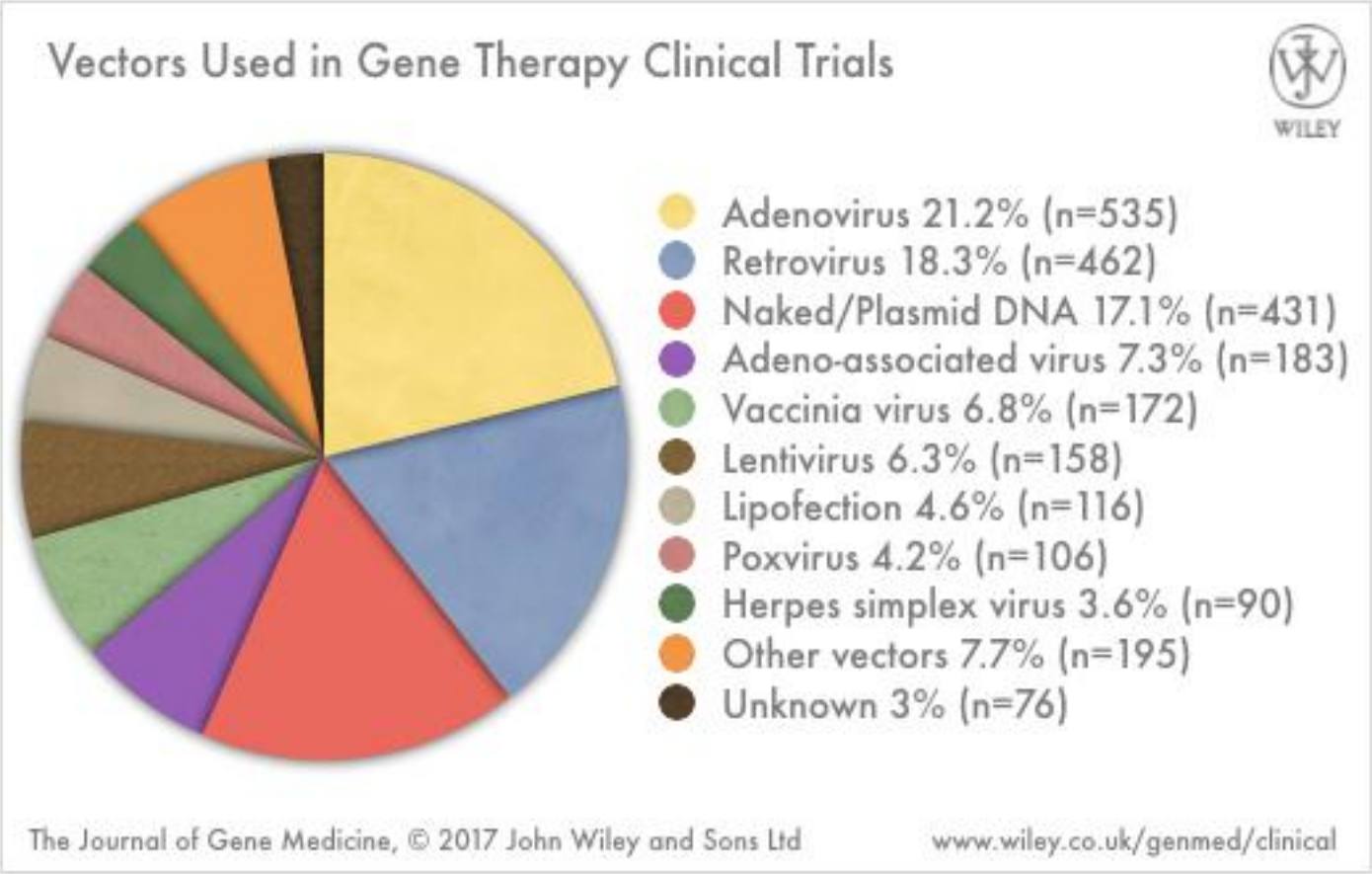
**Gene and Cell Therapy Laboratory, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona; †Congenital Coagulopathies Laboratory, Blood and Tissue Bank (BST); ‡Molecular Diagnosis and Therapy Unit, VHIR-UAB, Barcelona, Spain; §INSERM Unité Mixte de Recherche (UMR_S) 1193; ¶Université Paris-Sud; **Département Hospitalo-Universitaire Hepatinov, Paul Brousse Hospital, Villejuif, France; ††Center of Regenerative Medicine in Barcelona (CMRB); ‡‡Hemophilia Unit, Vall d'Hebron University Hospital, Barcelona, Spain; §§University of Piemonte Orientale, Novara, Italy; ¶¶Catalan Institution for Research and Advanced Studies (ICREA), Barcelona; ***Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain; and †††Biomedical Research Networking Center on Cardiovascular Diseases, Madrid, Spain*

Terapia génica: ensayos clínicos (2017)



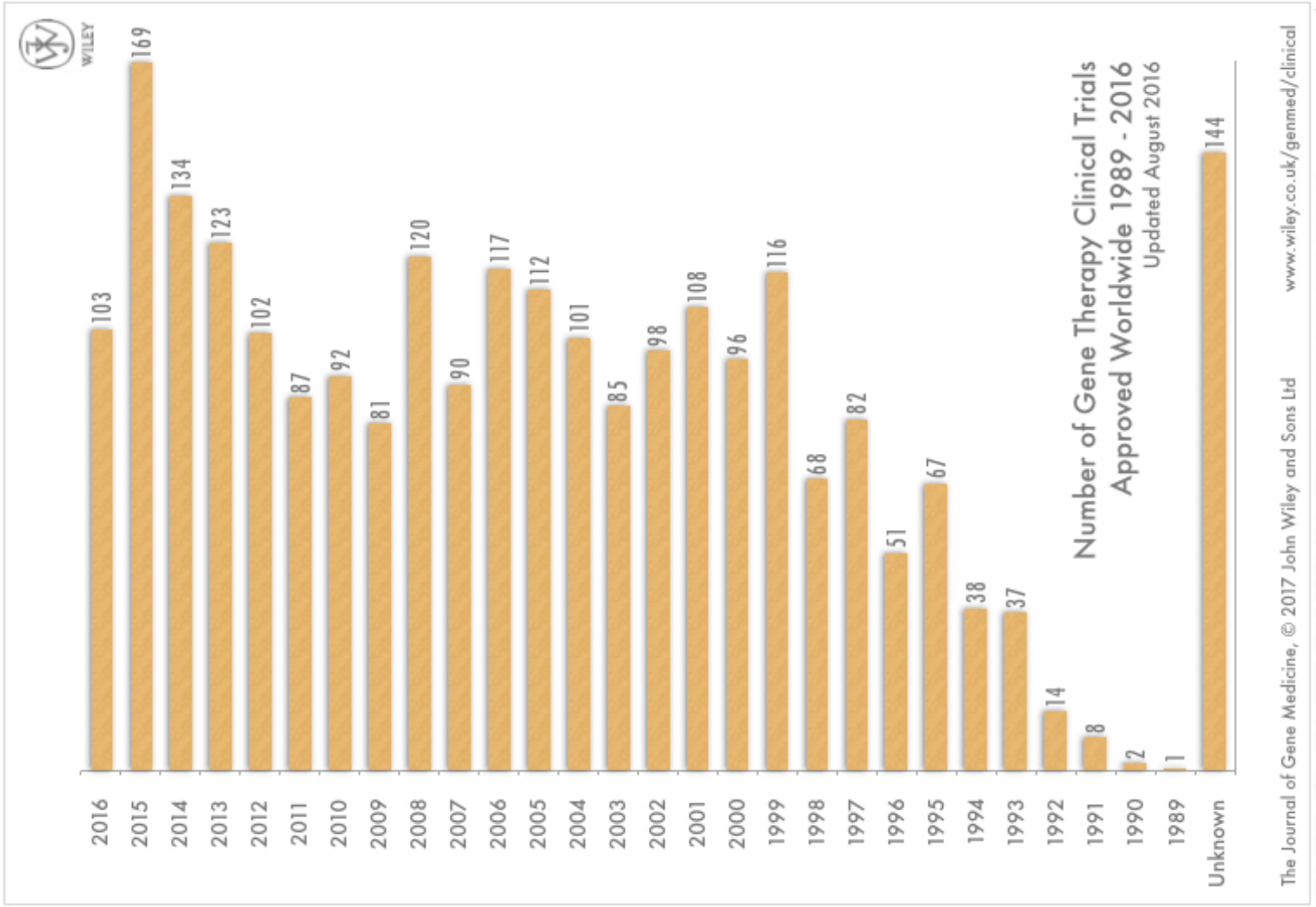
http://www.wiley.com/legacy/wileychi/genmed/clinical/

Terapia génica: vectores utilizados (2017)



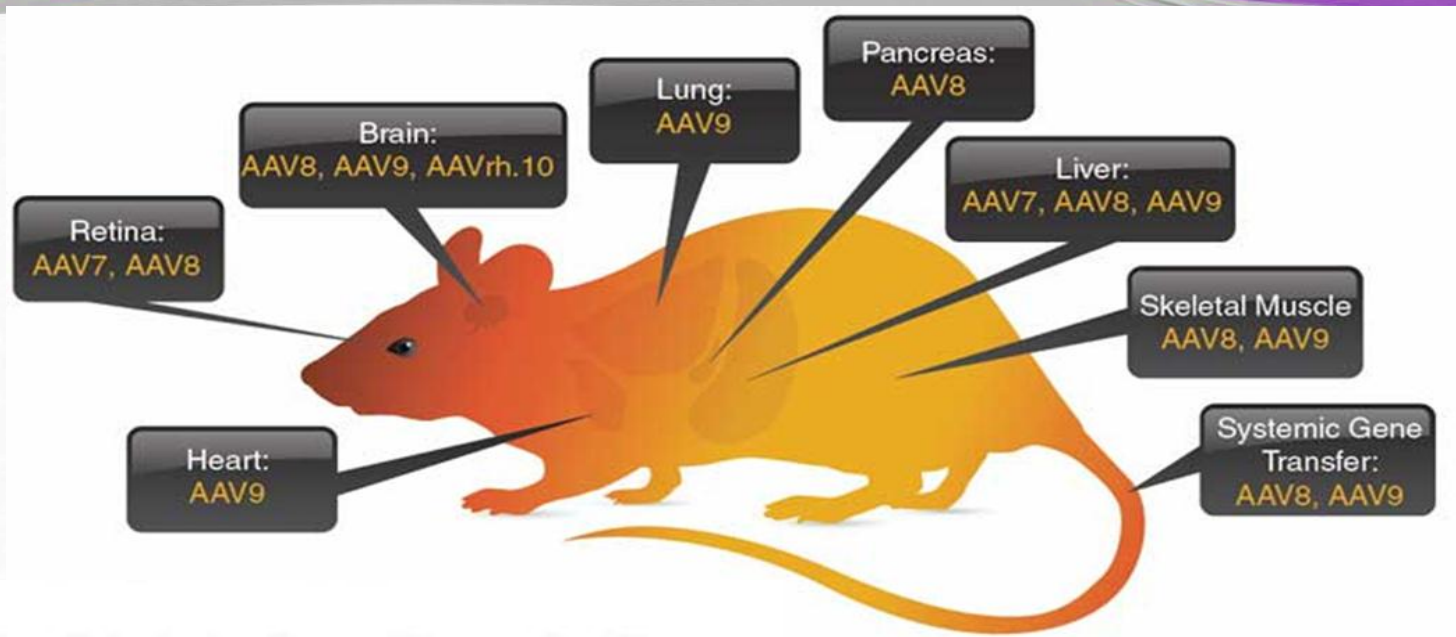
http://www.wiley.com/legacy/wileychi/genmed/clinical/

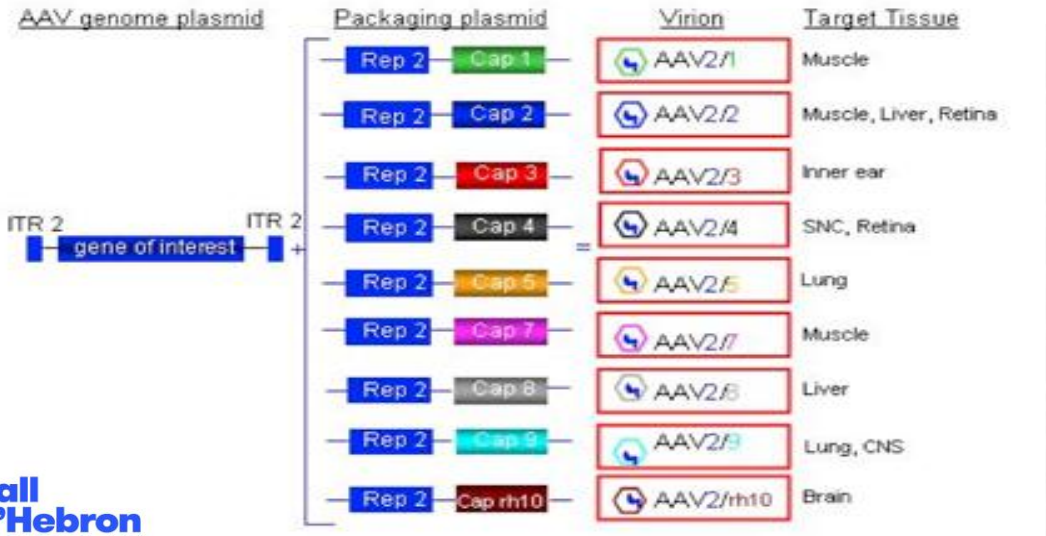
Terapia génica: ensayos por año



http://www.wiley.com/legacy/wileychi/genmed/clinical/

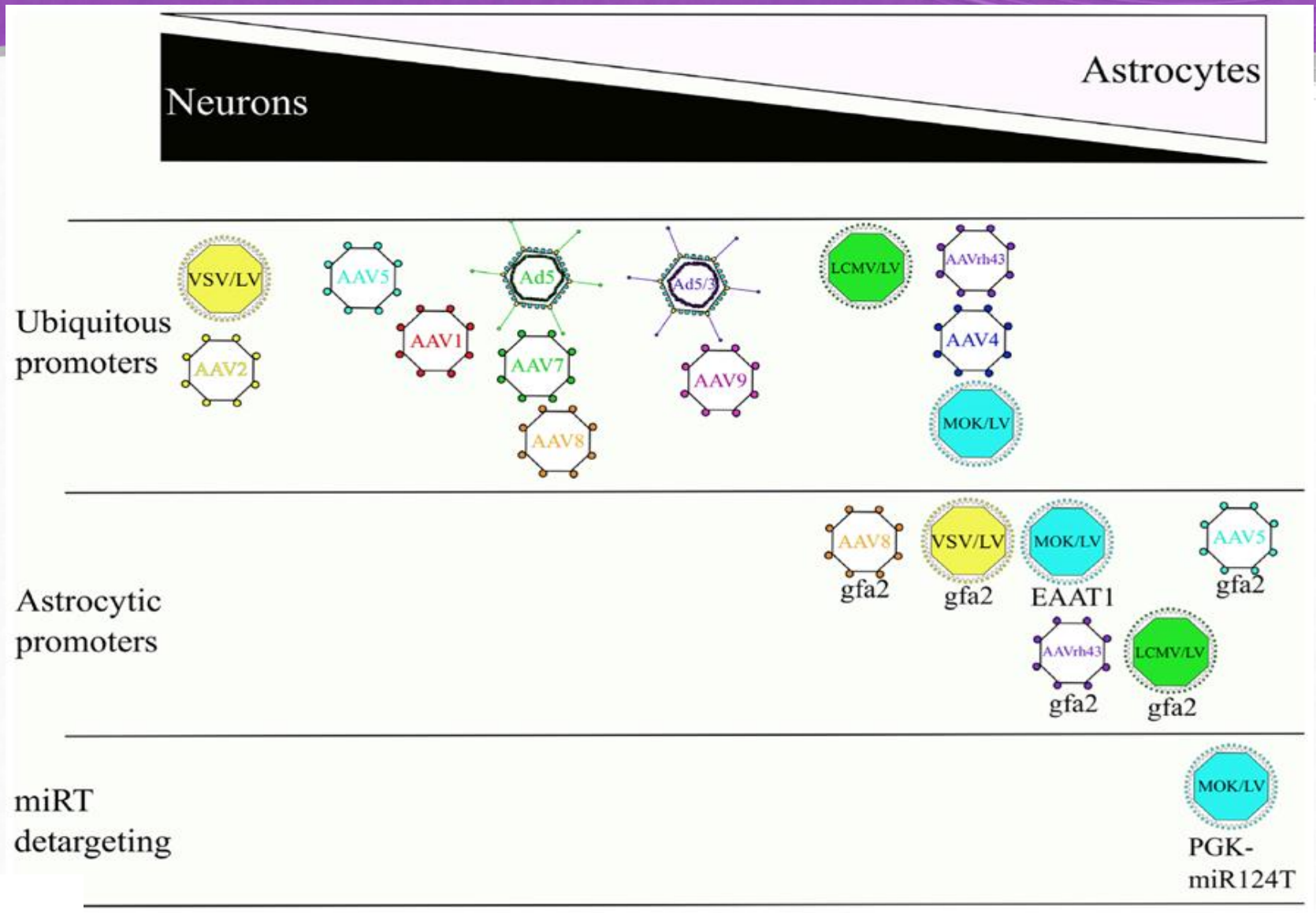
Targeting in GT: exploiting natural tropisms of vectors





Karen Kozarsky (ReGenX Biosciences)

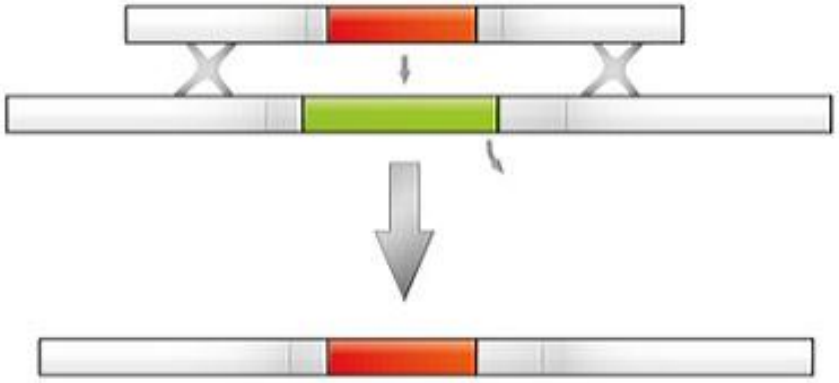
Transcriptional targeting in GT



TG: adición vs. edición

Genome editing

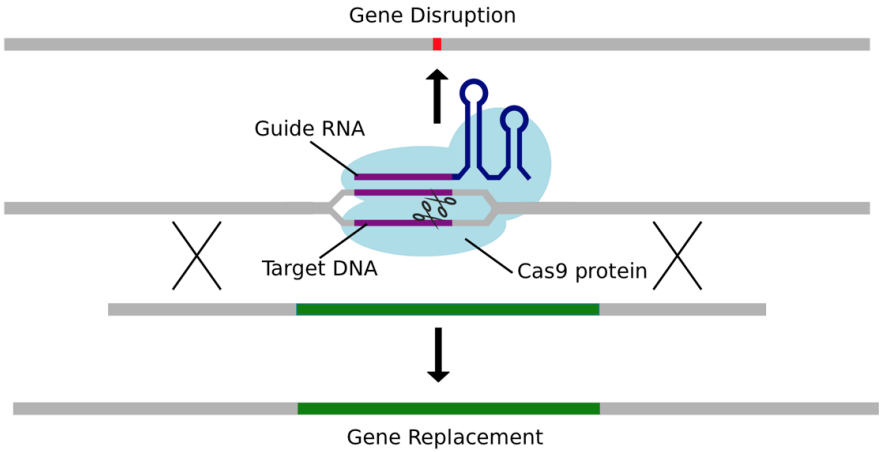
Homologous recombination



(+) Endogenous gene regulation

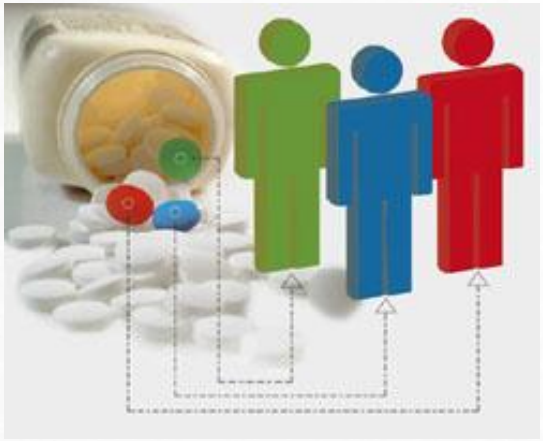
(-) Generally low efficiency

Can be stimulated through induction of Double Strand Breaks (DSBs)



Retos en TG para enfermedades minoritarias

- Hay más de 7000
- Modelo de mercado



1,4 millones de €

Vall d'Hebron
Institut de Recerca

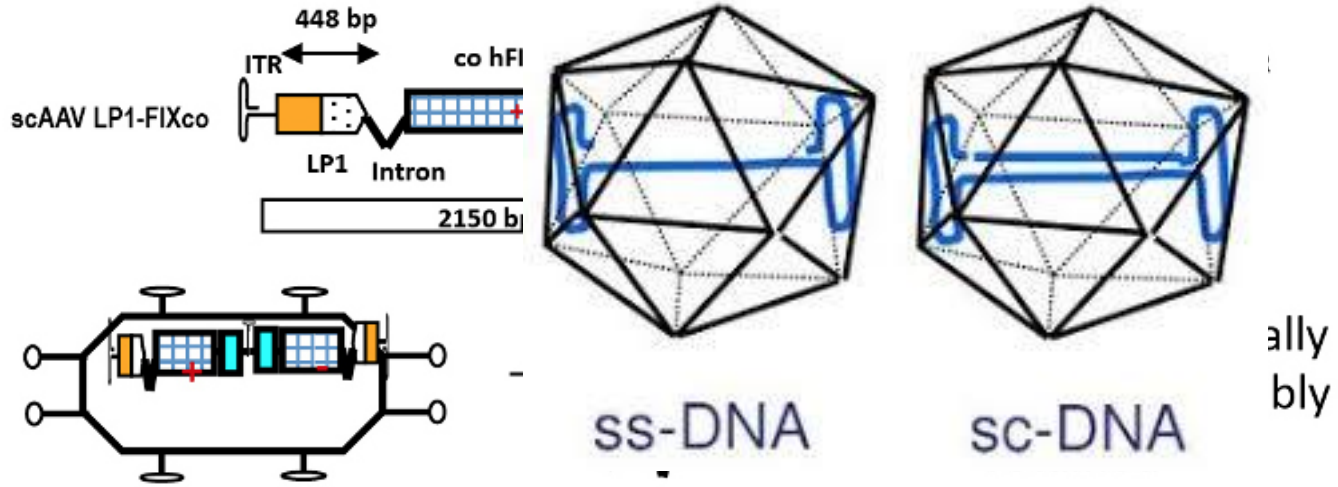
Excesiva regulación


SE HACE CAMINO AL ANDAR · COM

scAAV vectors

Vall d'Hebron
Institut de Recerca

Adeno Associated Virus (AAV)

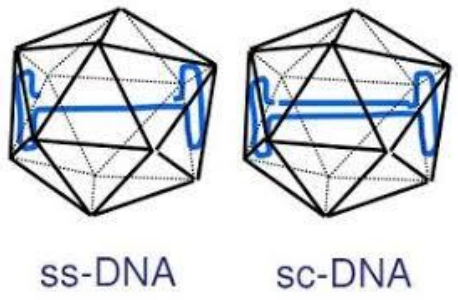


Alternative serotype (AAV8) with lower serum prevalence than wild type and a high tropism for the liver

Liver specific promoter

Codon optimisation of the FIX transgene to increase expression

Self-complementary from of the virus to over a rate limiting second strand synthesis



Hemofilia: modelos animales

Murino

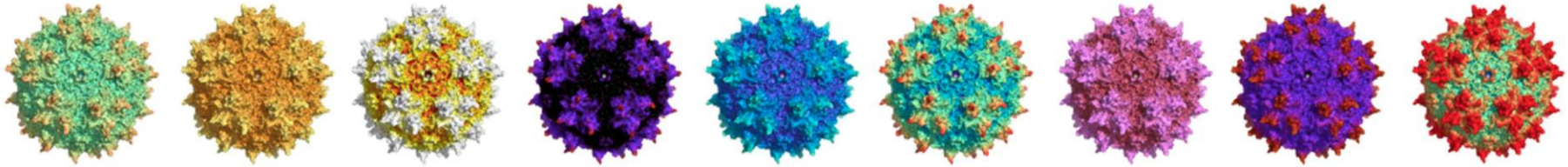
Hemofilia A: disrupción exón 16 ó 17 (neo)

Hemofilia B: disrupción promotor o exón 8 (neo)

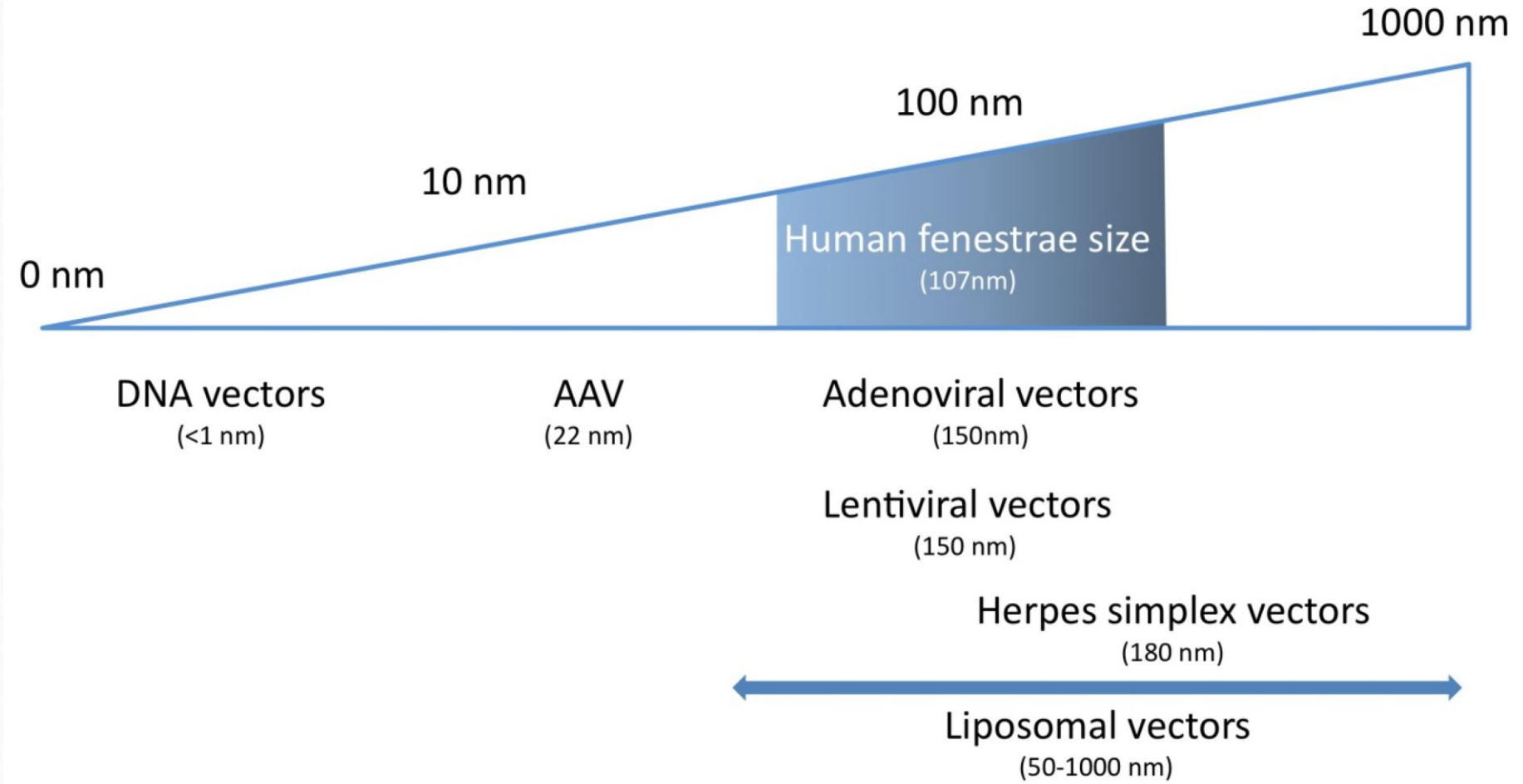
Canino

- Hemofilia A
  - Perro Chapel Hill: setter irlandés
  - Perro U. Queen's: Schnauzer miniatura

- Hemofilia B
  - Perro Chapel Hill: Keagle
  - Perro U. Auburn: Lhasa Apso



	rAAV1	rAAV2	rAAV3	rAAV4	rAAV5	rAAV6	rAAV7	rAAV8	rAAV9
Primary receptor	N-linked sialic acid	HSPG	HSPG	O-linked sialic acid	N-linked sialic acid	N-linked sialic acid; HSPG	unknown	unknown	N-linked galactose
Secondary receptor	unknown	FGFR1, HGFR, integrins, CD9, LamR	FGFR1, HGFR, LamR	unknown	PDGFR	EGFR	unknown	LamR	LamR


1000 nm
100 nm
10 nm
0 nm
Human fenestrae size
(107nm)
DNA vectors
(<1 nm)
AAV
(22 nm)
Adenoviral vectors
(150nm)
Lentiviral vectors
(150 nm)
Herpes simplex vectors
(180 nm)
Liposomal vectors
(50-1000 nm)