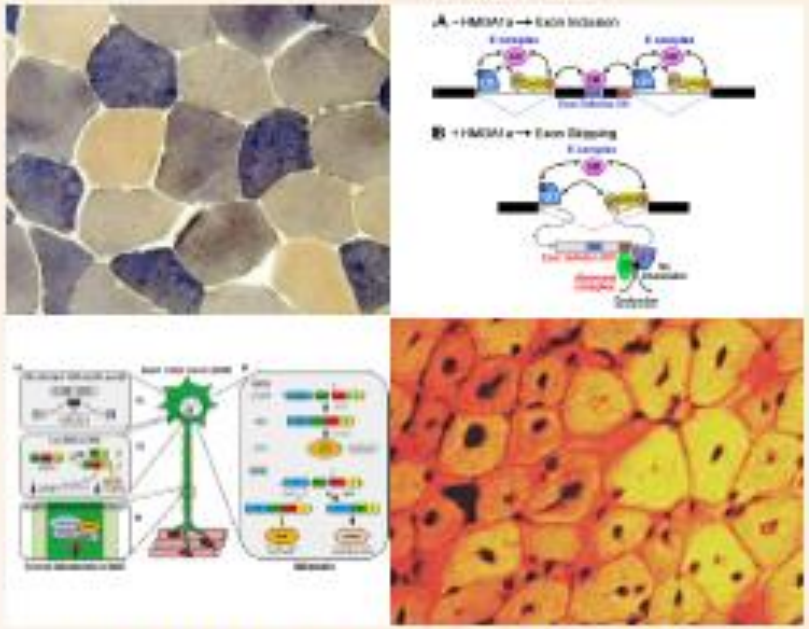
Hospital Universitario Ramón y Cajal

Enfermedades Musculares en la Infancia y Adolescencia(XV)

Organizado por:

Perspectivas terapéuticas en las enfermedades neuromusculares: diferentes mecanismos de intervención.

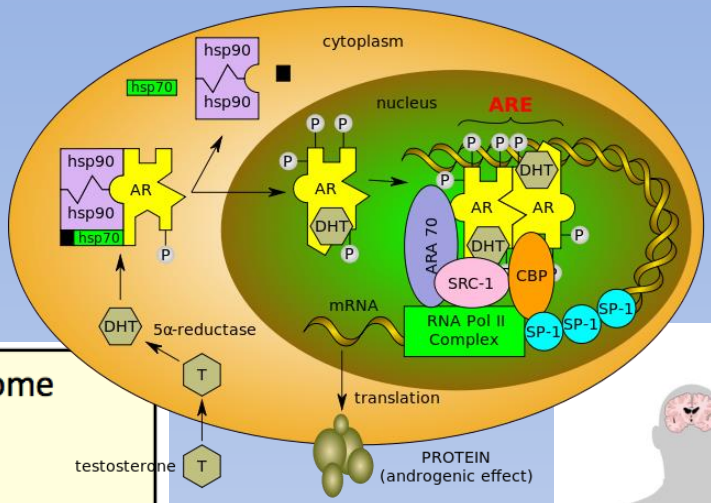
A. Jiménez Escrig. Servicio de Neurología. Hospital Universitario Ramón y Cajal

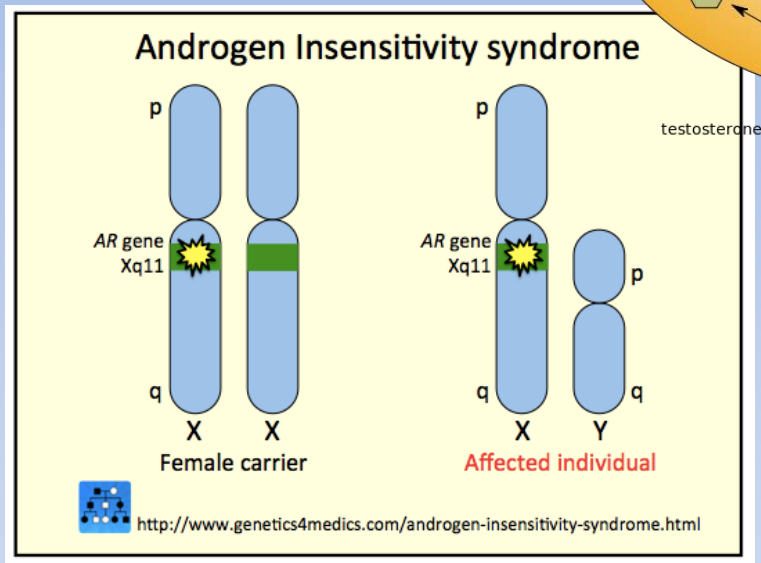


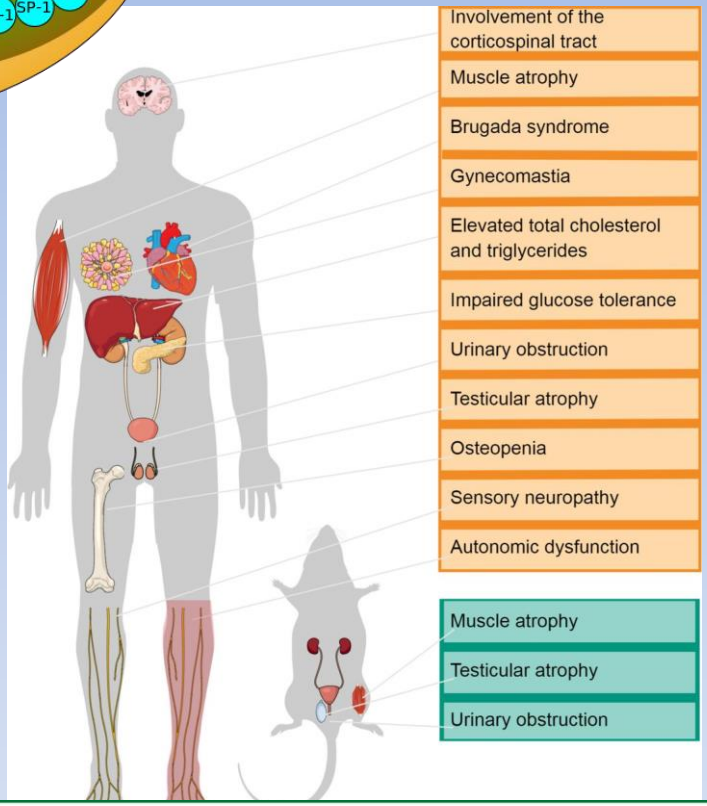
Servicio de Pediatría. B. García Cuartero. Jefe de Servicio

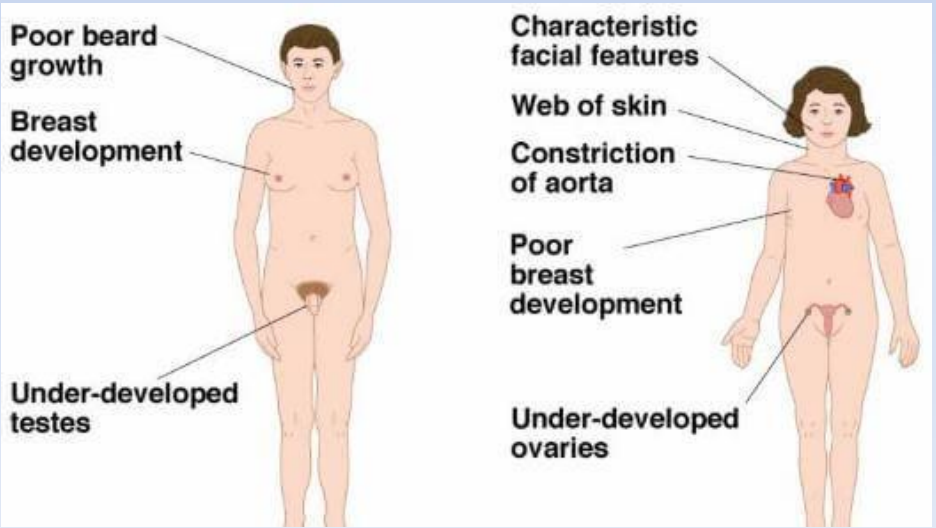
5 y 6 de Abril de 2018

Salón de Actos. Planta 0 centro.
Hospital Universitario Ramón y Cajal









REVIEW

Beyond motor neurons: expanding the clinical spectrum in Kennedy's disease

Raquel Manzano,[1] Gianni Sorarú,[2] Christopher Grunseich,[3] Pietro Fratta,[4] Emanuela Zuccaro,[5] Maria Pennuto,[5,6] Carlo Rinaldi[1]

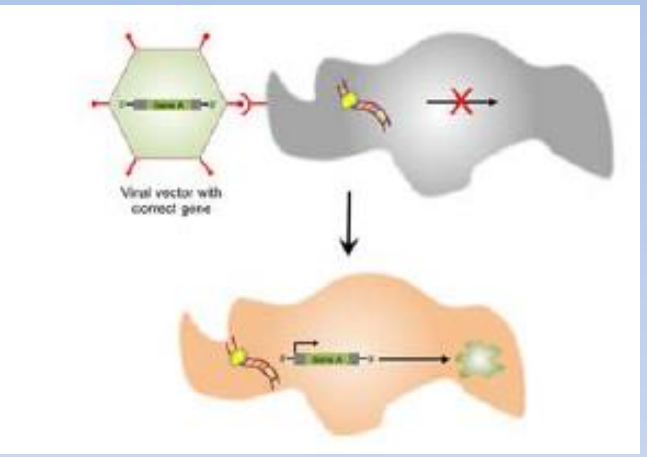
TERAPIA GENICA

REEMPLAZO

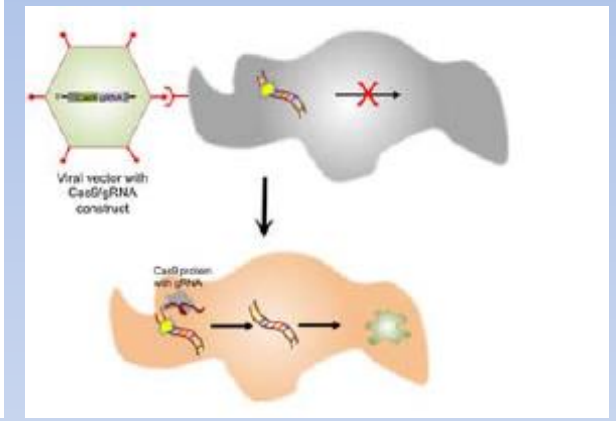
EDICION

MODIFICACION POSTRANSCRIPCION

APORTE SUSTITUTIVO y OTROS

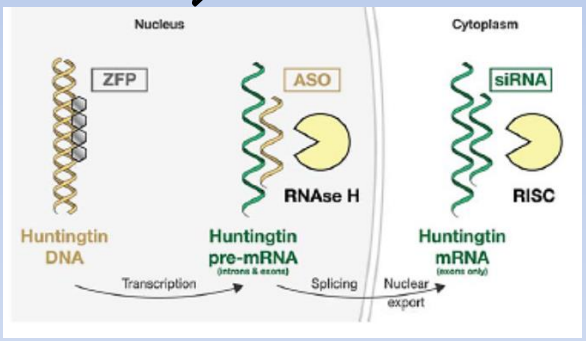


GEN



CRISPR

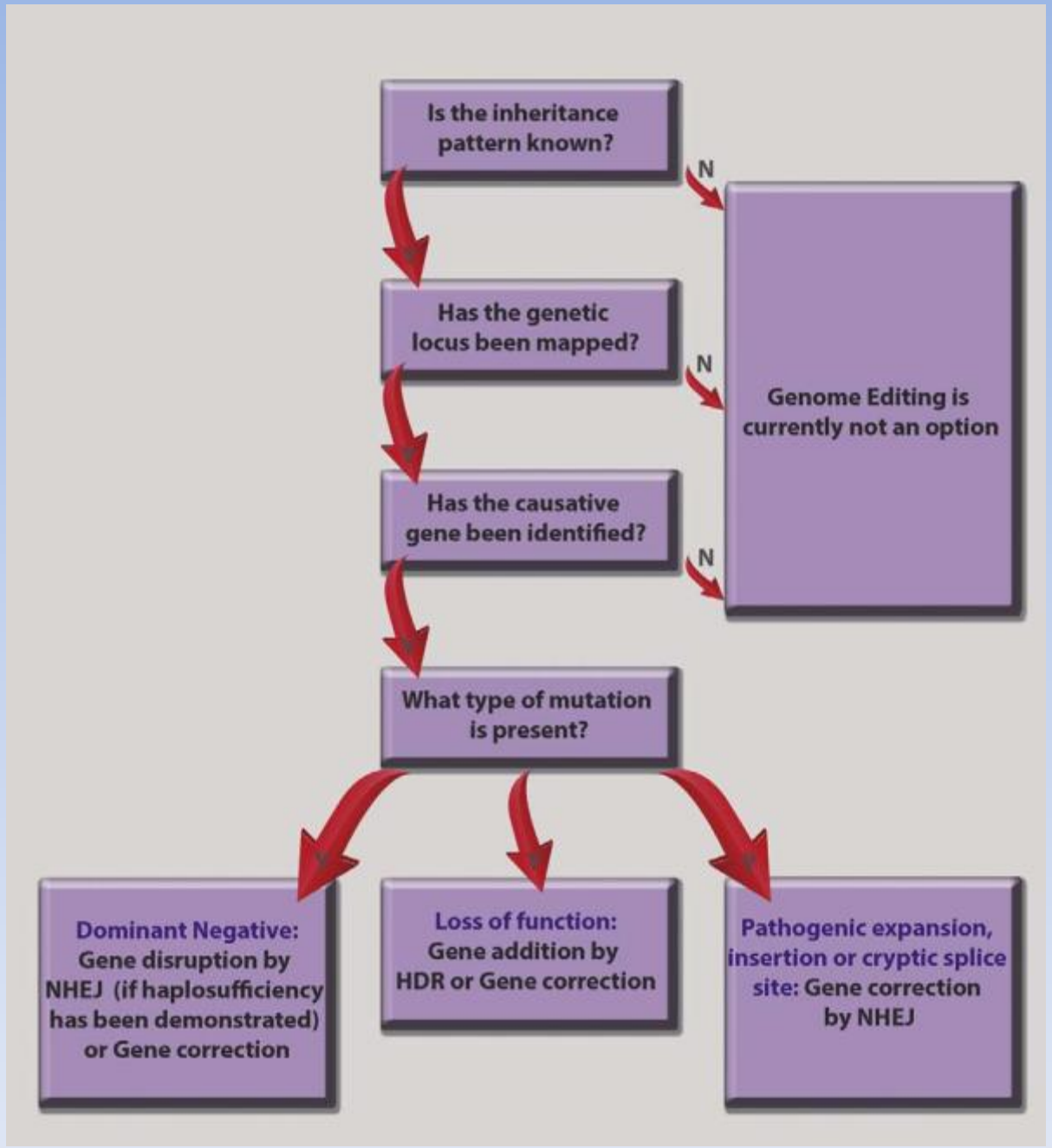
TALEN, ZINC FINGER NUCLEASES



ASO

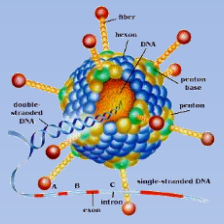
RNAi (siRNA, miRNA)

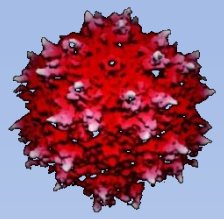
ASO

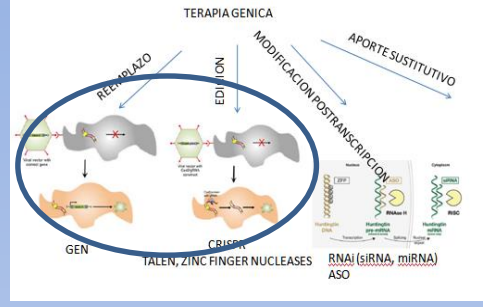

Is the inheritance pattern known?
N
Has the genetic locus been mapped?
N
Genome Editing is currently not an option
Has the causative gene been identified?
N
What type of mutation is present?
Dominant Negative: Gene disruption by NHEJ (if haplosufficiency has been demonstrated) or Gene correction
Loss of function: Gene addition by HDR or Gene correction
Pathogenic expansion, insertion or cryptic splice site: Gene correction by NHEJ

VECTORES:

NO VIRALES: endocitosis, microinyección, liposomas, electroforacion

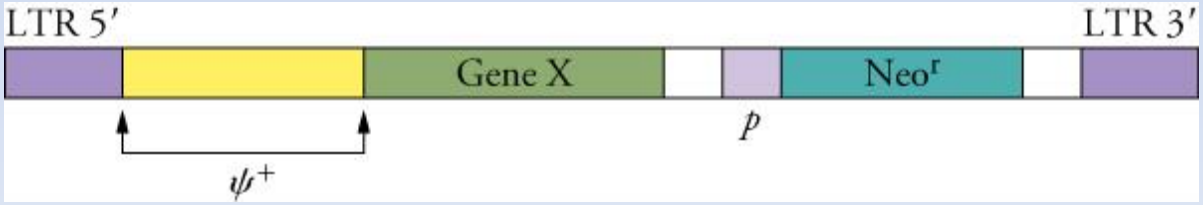






VIRALES	Adenovirus	AAV	Retrovirus	Lentivirus
Genome Type	dsDNA	ssDNA	ssRNA	ssRNA
Host genome integration	No	Reported at a very low frequency	Yes	Yes (integrase deficient versions available)
Transgene expression	Days/weeks	Months/years	Months/years	Months/years
Immunogenicity	High	Low	Moderate-high	Low-moderate
Packaging capacity	< 7.5kb	< 5kb	< 8kb	< 8kb

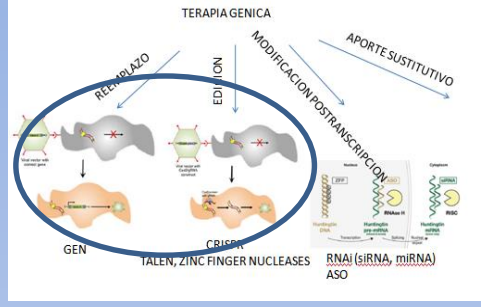
Vector retroviral



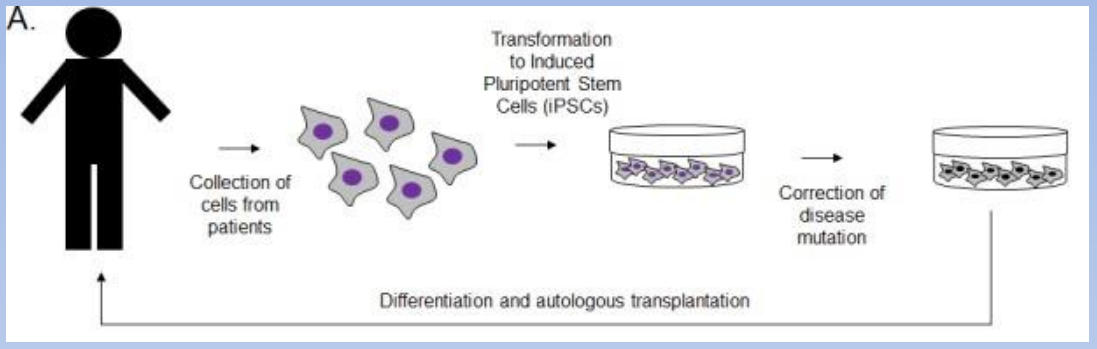
máximo: 8Kb

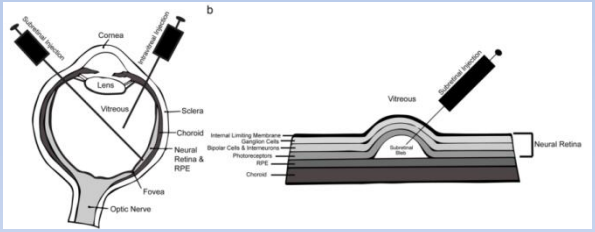
Sustituir gag, pol, env con gene X:
Marcador: gene Neo

TIPO DE TERAPIA

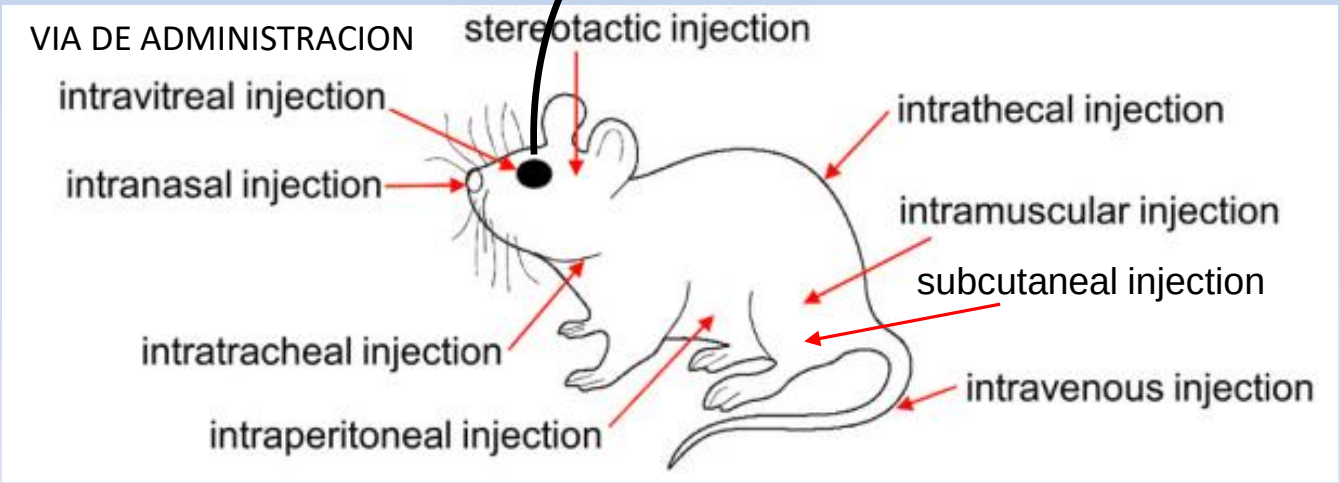


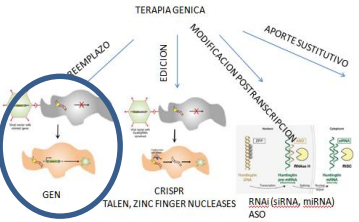
EXVIVO





INVIVO





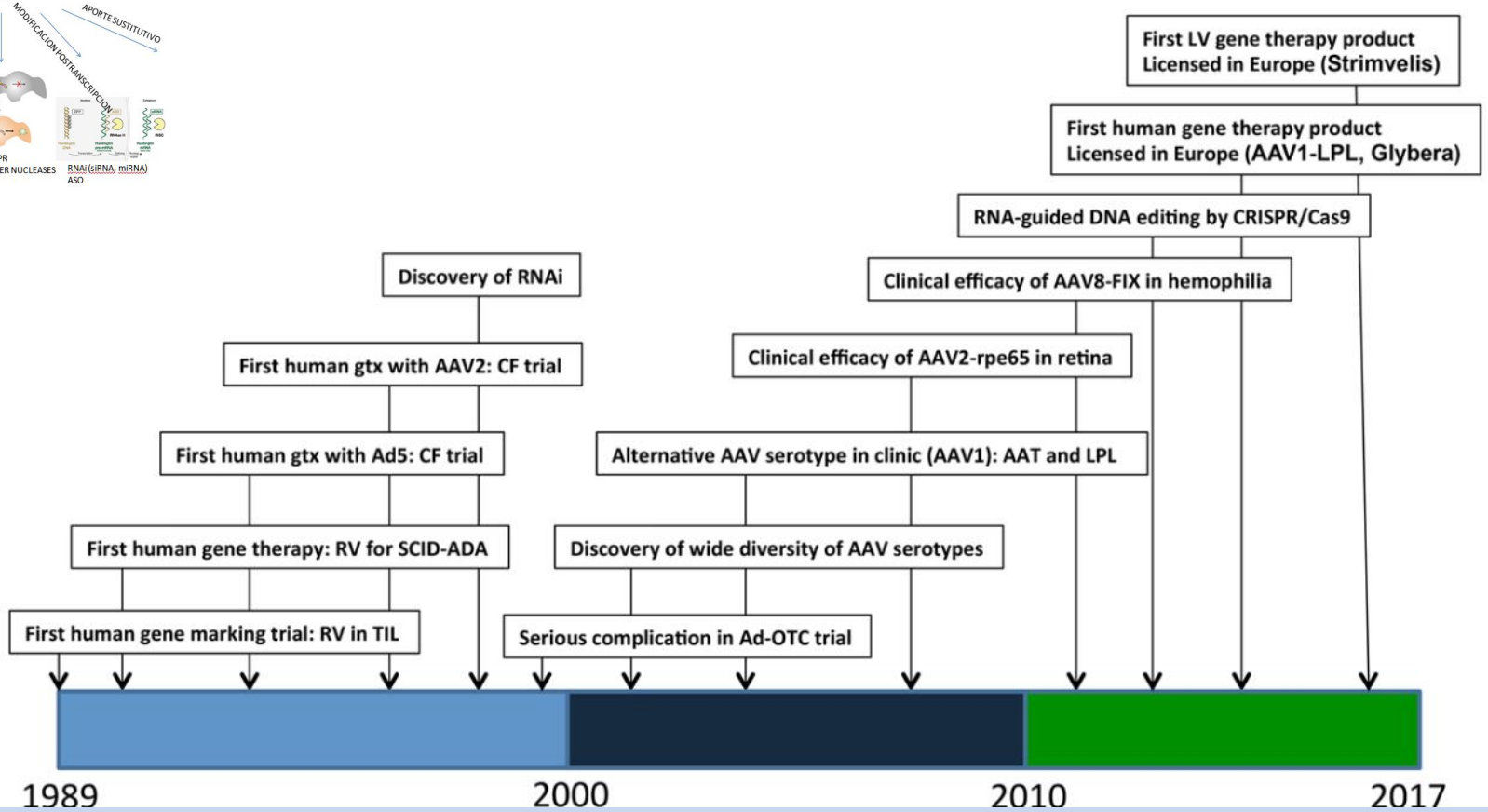
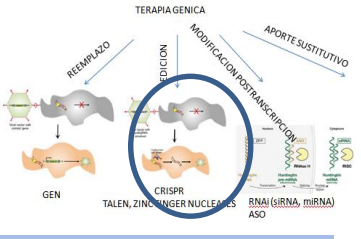


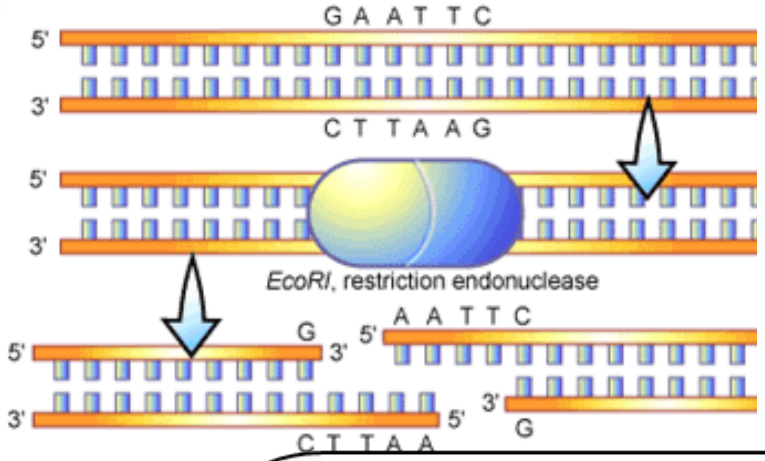
Table 1 Gene therapy clinical trials for monogenic diseases in phases III/IV

Disease	Vector	Outcomes	Location of the Trial
LPLD	AAV-Glybera	Improved lipid profile and decreased pancreatitis	The United States
X-linked adrenoleukodystrophy	Lentivirus	Improved neurologic development	France
Thalassemia major	Lentivirus	Decreased transfusion need	The United States
X-linked/ choroideremia rentinal disease (REP1)	AAV2	Improved vision	The United States- multiple countries
LCA	AAV2	Improved low-light vision	The United States
Leber hereditary optic neuropathy	AAV2	Improved vision	France - multiple countries including US

AAV, adeno-associated virus; LCA, Leber congenital amaurosis type 2; LPLD, lipoprotein lipase deficiency.

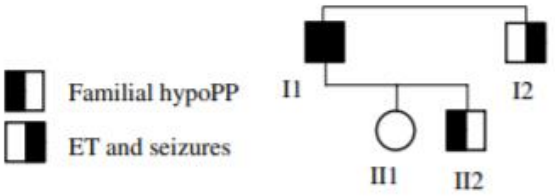
EDICION GENETICA





E. RESTRICCION

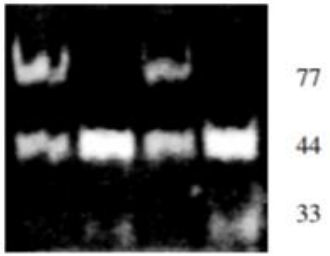
494

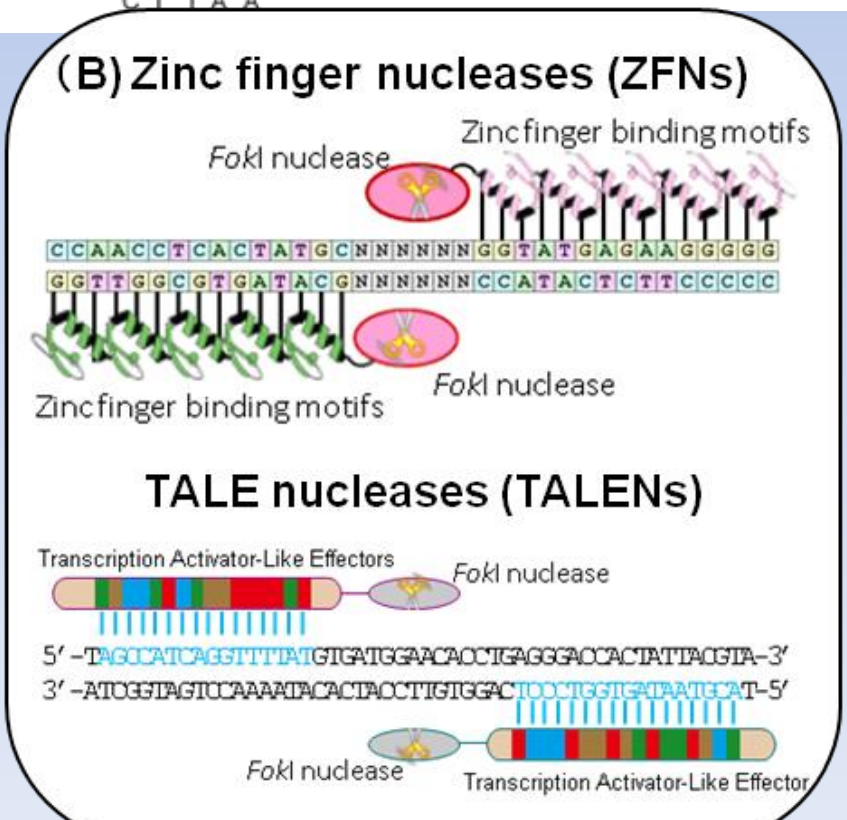


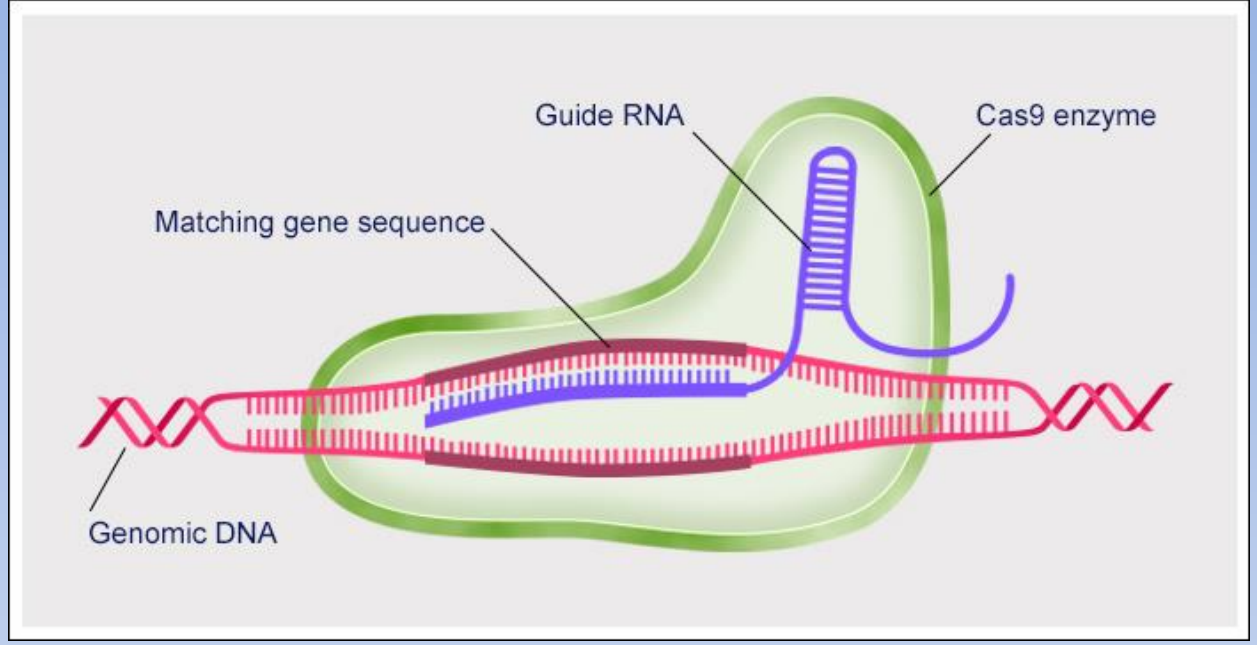
CACNL1A3

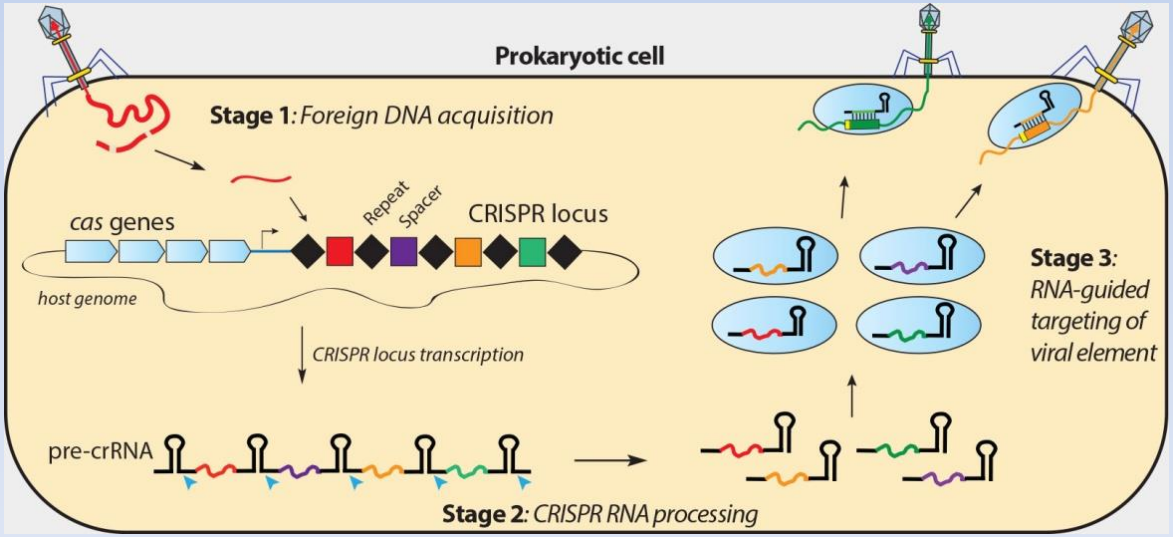
Arg528His

Bbv-I

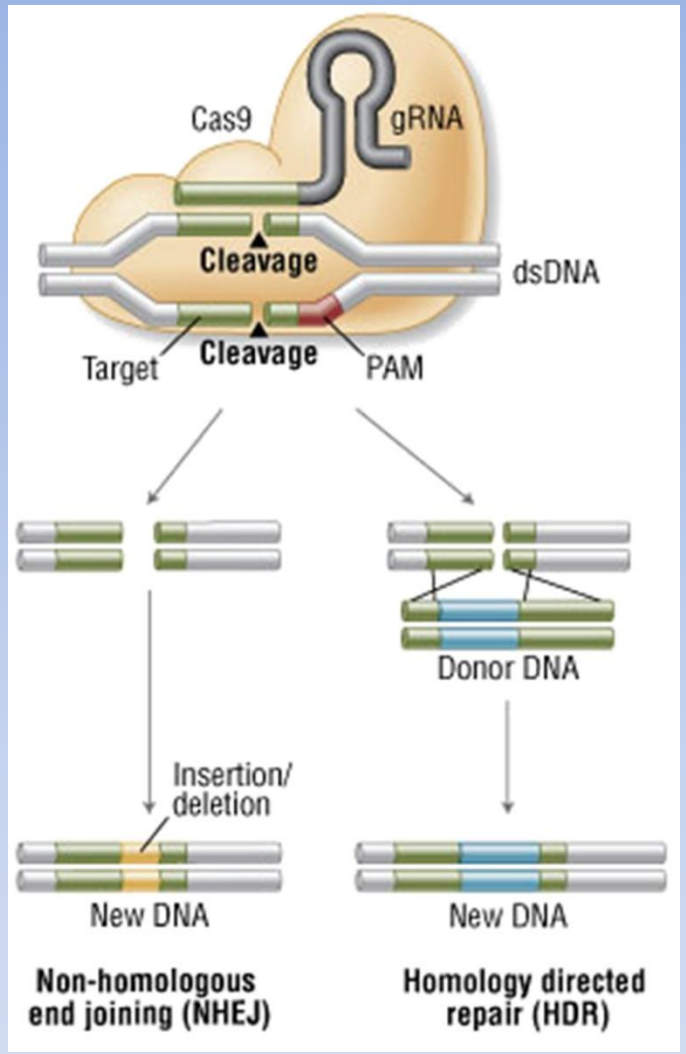




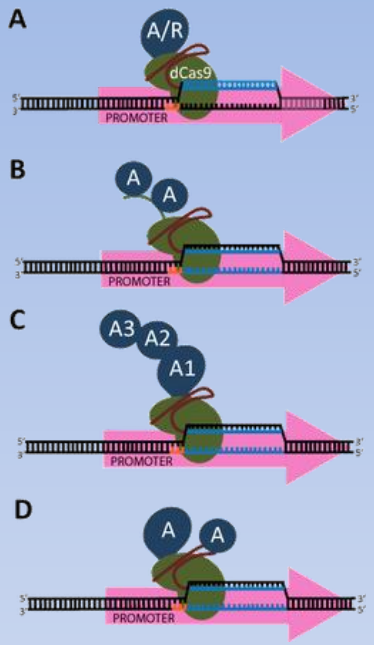

Guide RNA
Cas9 enzyme
Matching gene sequence
Genomic DNA

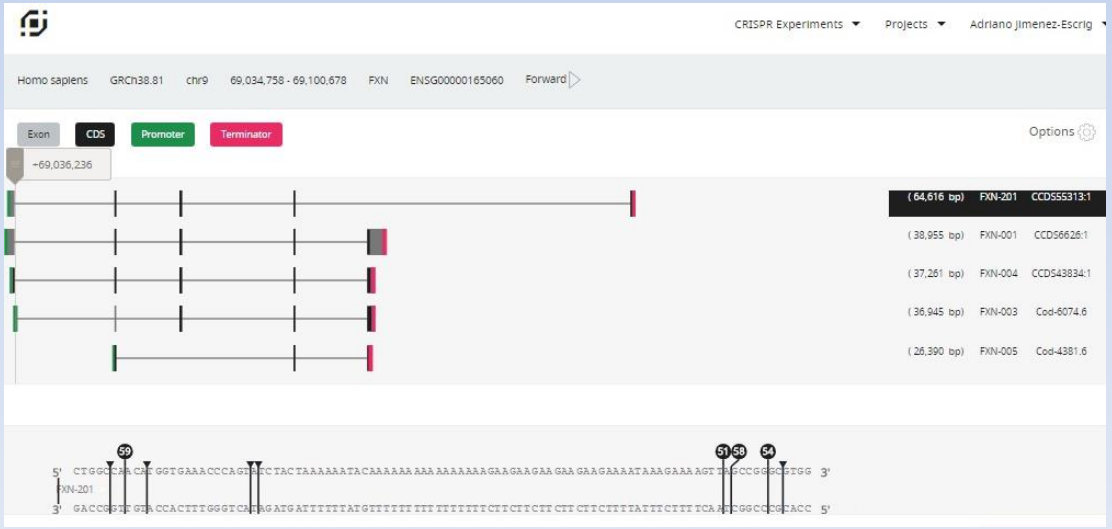

Prokaryotic cell
Stage 1: Foreign DNA acquisition
cas genes
Repeat
Spacer
CRISPR locus
host genome
CRISPR locus transcription
pre-crRNA
Stage 2: CRISPR RNA processing
Stage 3: RNA-guided targeting of viral element


Who is Who in the CRISPR-Cas World
This is a non-exhaustive collection of pictures with some of the many researchers that have contributed significantly with their studies to our current understanding of the CRISPR-Cas systems in prokaryotes and their applicaton for genome editing purposes in eukaryotes.
Francisco Mojica
University of Alicante, Spain
Rodolphe Barrangou
North Carolina State Univ, Raleigh, USA
Philippe Horvath
DuPont Nutrition and Health, France
Luciano Marraffini
The Rockefeller Univ., New York, USA
John van der Oost
Wageningen University, The Netherlands
Emmanuelle Charpentier
MPI for Infect. Biol., Berlin, Germany
Jennifer Doudna
Univ California Berkeley, CA, USA
Virginijus Siksnys
Vilnius University, Lithuania
Feng Zhang
BROAD-MIT, Cambridge, MA, USA
George Church
Harvard Med School, Boston, MA, USA
Rudolf Jaenisch
Whitehead Inst, Cambridge, MA, USA
J. Keith Joung
Mass Gen Hosp, Charlestown, MA, USA



ACTIVACION / SUPRESION DE LA EXPRESION GENICA





Gene Therapy (2017), 1–10

www.nature.com/gt

ORIGINAL ARTICLE

Deletion of the GAA repeats from the human frataxin gene using the CRISPR-Cas9 system in YG8R-derived cells and mouse models of Friedreich ataxia

DL Ouellet[1,2], K Cherif[1,2], J Rousseau[1,2] and JP Tremblay[1,2]

Steps in Using CRISPR/Cas	Resource	Website
Obtain the flanking sequence surrounding the mutation of interest	UCSC genome browser	https://genome.ucsc.edu
Design the guide RNA with the fewest off-target effects	CRISPR design tool	http://crispr.mit.edu/
	CasBLASTR	http://www.casblastr.org/
	Benchling	https://benchling.com/ editor
	Protospacer Workbench	http://www.protospacer.com/
Clone the guide RNA into Cas9 vector systems	Addgene	https://www.addgene.org/crispr/

"FA"

Interactive results: mouse over a guide or explore below for details

all guides

scored by inverse likelihood of offtarget binding
mouse over for details ... hide legend

- high quality guide
- mid quality guide
- low quality guide

	score	sequence	
Guide #1	90	TCCGGAGTTCAAGACTAACC	TGG
Guide #2	85	AAAGAAAAGTTAGCCGGGCG	TGG
Guide #3	74	GCCAGGTTAGTCTTGAACTC	CGG
Guide #4	62	CAGGCGCGCGACACCACGCC	CGG
Guide #5	45	TGTATTTTTTAGTAGATACT	GGG
Guide #6	43	CAAGACTAACCTGGCCAACA	TGG
Guide #7	42	TTGTATTTTTTAGTAGATAC	TGG
Guide #8	34	AAAATAAAGAAAAGTTAGCC	GGG
Guide #9	29	GATACTGGGTTTCACCATGT	TGG
Guide #10	20	GAAAATAAAGAAAAGTTAGC	CGG
Guide #11	6	TGGGTTTCACCATGTTGGCC	AGG

guide #1 quality score: 90

guide sequence: TCCGGAGTTCAAGACTAACC TGG
on-target locus: chr9:+71652127
number of offtarget sites: 47 (1 are in genes)

top 20 genome-wide off-target sites

sequence	score	mismatches
TCAGGAGTTCAAGACCAACCTGG	1.9	2MMs [3:16]
TCCAGAATTCAAAACTAACCCAG	0.7	3MMs [4:7:13]
TGGGGAGTTTAAGACTAACGAGG	0.6	4MMs [2:3:10:20]
CCCAGTGTACAAGACTAACCTAG	0.5	4MMs [1:4:6:9]
TCCTGTGGTCTAGACTAACCAAG	0.5	4MMs [4:6:8:11]
TCGAGATTTCAAGACTAACAAAG	0.5	4MMs [3:4:7:20]
TGCAGAGTCCAGGACTAACCTGG	0.4	4MMs [2:4:9:12]
TGCTGGGTTCAAGACTCACCGGG	0.4	4MMs [2:4:6:17]
TCCAGAATTTAACACTAACCAAG	0.3	4MMs [4:7:10:13]
CCAAGAGTTCAAGACTAGCCTGG	0.3	4MMs [1:3:4:18]
TGAGGAGTTCAGGACTAAACAGG	0.3	4MMs [2:3:12:19]
TCCGGAAGACAAGACTGACCTGG	0.2	4MMs [7:8:9:17]
CCCCGAGGTCAAGCCTAACCTGG	0.2	4MMs [1:4:8:14]

BIOTECNOLOGÍA

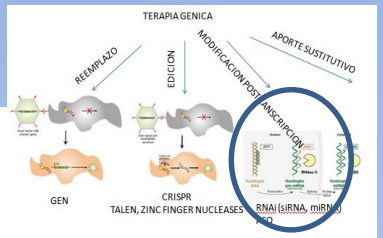
¿Tienes derecho a 'hackear' tu genoma?

Los intentos de los autodenominados "biohackers" de alterar la información genética de sus células han hecho saltar las alarmas entre las autoridades sanitarias y los expertos en bioética. Estas prácticas abren el debate sobre si existe el derecho a modificar nuestro organismo a la carta y qué consecuencias tendría.

CRISPR: ENSAYOS CLINICOS EN CURSO

Condition	Intervention	Phase	Type	Start date	Finish date
Metastatic non small cell lung cancer	*PD-1* knockout T cells from autologous origin	1	*Ex vivo*	2016	2018
Muscle-invasive bladder cancer stage IV	*PD-1* knockout T cells from autologous origin	1	*Ex vivo*	2016	2019
Hormone-refractory prostate cancer	*PD-1* knockout T cells from autologous origin	1	*Ex vivo*	2016	2020
Metastatic renal cell carcinoma	*PD-1* knockout T cells from autologous origin	1	*Ex vivo*	2016	2020
Advanced esophageal cancer	*PD-1* knockout T cells from autologous origin	2	*Ex vivo*	2017	2018
Gastric carcinoma stage IV, nasopharyngeal carcinoma stage IV, T-cell lymphoma stage IV, Hodgkin lymphoma stage IV, diff use large B-cell lymphoma stage IV	*PD-1* knockout T cells from autologous origin	1/2	*Ex vivo*	2017	2022
HIV-1-infection	*CCR5* modifed CD34+ hematopoietic stem/progenitor cells from donors	1	*Ex vivo*	2017	2021
B-cell leukemia, B-cell lymphoma	gene-disrupted allogeneic CD19-directed BBζ CAR-T cells (termed UCART019) will be generated by combining the lentiviral delivery of CAR and CRISPR RNA electroporation to disrupt endogenous *TCR* and *B2M* genes	1/2	*Ex vivo*	2017	2022
Human papillomavirus related malignant neoplasm	TALEN and CRISPR/Cas9	1	*In vivo*	2018	2019
Neurofbromatosis type 1	establish isogenic *NF1* wild-type (*NF1*+/+), *NF1* heterozygous (*NF1*+/-), and *NF1* homozygous (*NF1*-/-) patient-specifc iPSC lines using CRISPR/Cas9 technology	1	*Ex vivo*	2017	2019
Gastrointestinal infection	knockout CRISPR and gain-of-function CRISPR SAM Procedure: duodenal biopsy	1	*Ex vivo*	2018	2020

RNAi: siRNA



dsRNA

Dicer binds and cleaves dsRNA into siRNAs

Dicer

B

Direct introduction of synthetic siRNAs (~21 nt) into cells

RISC

siRNA-mediated target recognition

RISC

RISC

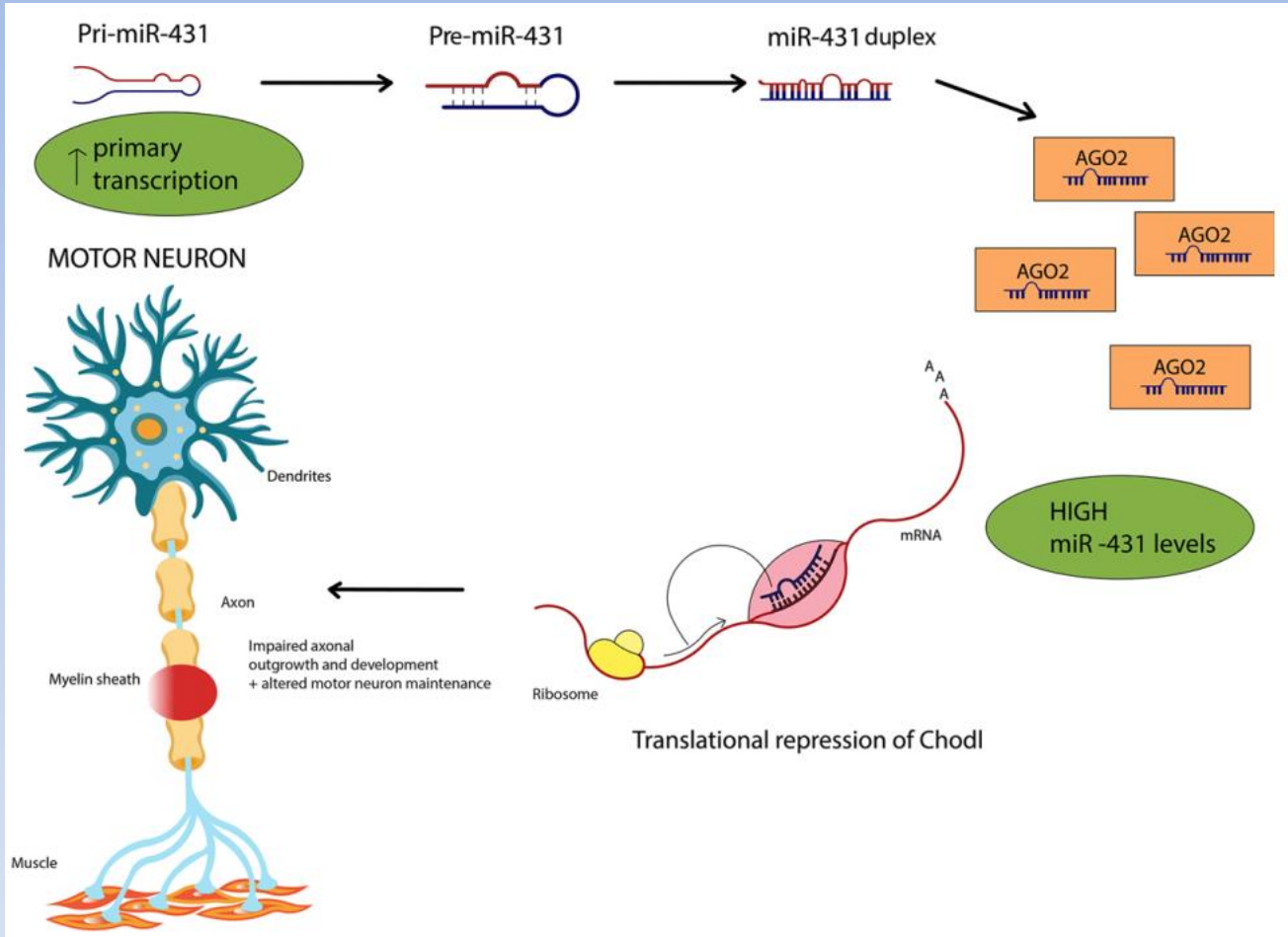
mRNA
m7G

$(AAA)_n$

Targeted mRNA is destroyed

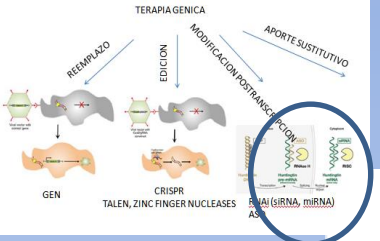
Compound	Application route	Formulation/ modification	Target	Indication	Company	Clinical status
Bevasiranib	intravitreal	–	veGF	Age-related macular degeneration	Opko Health inc.	Phase III, terminated
AGN-745 (Sirna027)	intravitreal	–	veGF	Age-related macular degeneration	Allergan/Sirna	Phase II, terminated
ALN-RSv01	inhalation	–	RSv nucleocapsid	RSv infection after gene lung transplantation	Alnylam Pharmaceuticals	Phase II, completed
RXi109	intradermal	Asymmetric siRNA with phosphorothioates and lipophilic ligands	Connective tissue growth factor	Dermal scarring after surgery	RXi Pharmaceuticals	Phase II
QPi-1002	intravenous	Modified siRNA (alternating 2′-0-Me)	p53	Delayed graft function and acute kidney injury	Quark Pharmaceuticals/ Novartis	Phase II
CALAA-01	intravenous	RONDeLTM (cyclodextrin-based formulation with PeG and transferrin)	M subunit of ribonucleotide reductase	Solid tumors	Arrowhead Research Corporation	Phase I completed
Patisiran (ALN-TTR02)	intravenous	SNALP	TTR	TTR amyloidosis (FAP)	Alnylam Pharmaceuticals	Phase III
ALN-TTRsc	Subcutaneous	GalNAc conjugate	TTR	TTR amyloidosis (FAC)	Alnylam Pharmaceuticals	Phase II
ARC-520	intravenous	Dynamic polyconjugate (co-injection with siRNA)	Coagulation factor 7	Hepatitis B	Arrowhead Research Corporation	Phase II73,75
siRNA-ephA2-DOPC	intravenous	Liposome (DOPC)	ePHA2	Advanced cancers	MD Anderson Cancer Center	Phase II
TD101	intradermal injection/ microneedle	Modified siRNA (“Accell”: 2′0-Me, cholesterol, phosphorothioates)	Keratin 6a	Pachyonychia congenita	Trans Derm	Phase I completed
Atu027	intravenous	Atuplex® (liposome) with AtuRNA® (2′-O-Me)	Protein kinase N3	Advanced solid cancer	Silence Therapeutics	Phase II
Atu111	intravenous	DACC lipoplex	Angiopoietin-2	Lung indications	Silence Therapeutics	Phase II
PF-655	intravitreal	AtuRNA® (2′-O-Me)	RTP801	Diabetic macular edema/age-related macular degeneration	Quark Pharmaceuticals/ Pfizer	Phase II
QPi-1007	intravitreal	Modified siRNA (alternating 2′-0-Me)	Caspase 2	Optic nerve atrophy and non-arteritic ischemic optic neuropathy	Quark Pharmaceuticals	Phase I/IIa
siG12D LODeR	intratumoral	LODeRTM (PLGA matrix)	Mutant K-Ras G12D	Pancreatic ductal adenocarcinoma	Silenseed	Phase I/II
TKM-PLK1	Hepatic intraarterial/ intravenous	SNALP	PLK1	Liver cancer	Tekmira Pharmaceuticals	Phase I/II
ND-L02-S0201	intravenous	vitamin A-coupled lipid nanoparticles	HSP47	Fibrosis	Nitto Denko Corporation	Phase I
DCR-MYC	intravenous	Lipid anoparticles (enCore)	MYC	Hepatocelluar carcinoma	Dicerna Pharmaceuticals	Phase I

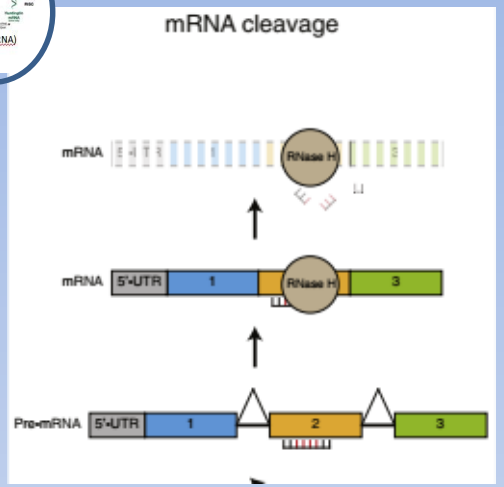
RNAi: miRNA

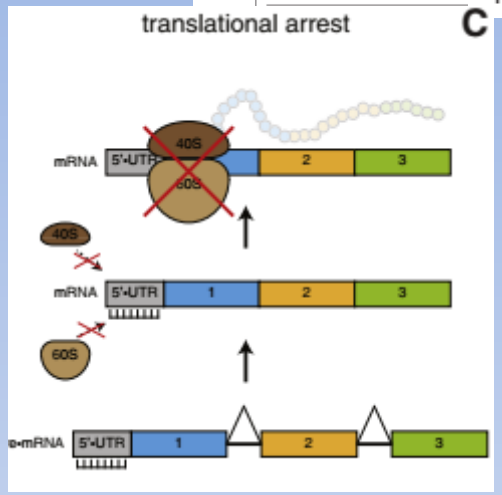

Pri-miR-431
Pre-miR-431
miR-431 duplex
primary transcription
AGO2
AGO2
AGO2
AGO2
MOTOR NEURON
Dendrites
Axon
Myelin sheath
Impaired axonal outgrowth and development + altered motor neuron maintenance
Muscle
A A A
mRNA
Ribosome
HIGH miR -431 levels
Translational repression of Chodl

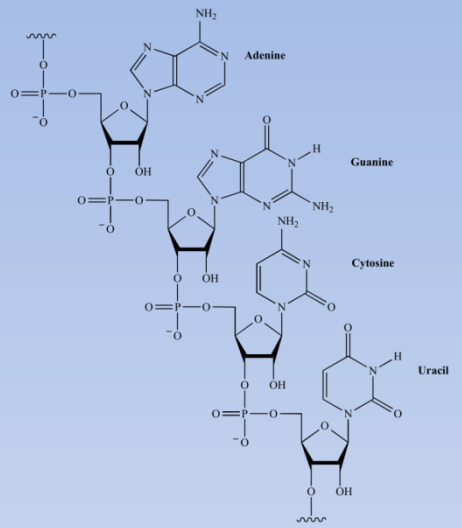
Antisense oligonucleotides: the next frontier for treatment of neurological disorders

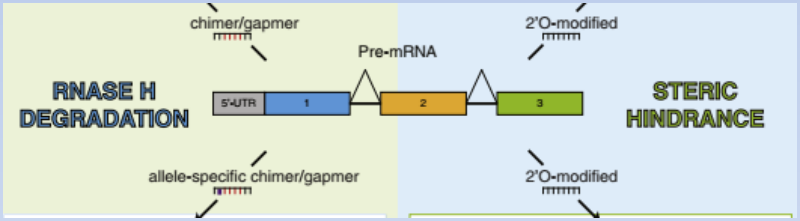
NATURE REVIEWS | NEUROLOGY

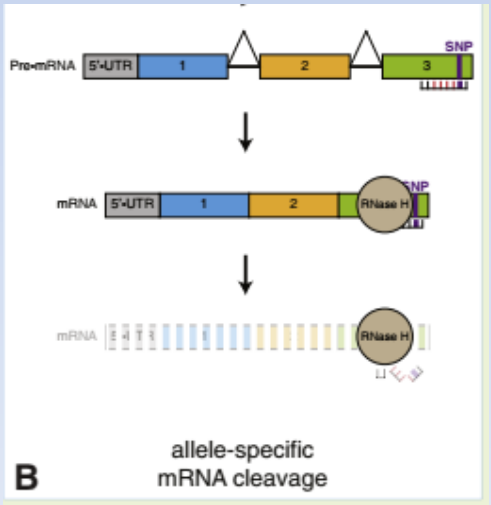


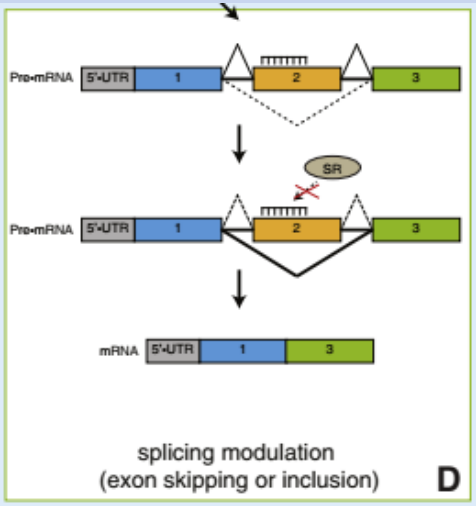


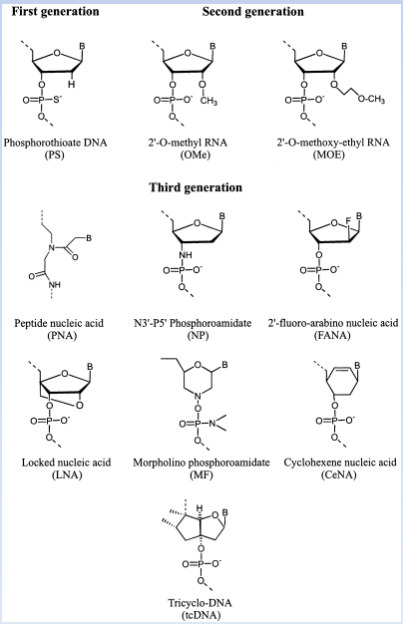


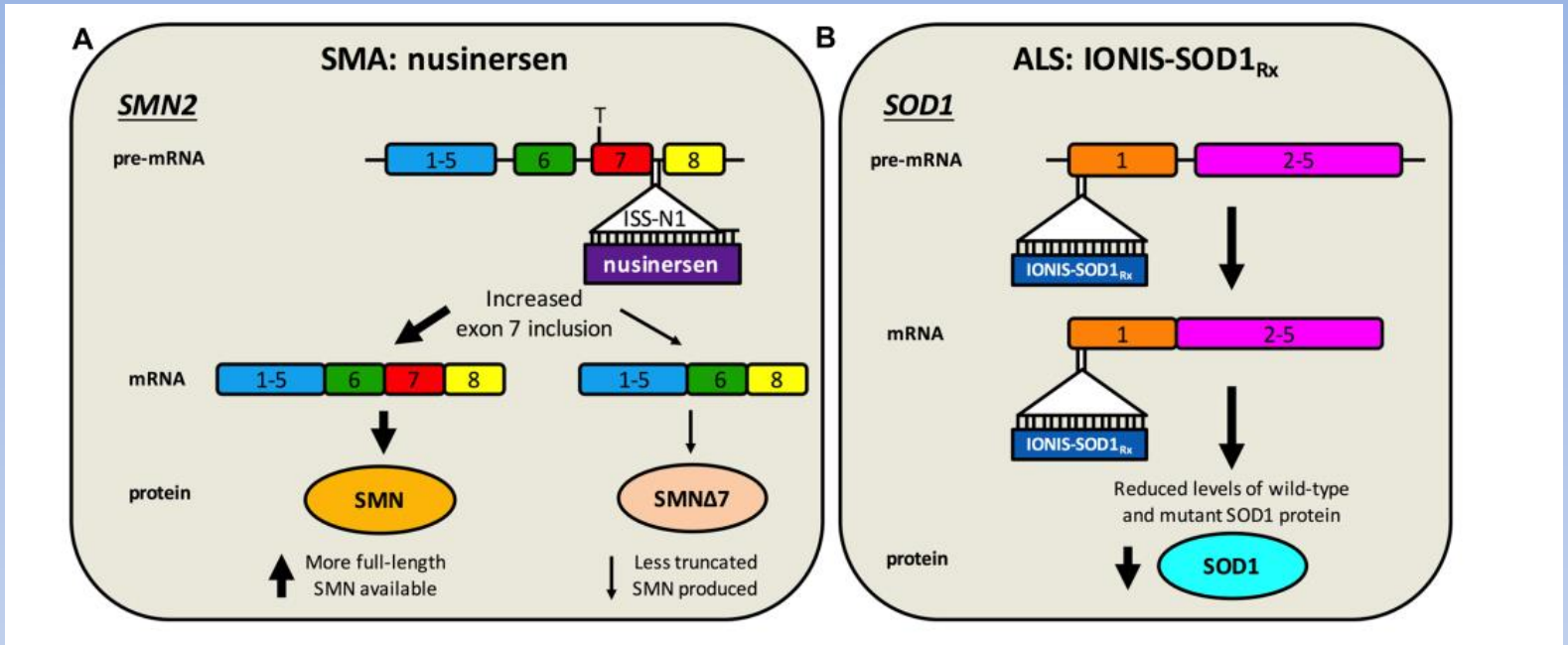


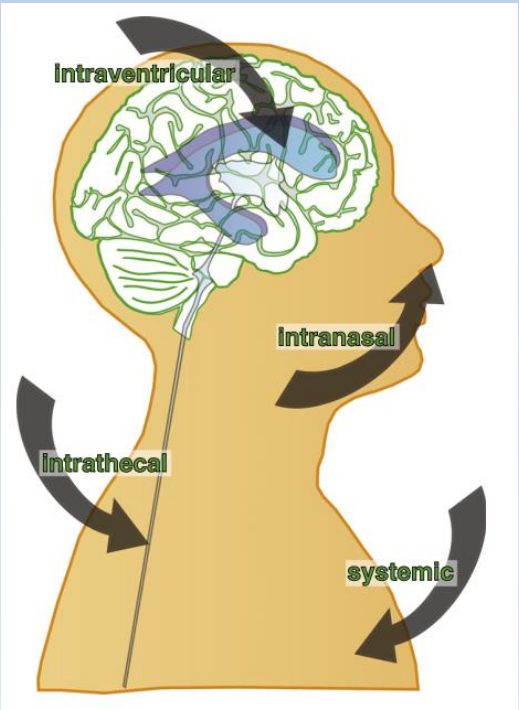










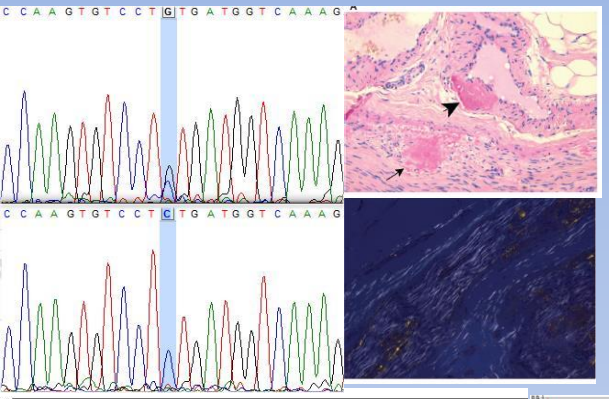


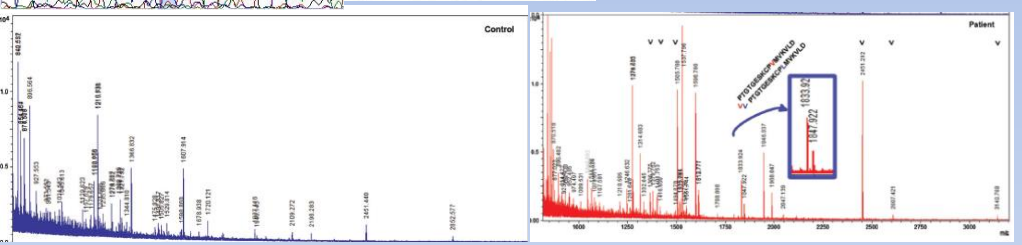
- Amplia distribucion tisular, excepto SNC
- Acumulación en túbulo renal proximal
- No atraviesan BHE, pero si se distribuyen por el SNC tras administracion intratecal (bolo)
- Efecto acumulativo en músculo
- E. secundario: trombocitopenia ->sangrado

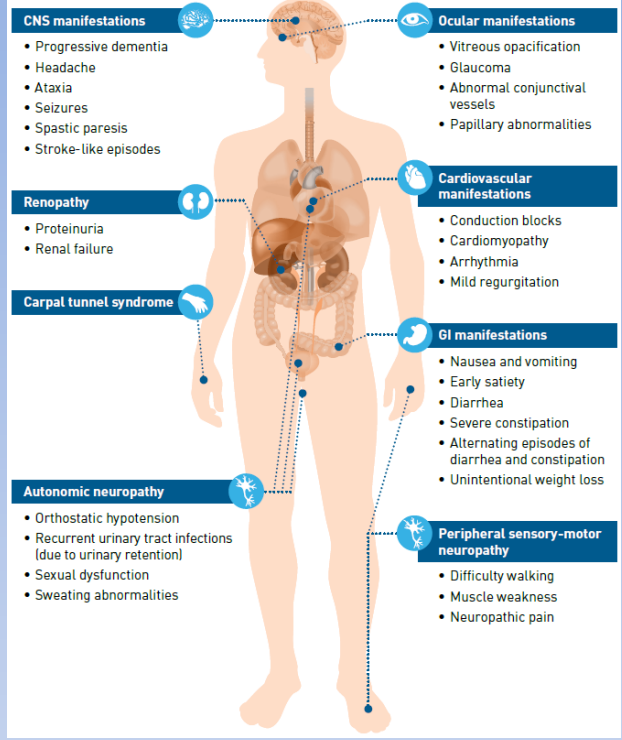
ASO: ENSAYOS CLINICOS

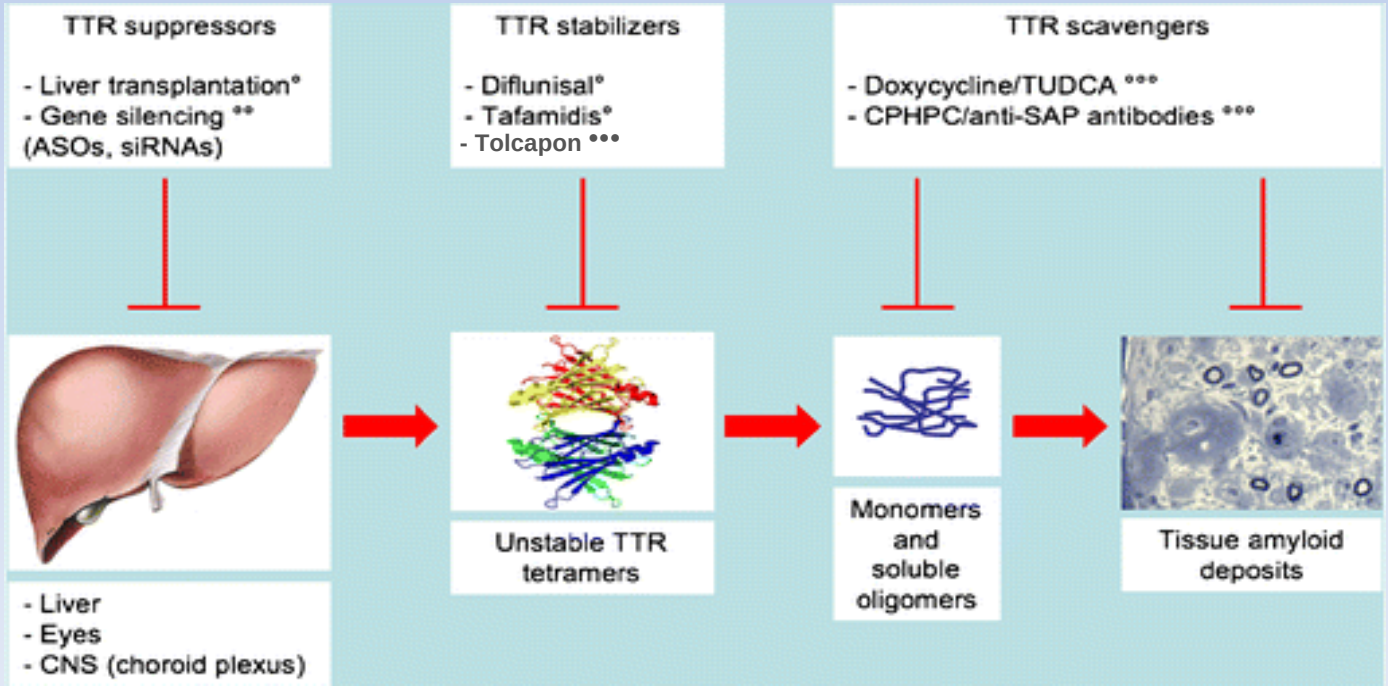
	Target gene	Chemistry	Mechanism	Treatment route	Design (phase)
Drisapersen	DMD (exon 51)	2′-OMe	Splicing modulation	Subcutaneous or IV	Open label (I–II)
				Subcutaneous	Placebo-controlled (I)
					Placebo-controlled (II)
					Placebo-controlled (III)
					Open label (III)
Eteplirsen	DMD (exon 51)	PMO	Splicing modulation	Intravenous	Single blind (I–II)
					Open label (I–II)
					Placebo-controlled (II)
					Open label (II)
					Open label (III)
PRO044	DMD (exon 45)	2′-OMe	Splicing modulation	Subcutaneous or IV	Open label (II)
PRO045	DMD (exon 45)	2′-OMe	Splicing modulation	Subcutaneous	Open label (II)
SRP-4045	DMD (exon 45)	PMO	Splicing modulation	Intravenous	Placebo-controlled (III)
SRP-4053	DMD (exon 53)	PMO	Splicing modulation	Intravenous	Placebo-controlled (III)
Nusinersen	SMN2	2′MOE-PS	Splicing modulation	Intrathecal	Open label (I)
					Open label (I–II)
					Open label (II)
					Sham-controlled (III)
					Open label (II)
					Sham-controlled (II)
					Open label (III)
IONIS-TTR	TTR	2′-MOE-PS	RNase H-mediated degradation	Subcutaneous	Placebo-controlled (III)
IONIS-HTT	HTT	2′-MOE-PS	RNase H-mediated degradation	Intrathecal	Placebo-controlled (I–II)
IONIS-SOD1	SOD1	2′-MOE-PS	RNase H-mediated degradation	Intrathecal	Placebo-controlled (I)
ISIS-DMPK	DMPKS	2′-MOE-P	RNase H-mediated degradation	Subcutaneous	Placebo-controlled (I–II)
TL1102	CD49d	2′-MOE-PS	RNase H-mediated degradation	Subcutaneous	Placebo-controlled (I–II)

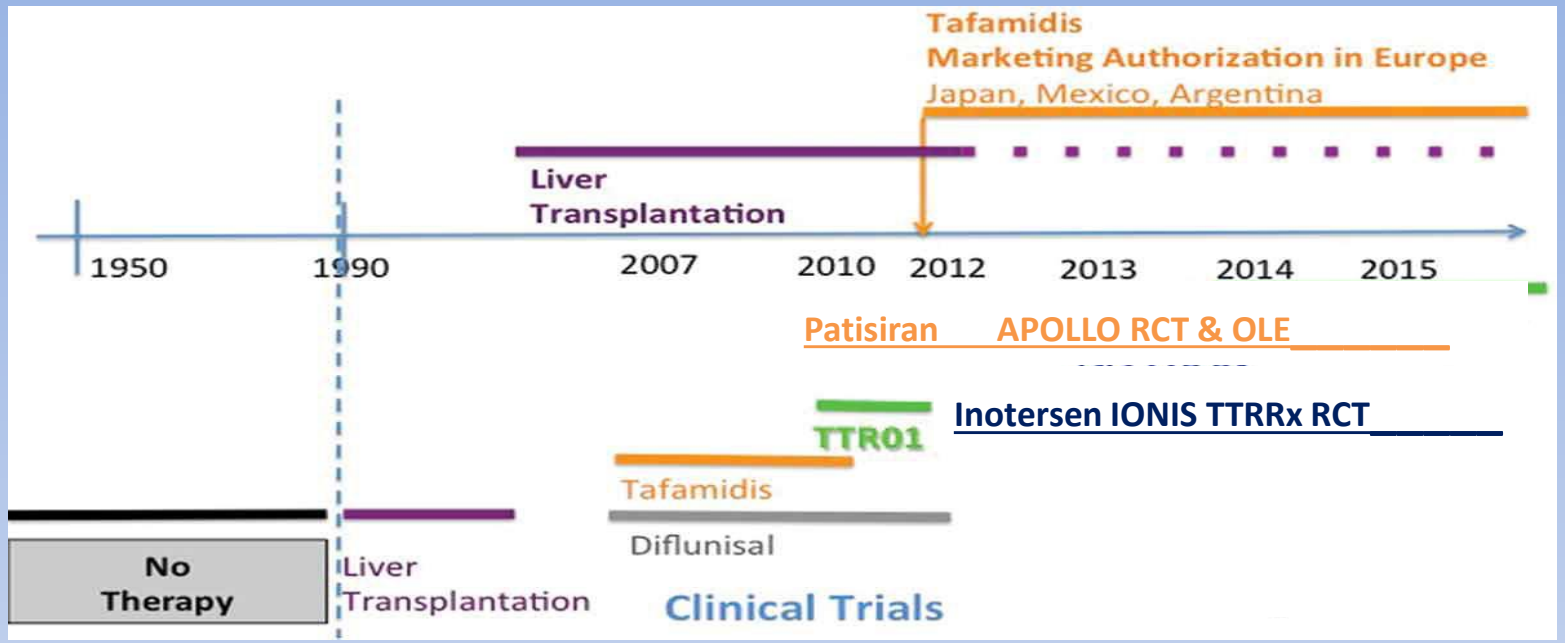
POLINEUROPATIA AMILOIDEA FAMILIAR (TTR)

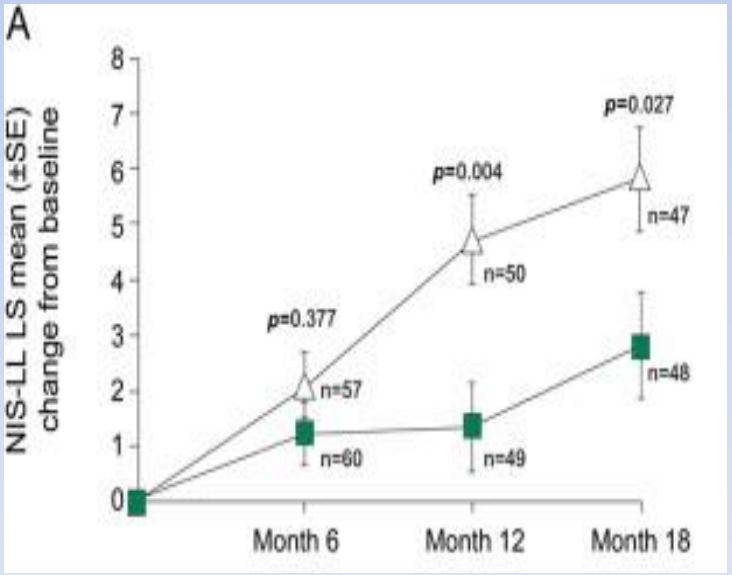


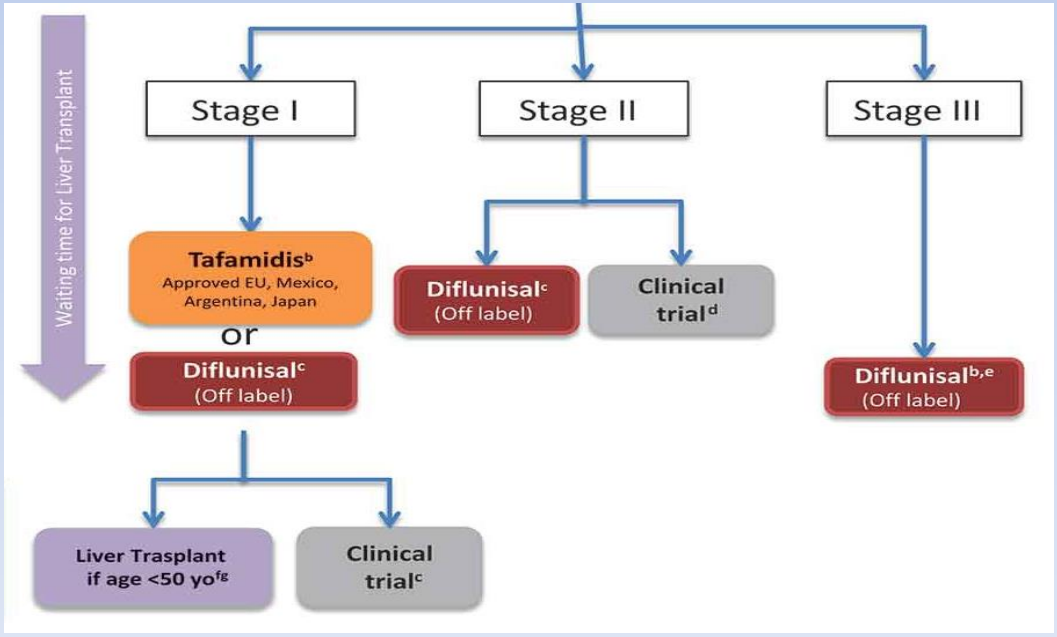




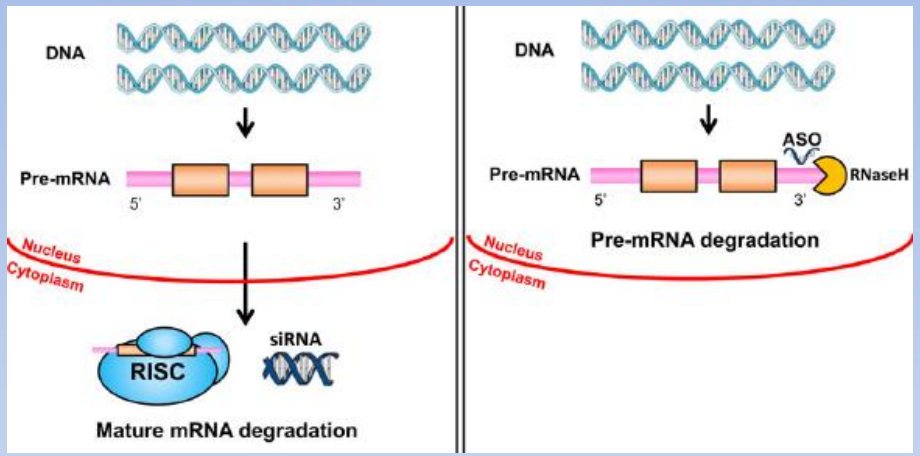


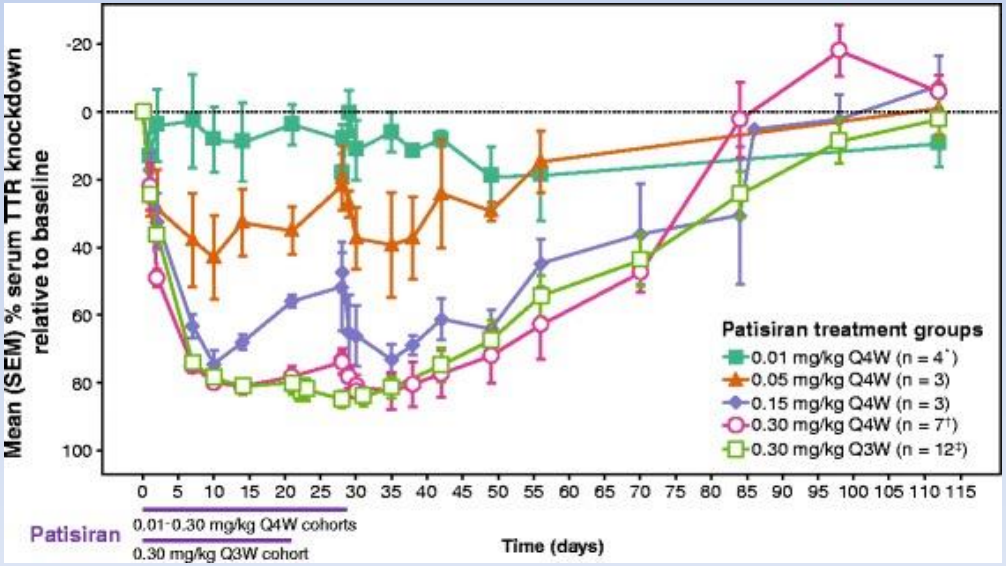

Tafamidis
Marketing Authorization in Europe
Japan, Mexico, Argentina
Liver
Transplantation
1950
1990
2007
2010
2012
2013
2014
2015
Patisiran APOLLO RCT & OLE
Inotersen IONIS TTRRx RCT
TTR01
Tafamidis
Diflunisal
No
Therapy
Liver
Transplantation
Clinical Trials


A
NIS-LL LS mean (±SE)
change from baseline
8
7
6
5
4
3
2
1
0
p=0.027
p=0.004
p=0.377
n=47
n=50
n=57
n=48
n=60
n=49
Month 6
Month 12
Month 18


Waiting time for Liver Transplant
Stage I
Stage II
Stage III
Tafamidis[b]
Approved EU, Mexico, Argentina, Japan
or
Diflunisal[c]
(Off label)
Diflunisal[c]
(Off label)
Clinical trial[d]
Diflunisal[b,e]
(Off label)
Liver Trasplant if age <50 yo[f,g]
Clinical trial[c]

- Genetic modifying therapy
  - siRNA ASO
    patisiran inotersen



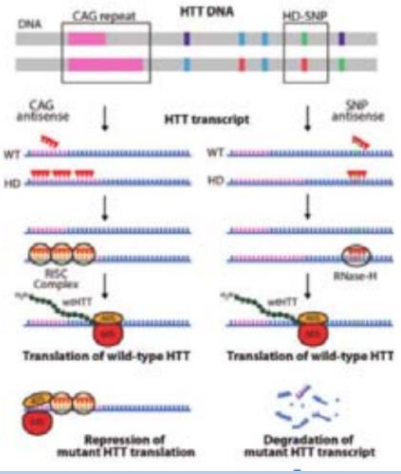


- at 2 yrs: 14/27 (71%) no progression
- APOLLO RCT: effectiveness at 1.5 yr on all endpoints

ENFERMEDAD DE HUNTINGTON

WAVE LifeScience ASO

- WSE-120101 will be an ASO that targets the single nuclear polymorphism (SNP) rs362307
- Attempt to be allele-specific silencing

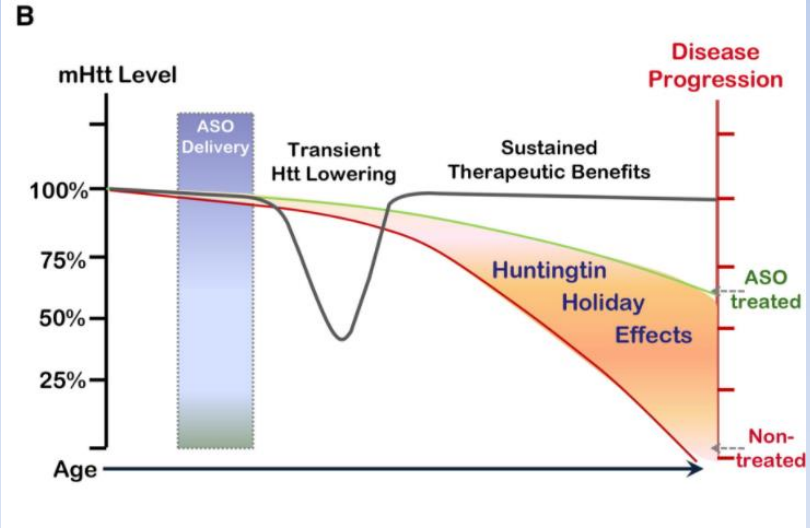


IONIS Antisense Oligonucleotides Roche

- Phase1b **safety** study began late 2015!! (Ionis-HttRx)
- Efficacy of ASO will NOT be determined in this trial
- Ionis-HttRx is an ASO that lowers normal and mutant *huntingtin*
- Duration of action ~ 4months
- Drug to be delivered intrathecally
- Due to complete Sept. 2017
  - No major safety events thus far…

36 patients + placebo controls
4 doses (10, 30, 50 and 70 mg)
14 week study at clinical sites in UK, Germany and Canada only
Post-treatment observation up to 29 weeks

- ZFN's create double stranded breaks in DNA at specific points
  - Requires creation of custom targeted DNA sequence
- Clustered regularly interspaced short palindromic repeats (CRISPR)
  - Combines existing defense mechanism against viral invasion with RNA guide to target and excise DNA sequences (ie could shorten a 42 to a 22)
- uniQure developing AAV5-miRNA model to knockdown mHtt production
  - Would require only single dose

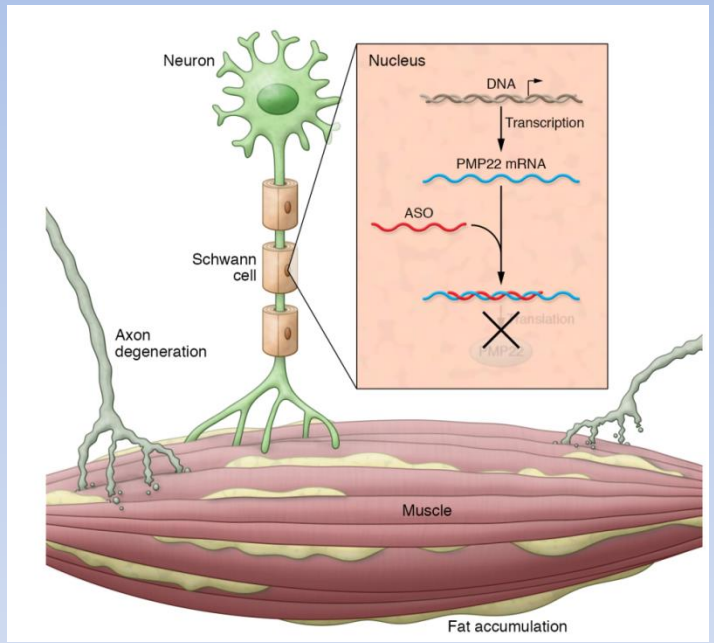


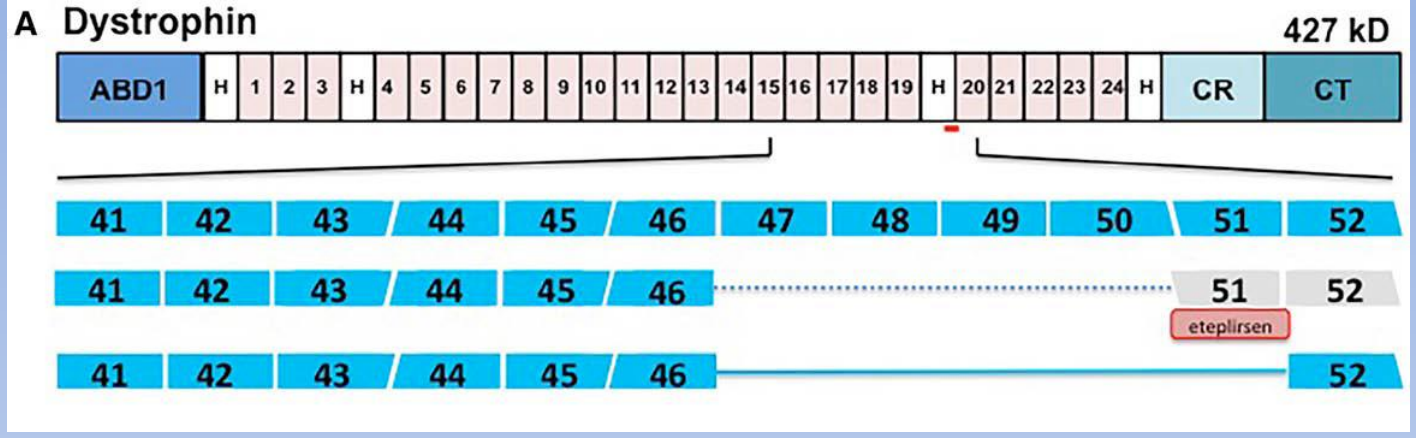
The Journal of Clinical Investigation RESEARCH ARTICLE

PMP22 antisense oligonucleotides reverse Charcot-Marie-Tooth disease type 1A features in rodent models

Hien Tran Zhao,[1] Sagar Damle,[1] Karli Ikeda-Lee,[1] Steven Kuntz,[1] Jian Li,[2] Apoorva Mohan,[1] Aneeza Kim,[1] Gene Hung,[1] Mark A. Scheideler,[3] Steven S. Scherer,[2] John Svaren,[4] Eric E. Swayze,[1] and Holly B. Kordasiewicz[1]

- Ratas: 6 semanas (inicio déficit motor a 5 semanas
- Reducción niveles PMP22 50%
- Mejoria motor y neurofisiológica

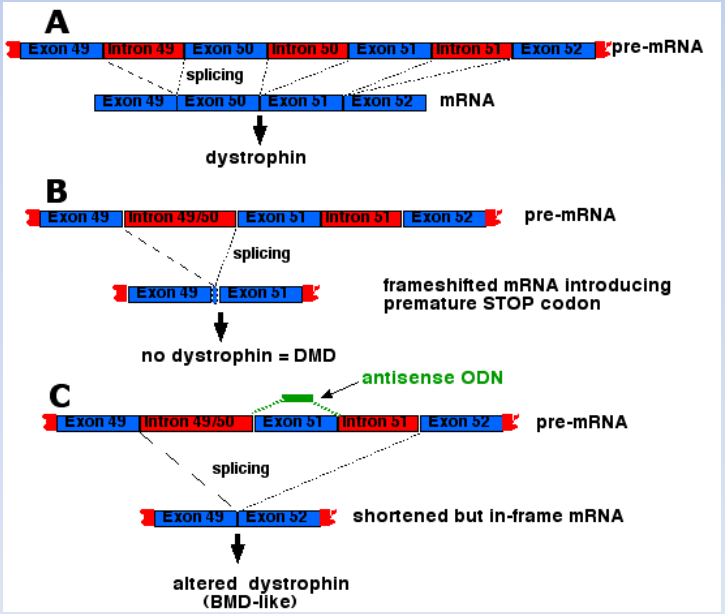


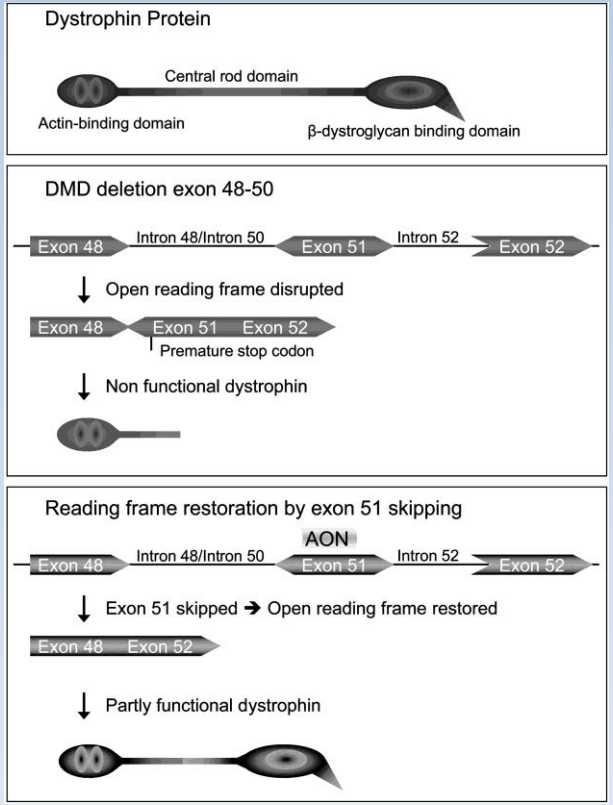


79 exones, mRNA 14 kb → excede capacidad de vectores; se requieren 3 vectores o minidistrofinas

Inyeccion IV, intraarterial, intraperitoneal

Efecto variable. Musculo cardiaco es más resistente → insuficiencia cardiaca por aumento de movilidad



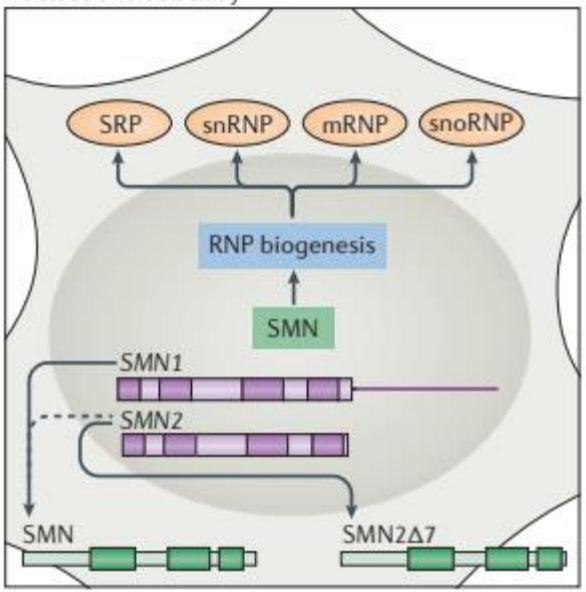


Age	Intervention	Clinical trial	outcomes	main finding
5–19	None	Observational	I: resting energy expenditure (REE) II: BMIZ-score	High prevalence of obesity and no difference between REE in ambulators and non-ambulators.
7–25	eplerenone	Phase I	I: myocardial damage by late gadolinium enhancement cardiac magnetic resonance (LGE)	Effective and safe cardioprotection, especially when started at younger age.
5 and older	ataluren	Phase II	I: 6MWD II: meaningful differences between treatment and placebo	Promising improvement as a treatment for DMD.
7–14	tadalafil	Phase III	I: 6MWD II: North Start Ambulatory Assessment and timed function tests state.	No significant effects on disease. Adverse events consistent with known safety profile and disease
10–18	ibedenone	Phase III	I: changes from baseline in FVC and FVC% by spirometry	Fewer treated patients with decline by a margin of 10%, 30%, 40% or 50% compared with placebo.
7–11	givinostat	N/A	I: histological evidence	Reduced amount of fibrotic tissue and increased fraction of muscle tissue.
5–11	AAV minidystrophin	Phase I	I: safety, response and therapeutic transgene expression measurement	Well-tolerated vector, but overall low minidystrophin transgene expression.
24–37	AVV follistatin	Phase I/II	I: 6MWD II: histology	Improved 6MWD. Reduced endomysial fibrosis, central nucleation and increasednormal fibre size distribution with hypertrophy.
N/A	drisapersen	Phase I/II	I: pharmacokinetics and safety II: dystrophin expression, muscle strength and function (6MWD)	Well-tolerated over 188 weeks with possible renal effects, thrombocytopaenia and injection-site reactions. Improvement in 6MWD at 12 weeks and sustained after 3.4 years of dosing.
5 and older	drisapersen	Phase II	I: 6MWD II: safety, and renal, hepatic and haematological monitoring	Some benefit in 6MWD. No serious adverse events reported.
7–18	eteplirsen	N/A	I: change in number (%) if dystrophin positive fibres II: 6MWD and adverse events	Statistically significant progress in 6MWD in treated group and lower incidence of loss of ambulation. Well-tolerate dosage.

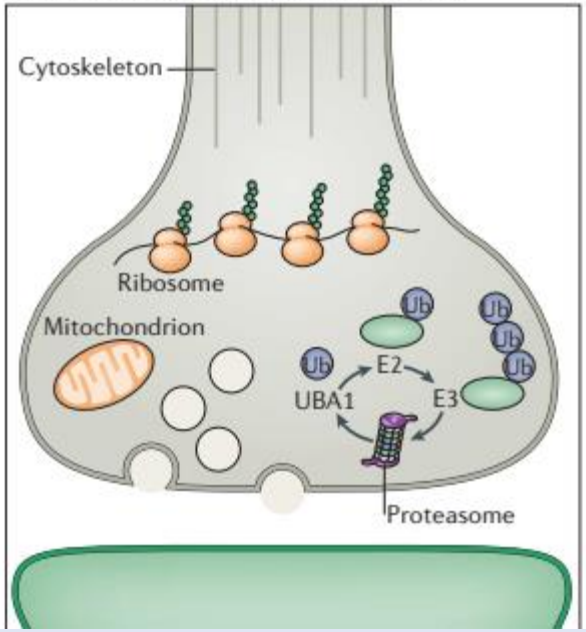
AME

SMA pathways

Nucleus and cell body



Cell body, axon or synapse (including NMJ)



Therapeutic targets

SMN-targeted therapies

SMN1 replacement
- Gene therapy

SMN2 splicing
- First-generation and second-generation ASOs
- Small molecules

Increase SMN
- HDAC inhibitors (e.g. VPA)
- STAT5 activators (e.g. prolactin)
- SMN ubiquitylation inhibitors (e.g. ML372)

SMN-independent therapies and modifiers

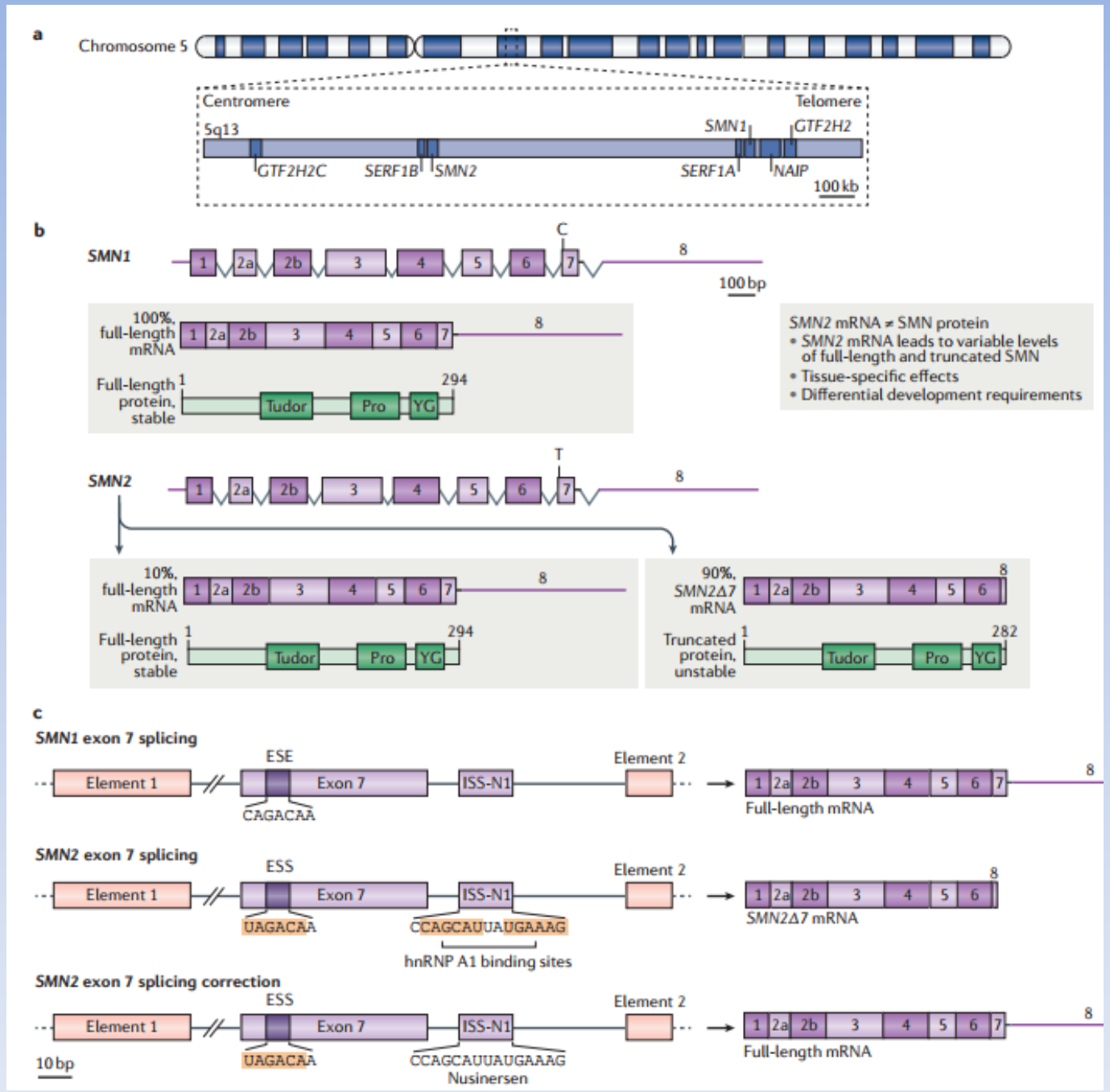
Cytoskeleton
- ROCK (fasudil)
- RHOA
- Profilin

Local translation
- Ribosomal proteins

Ubiquitin homeostasis
- UBA1
- Targets of UBA1, e.g. CTNNB1 (quercetin)

Mitochondria
- Olesoxime
- PGK1 (terazosin)

Endocytosis
- PLS3
- NCALD


a
Chromosome 5
Centromere
Telomere
5q13
SMN1
GTF2H2
GTF2H2C
SERF1B
SMN2
SERF1A
NAIP
100 kb
b
SMN1
C
8
100 bp
100%, full-length mRNA
Full-length protein, stable
1
294
Tudor
Pro
YG
SMN2 mRNA ≠ SMN protein
• SMN2 mRNA leads to variable levels of full-length and truncated SMN
• Tissue-specific effects
• Differential development requirements
SMN2
T
10%, full-length mRNA
90%, SMN2Δ7 mRNA
Truncated protein, unstable
282
c
SMN1 exon 7 splicing
ESE
Element 1
Exon 7
ISS-N1
Element 2
CAGACAA
Full-length mRNA
SMN2 exon 7 splicing
ESS
UAGACAA
CCAGCAUUAUGAAAG
hnRNP A1 binding sites
SMN2Δ7 mRNA
SMN2 exon 7 splicing correction
10 bp
Nusinersen

Therapeutic approach	Therapy	Target	Stage of development
SMN-targeted therapies	Nusinersen (ASO)	SMN2 splicing	Approved
	Other experimental ASOs	SMN2 splicing	Clinical trial (phase I, II and III), preclinical
	Small molecules: RG7910, LMI070	SMN2 splicing	Clinical trial (phase I and II)
	Gene therapy: AVXS-101	SMN1 replacement	Clinical trial (phase I)
Neuroprotection	Olesoxime	Mitochondria	Clinical trial (phase II and III)
Muscle enhancement	CK2127107	Fast troponin (activator)	Clinical trial (phase I and II)
	SRK-015	Myostatin (inhibitor)	Clinical trial (phase I)
	Pyridostigmine, 4-aminopyridine	Fatigability and endurance	Clinical trial (phase II and III)
	Exercise and/or physiotherapy	Overall muscle strength	NA
Modifiers of SMA	Upregulation of UBA1: possible gene therapy or small-molecule therapy	Ubiquitin homeostasis	Preclinical
Upregulation of PLS3: possible gene therapy or small-molecule therapy	Actin dynamics	Preclinical	
Downregulation of NCALD: possible gene therapy or small-molecule therapy	Endocytosis	Preclinical	
Quercetin-mediated inhibition of CTNNB1	Motor neuron stability	Preclinical	
Fasudil-mediated inhibition of ROCK	Actin dynamics	Preclinical	

CONTROVERSIAS:

DE LA TECNICA: VEHÍCULO DE APLICACIÓN, VIA DE ADMINISTRACION

OFF TARGETS/ON TARGETS, OTROS

ETICOS: USO DE IPSC, CAMBIOS EN EL GENOMA

LEGALES: USOS CLINICOS, PATENTES,..

ECONOMICOS

ENFERMEDADES RARAS DE BAJA FRECUENCIA

VENTANA TERAPEUTICA