Atrofia muscular espinal

Presente y futuro de la investigación

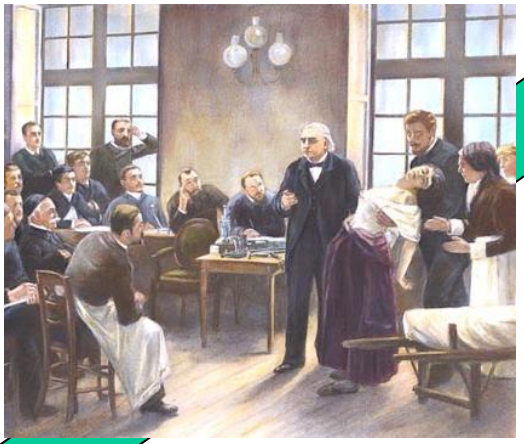
Knowledge on SMA on the last 150 years

Protocols of treatments

???

Translational research

2000 →



Genetic diagnosis

1990-1999

Gene cloned in 1995!

Clinical diagnosis

1950-80

Disease description

1850-1890

Características de la AME

- Es una enfermedad de dos genes, la mutación en el SMN1 la determina y el número de copias de SMN2 modifica el fenotipo.
- La disminución de la proteína SMN en neuronas motoras parece la causa inicial de la enfermedad.
- Existen fenotipos discordantes intrafamiliar y en no emparentados.
- La presencia y estudio del gen SMN2 puede servir de herramienta terapéutica.

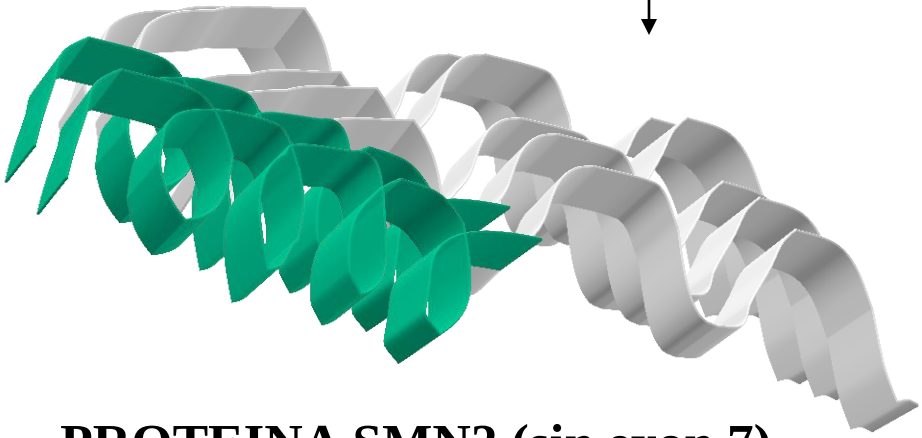
ADN

SMN 2

SMN 1

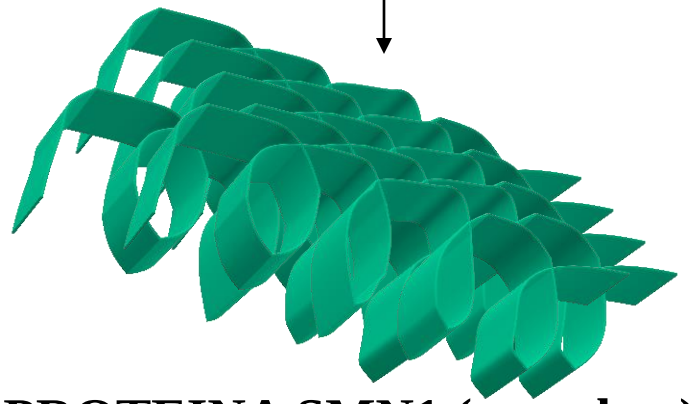
EXONES

ARN mensajero



PROTEINA SMN2 (sin exon 7)

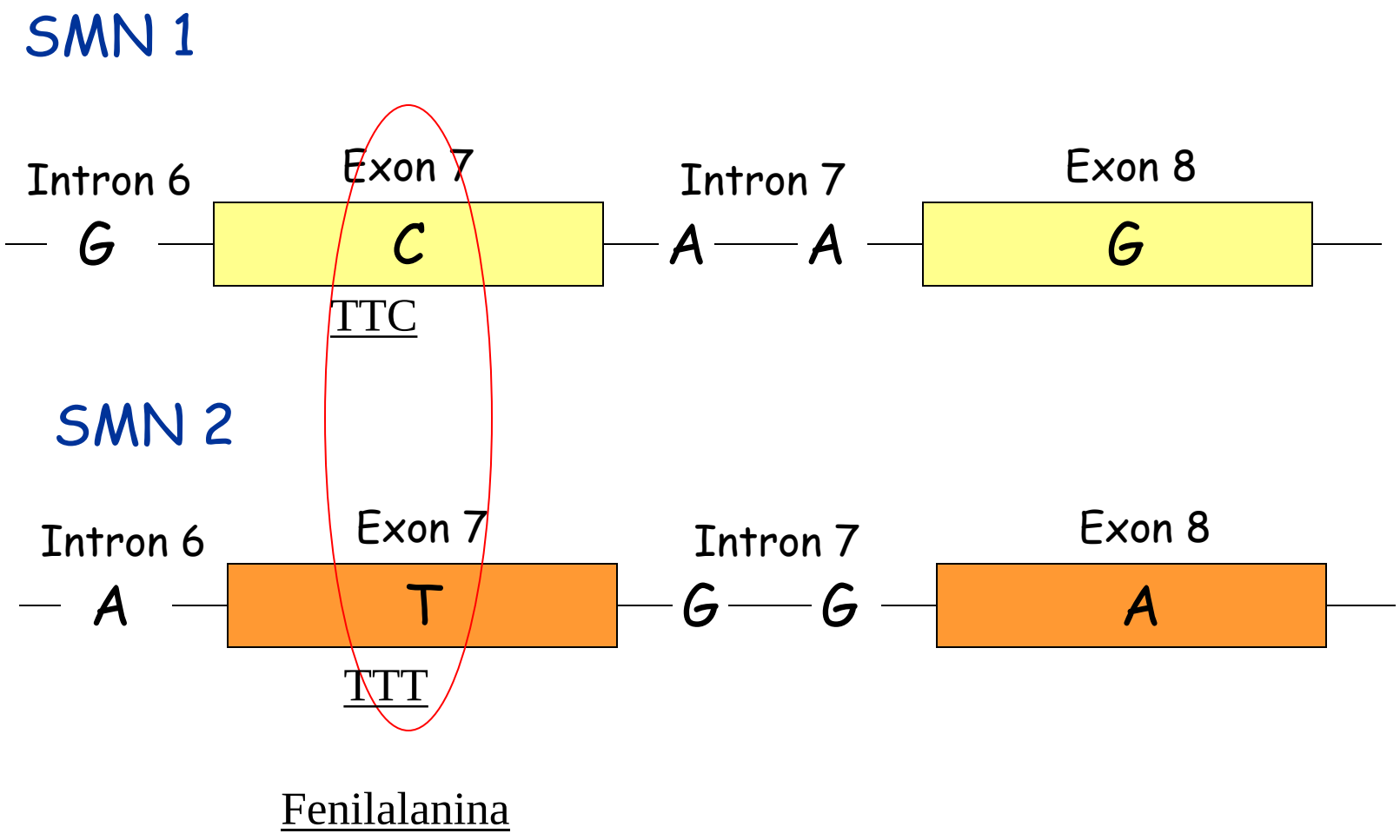
inestable y parcialmente funcionante



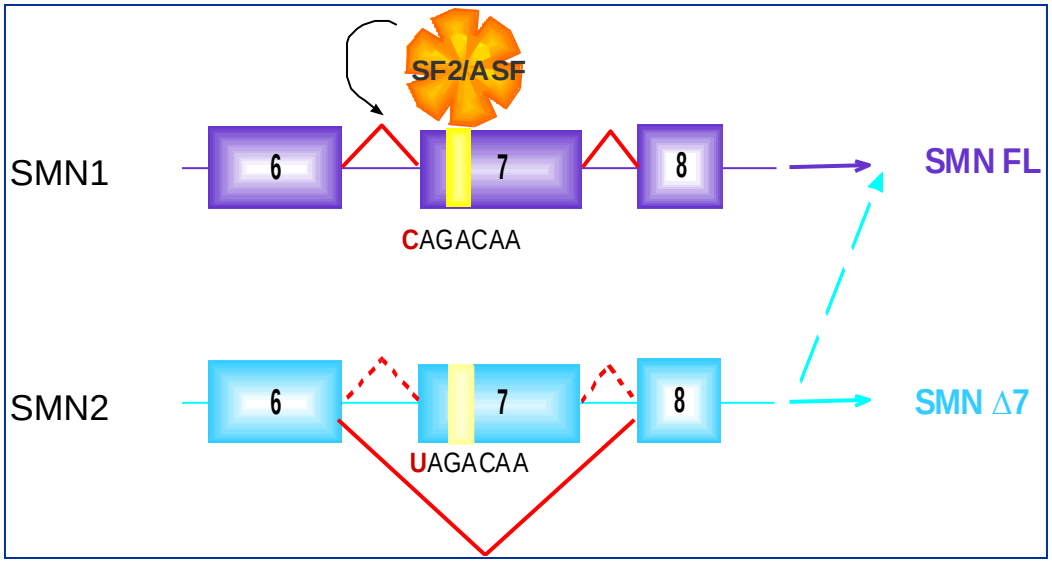
PROTEINA SMN1 (completa)

totalmente funcionante

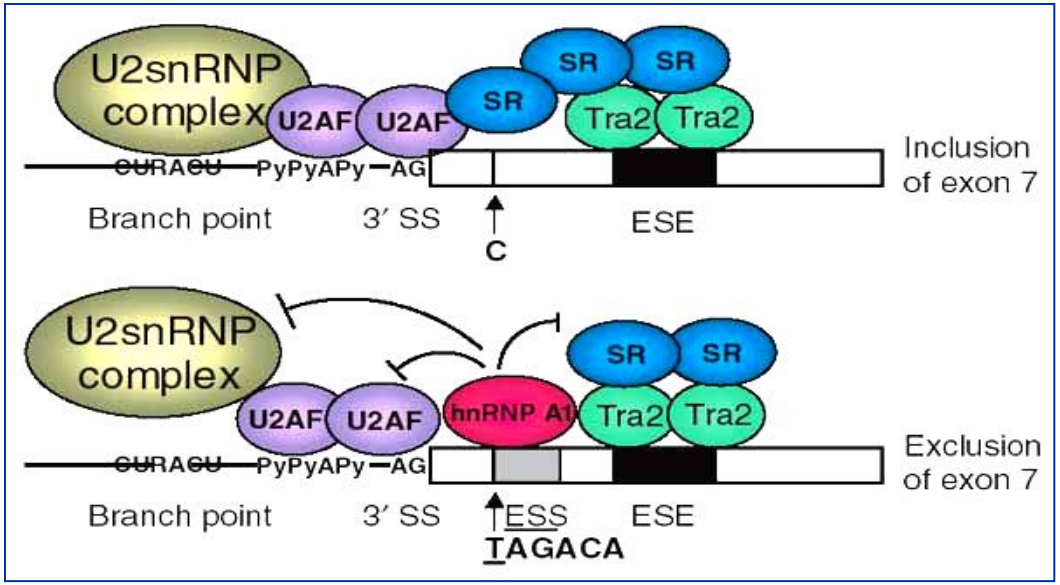
Diferencias entre SMN 1 y SMN2



EXONIC SPLICING ENHANCER (ESE)



EXONIC SPLICING SILENCER (ESS)

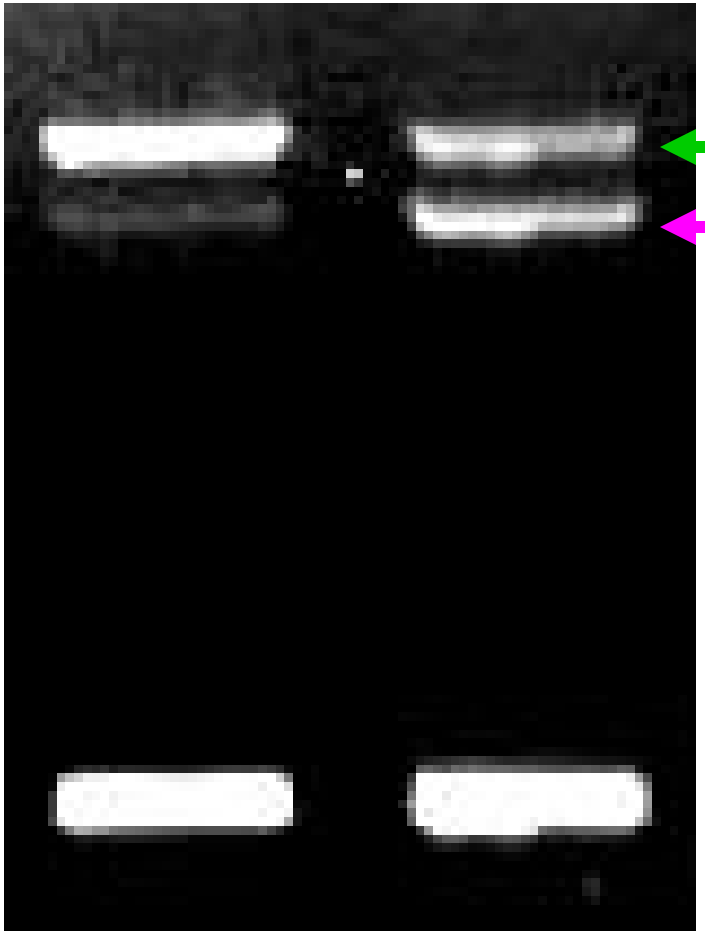


Kashima et Manley, Nat Genet 2003

RNA MEDULA ESPINAL

Control
SMN1

AME
SMN2



SMN COMPLETO

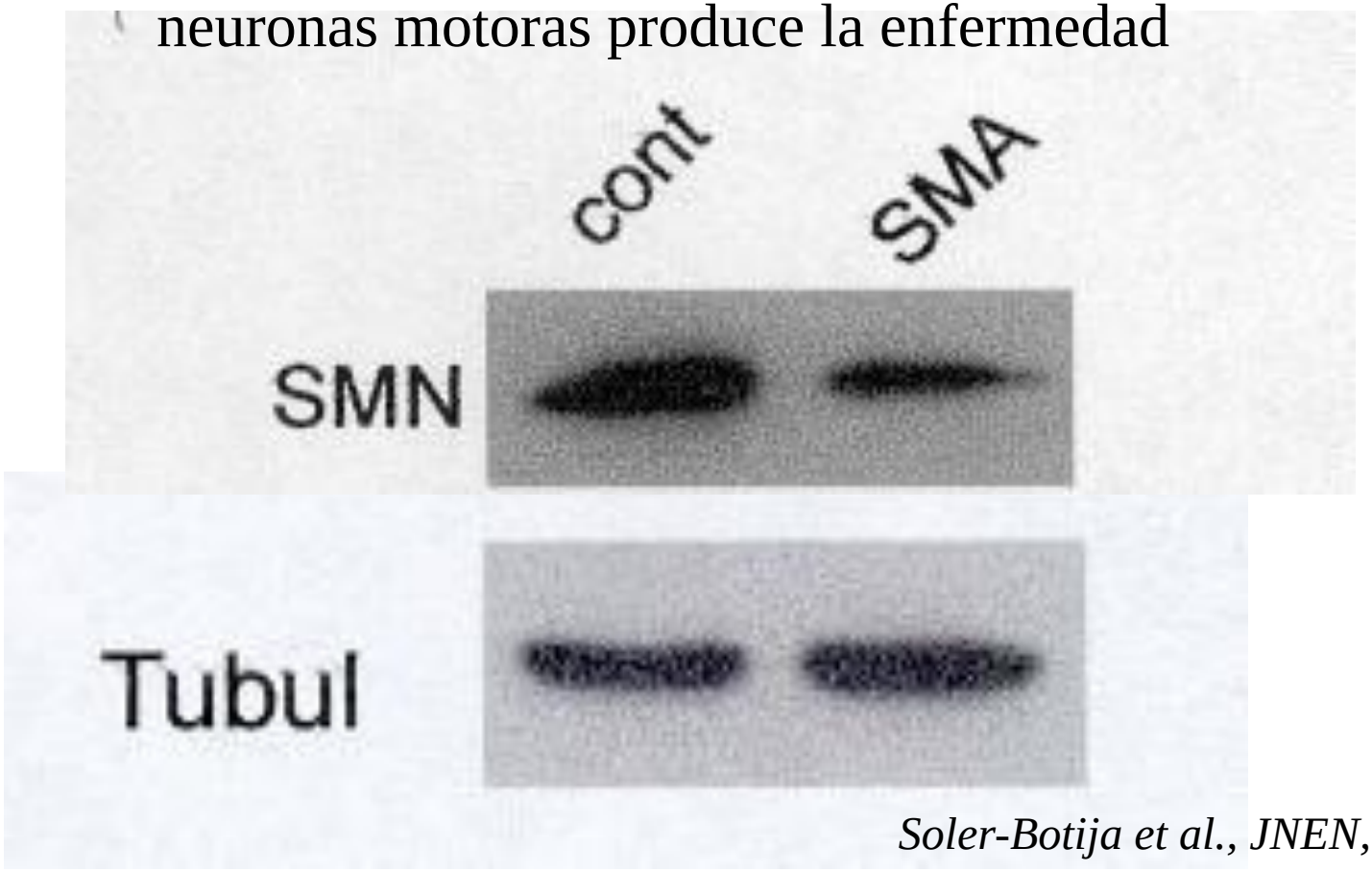
SMN delta 7

β actina

Soler-Botija et al., JNEN, 2005

PROTEINA MEDULA ESPINAL

Una disminución de la cantidad de proteína SMN en las neuronas motoras produce la enfermedad

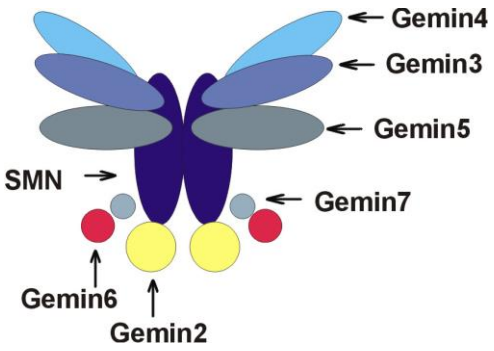


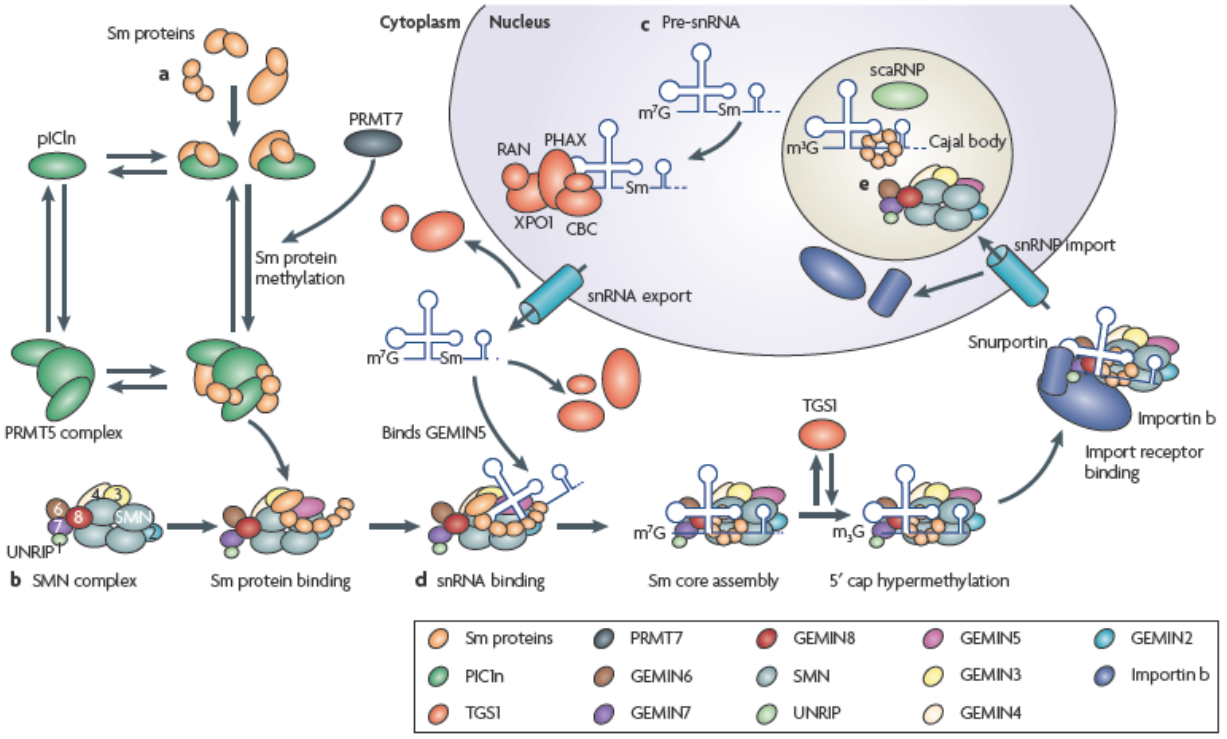
Soler-Botija et al., JNEN, 2005

El gen SMN2 es capaz de producir algo de proteína SMN funcional pero no alcanza para evitar la enfermedad

Funciones

- *Generales?*
  - Biogénesis y ensamblaje de las partículas snRNPs
  - *Splicing* del pre-mRNA
  - Transcripción génica
  - Metabolismo del RNA ribosómico
- *Neuronales?*
  - Apoptosis
  - Transporte axonal mRNA
  - Formación de las neuritas y uniones neuromusculares





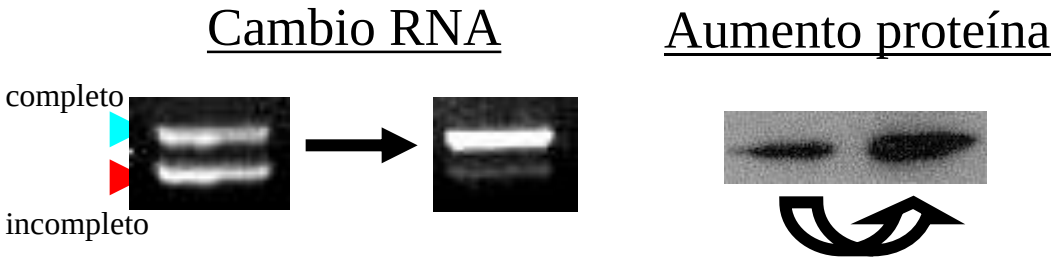

Rehabilitación
Fisioterapia
Nutrición
Respiratorio
Cirugía

Estrategias terapéuticas AME

- 1. Incrementar la cantidad de proteína producida por el gen SMN2.
- 2. Proteger las neuronas motoras del daño de la enfermedad.
- 3. Incrementar la fuerza y resistencia muscular.
- 4. Transferir copias normales del gen SMN1 a la médula espinal (terapia génica).
- 5. Sustituir las neuronas motoras por medio de la terapia celular (células troncales).

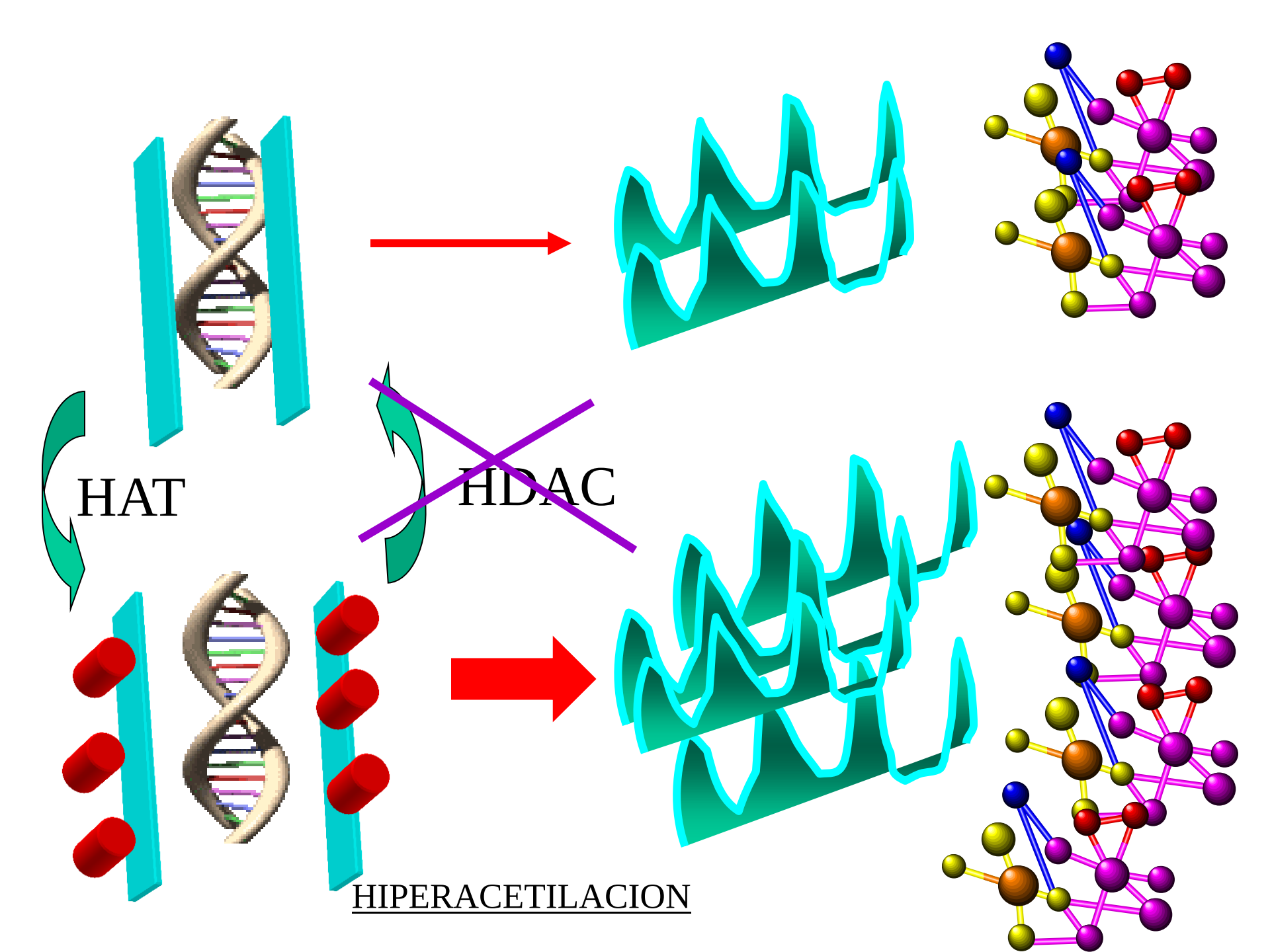
Estrategias terapéuticas AME

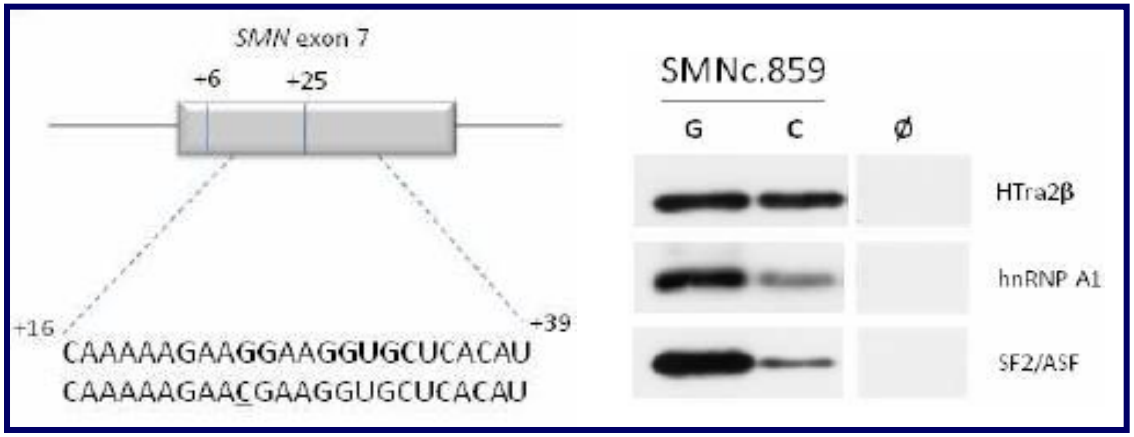
- 1. Incrementar la cantidad de proteína producida por el gen SMN2.
  - Con medicamentos que regulen el SMN2
    - Ac. Valproico, Fenilbutirato, Hidroxiurea, Salbutamol



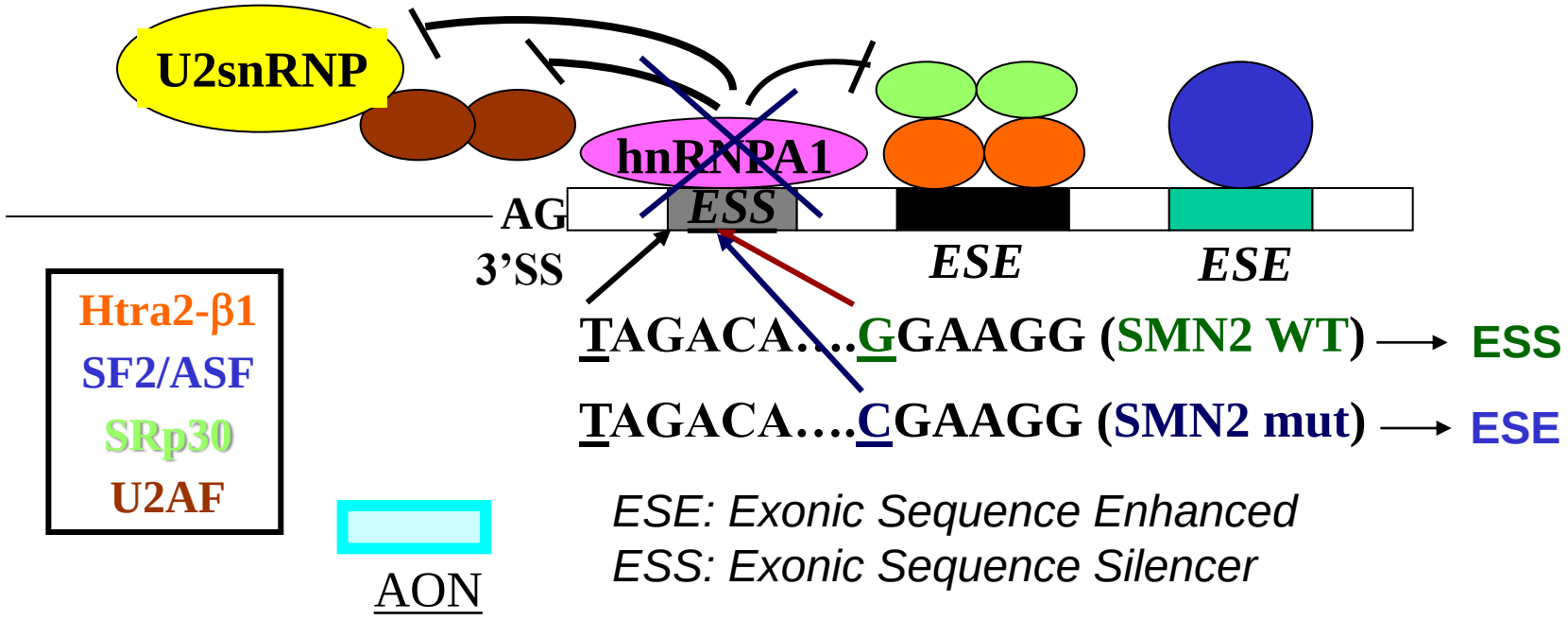
  - Incrementando la inclusión del exón 7 en los tránscritos generados por SMN2.
    - Pequeñas moléculas que se pegan al RNA y hacen incluir el exón 7 (Oligonucleótidos antisense o antisentido)

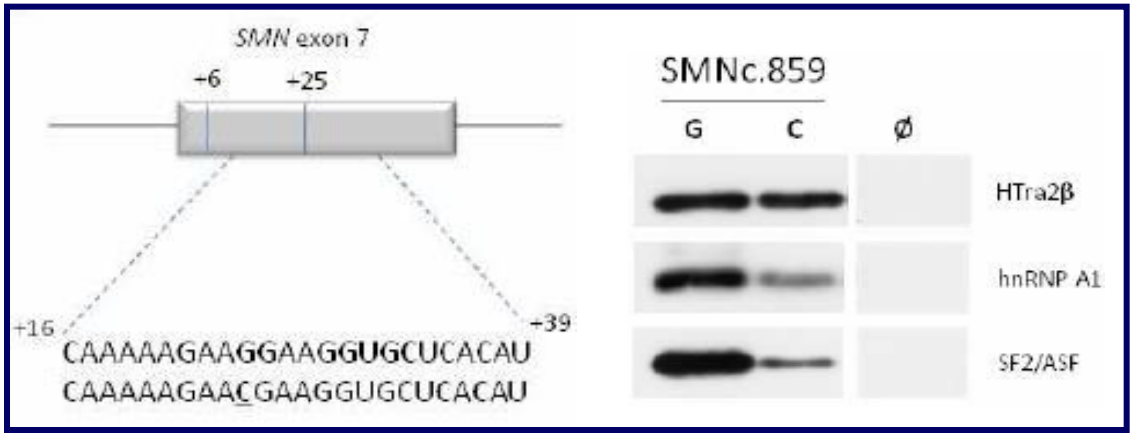
Otros fármacos en investigación: PTC SSN/Q/X (PTC Therapeutics) RG3039 (Repligen Corporation).


HAT
HDAC
HIPERACETILACION

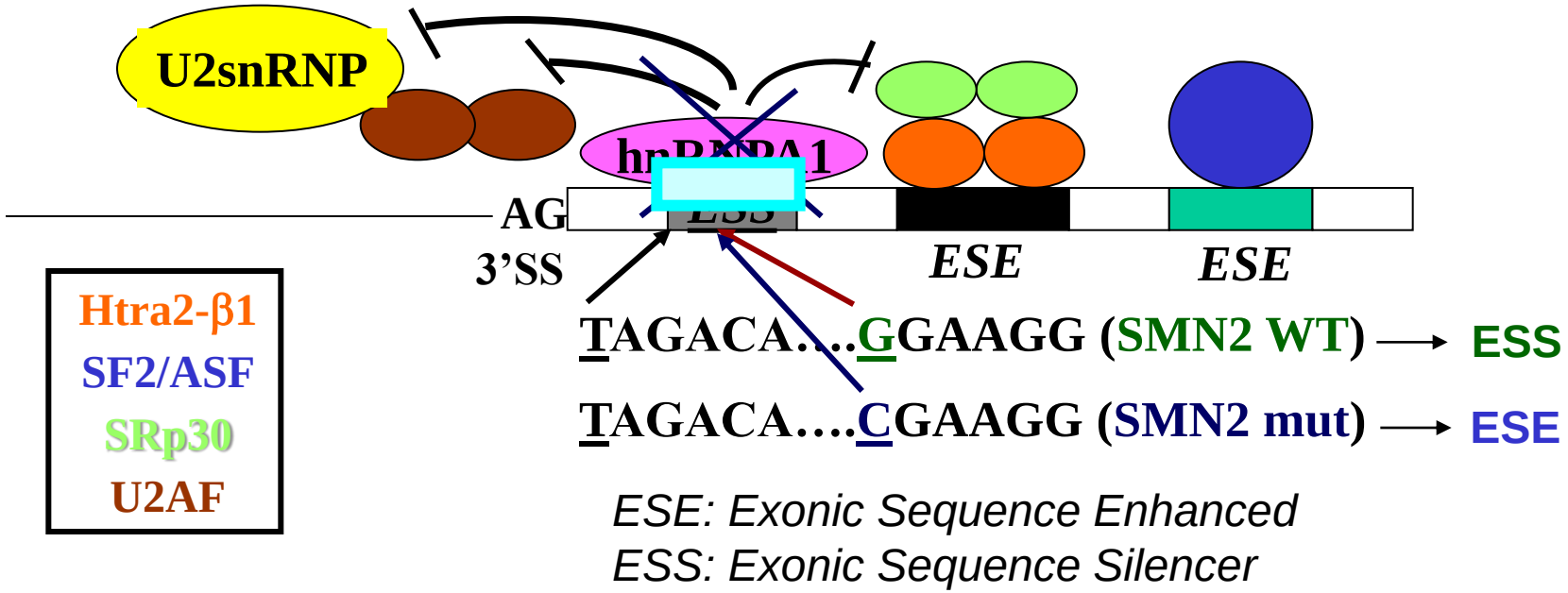


Inclusion exon 7 **(SMN2)** / exclusion exon 7 **(SMN2)**



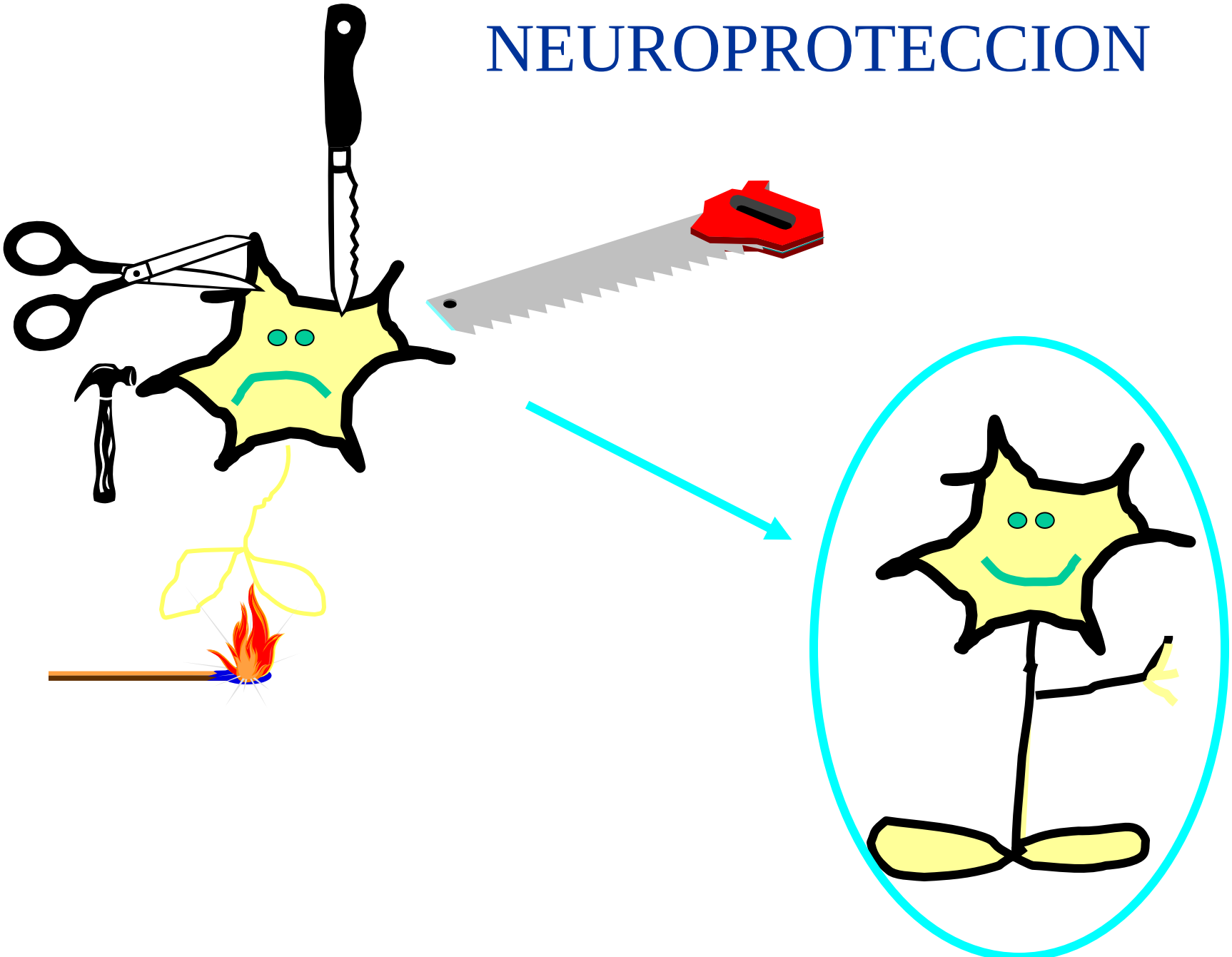


Inclusion exon 7 **(SMN2)** / exclusion exon 7 **(SMN2)**



ESE: Exonic Sequence Enhanced
ESS: Exonic Sequence Silencer

NEUROPROTECCION



Estrategias terapéuticas AME

2. Proteger las neuronas motoras del daño de la enfermedad.

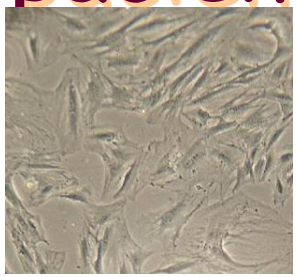
   – Independientemente del SMN, existen medicamentos neuroprotectores que podrían detener o enlentencer el proceso de muerte neuronal.
     - Riluzol, Olesoxime (Trophos), Carnitina.

Aumentar la fuerza muscular

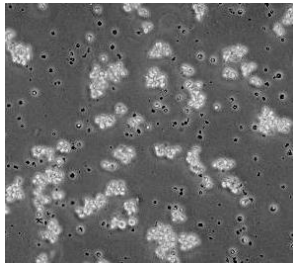
Estrategias terapéuticas AME

3. Incrementar la fuerza y resistencia muscular.
   - Fisioterapia, ejercicio moderado.
   - Folistatina incrementa el crecimiento muscular dado que es un antagonista de la miostatina (que inhibe el crecimiento). El ejercicio estimula la producción de folistatina y disminuye la miostatina.
   - Salbutamol puede estimular la fuerza muscular.
   - Carnitina está disminuída en el músculo AME.

Tratamiento in vitro en células de pacientes

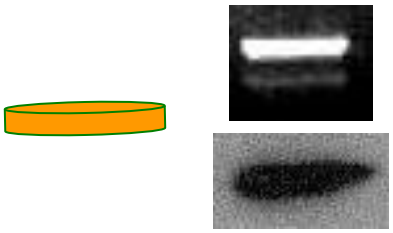


Fibroblastos

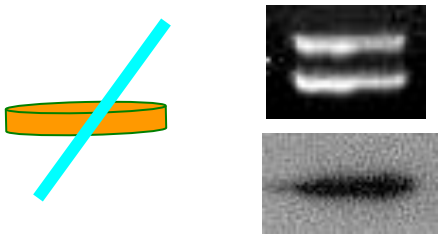


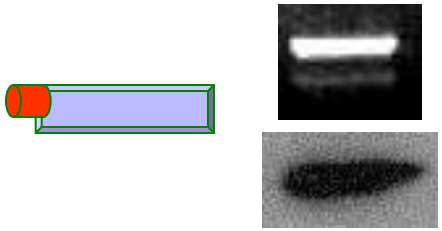
Linfoblastos

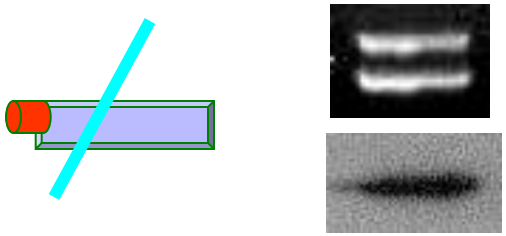
Responden



NO Responden







VARIABILIDAD INDIVIDUAL

Responden a uno si y a otro no

Responden en una célula pero no en otra

Algunos casos necesitan más dosis

Algunas casos necesitan dos fármacos

Hermanos responden diferente

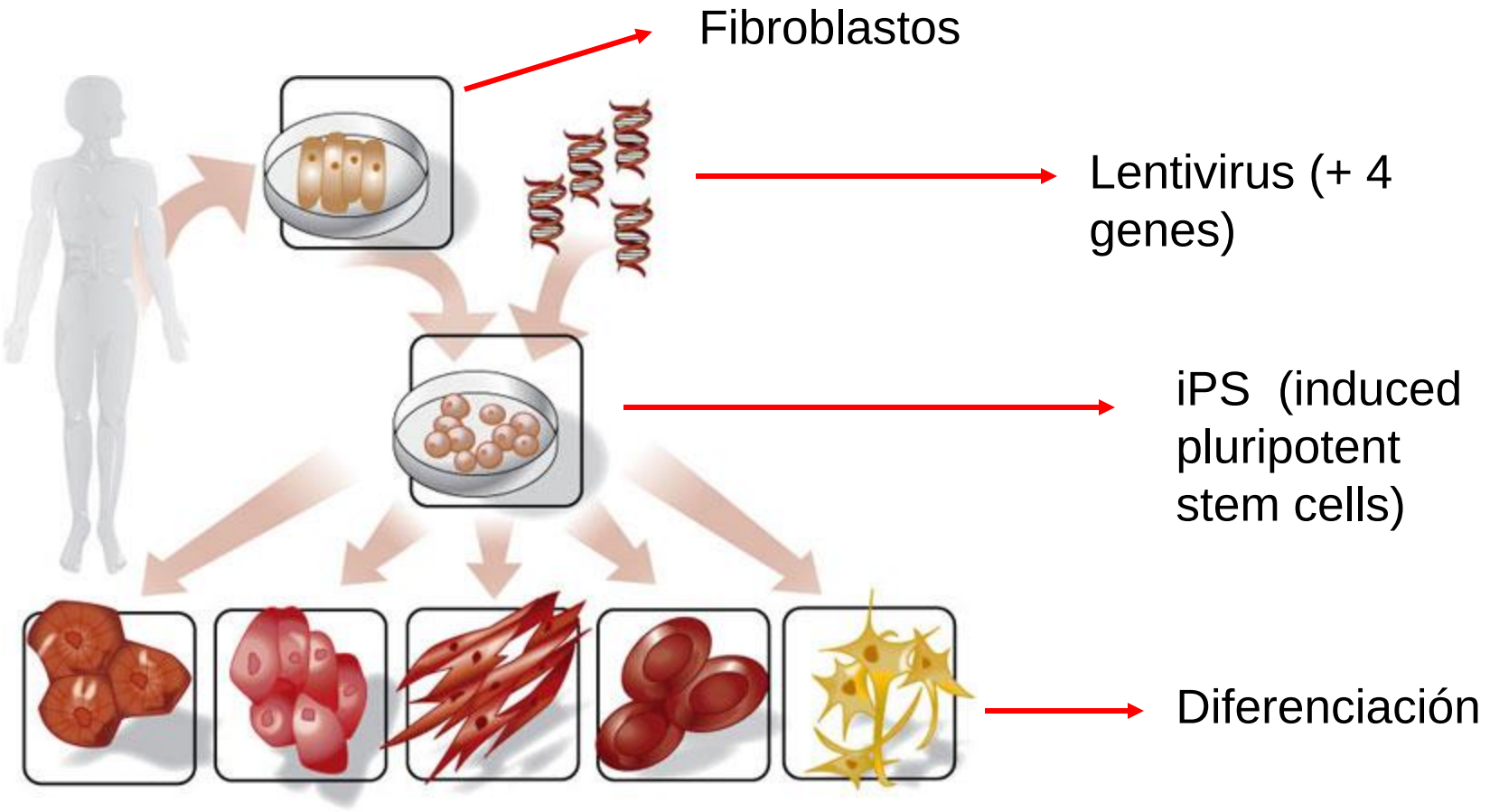
Also et al.,EJHG, 2011

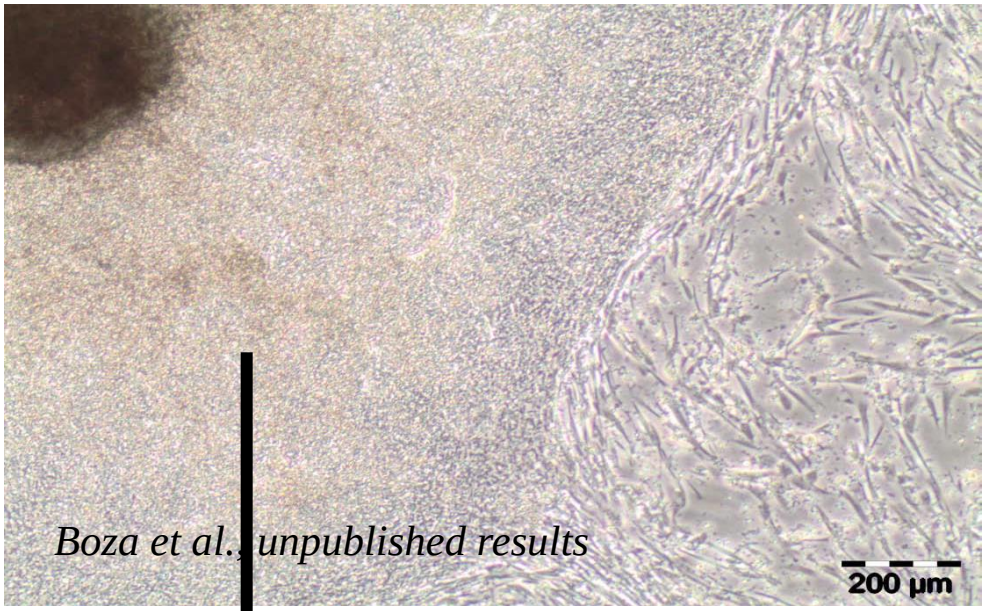
Conclusiones

- Existen responders y no responders a la terapia de incremento del SMN
- Hay diferencias intrapaciente según el tipo celular
- La región que condiciona esta respuesta no está en el gen SMN2
- Estratificar los pacientes según la respuesta in vitro
- Necesidad de establecer modelos neuronales de pacientes

Induced pluripotent stem cells from a spinal muscular atrophy patient

Allison D. Ebert[1,2], Junying Yu[3], Ferrill F. Rose Jr[4], Virginia B. Mattis[4], Christian L. Lorson[4], James A. Thomson[2,3,5] & Clive N. Svendsen[1,2,5,6]





Boza et al., unpublished results

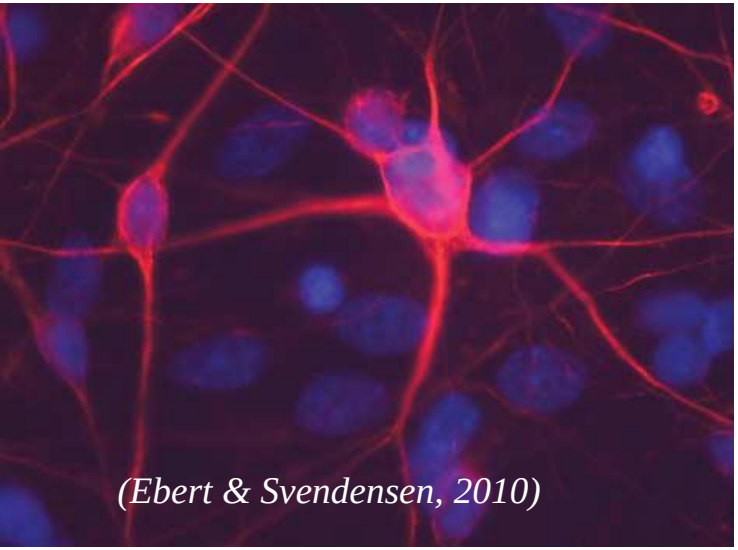
In collaboration with Royal Holloway Hospital London.

Behaviour and phenotype of motor neurons

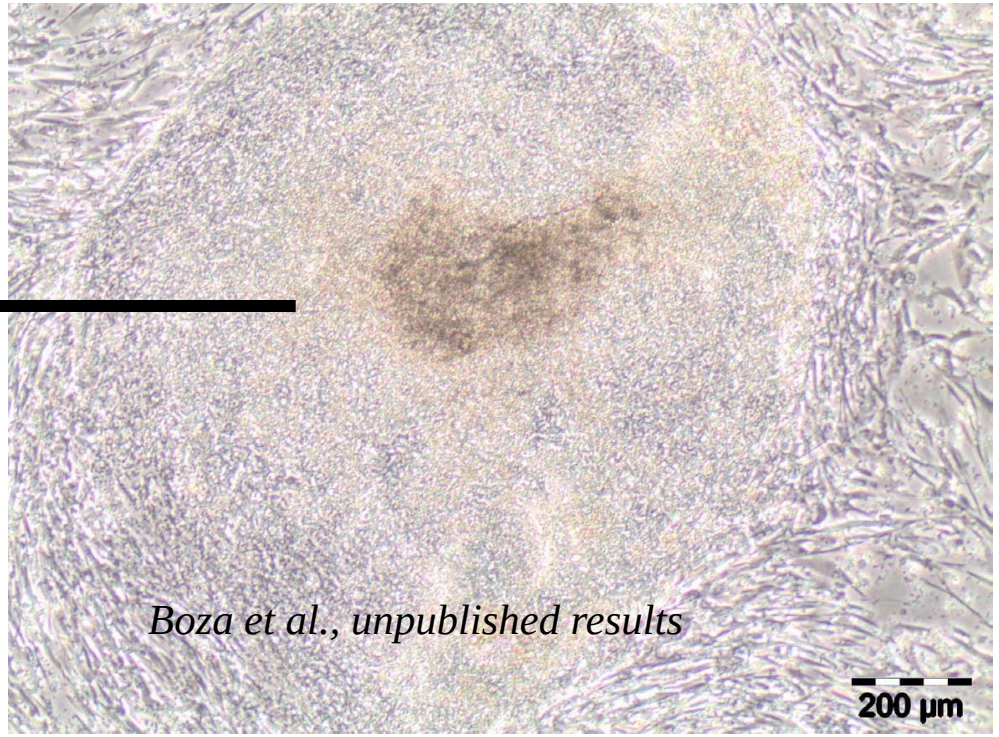
Response to drugs

Expression analysis

RNA NGS



(Ebert & Svendensen, 2010)



Boza et al., unpublished results

Estrategias terapéuticas AME

4. Transferir copias normales del gen SMN1 a la médula espinal (terapia génica).

   – AAV9 que pasa la barrera hematoencefálica
   – Aumenta la supervivencia cuando se adminstra muy precozmente al ratón SMA.
   – Se está estudiando en primates

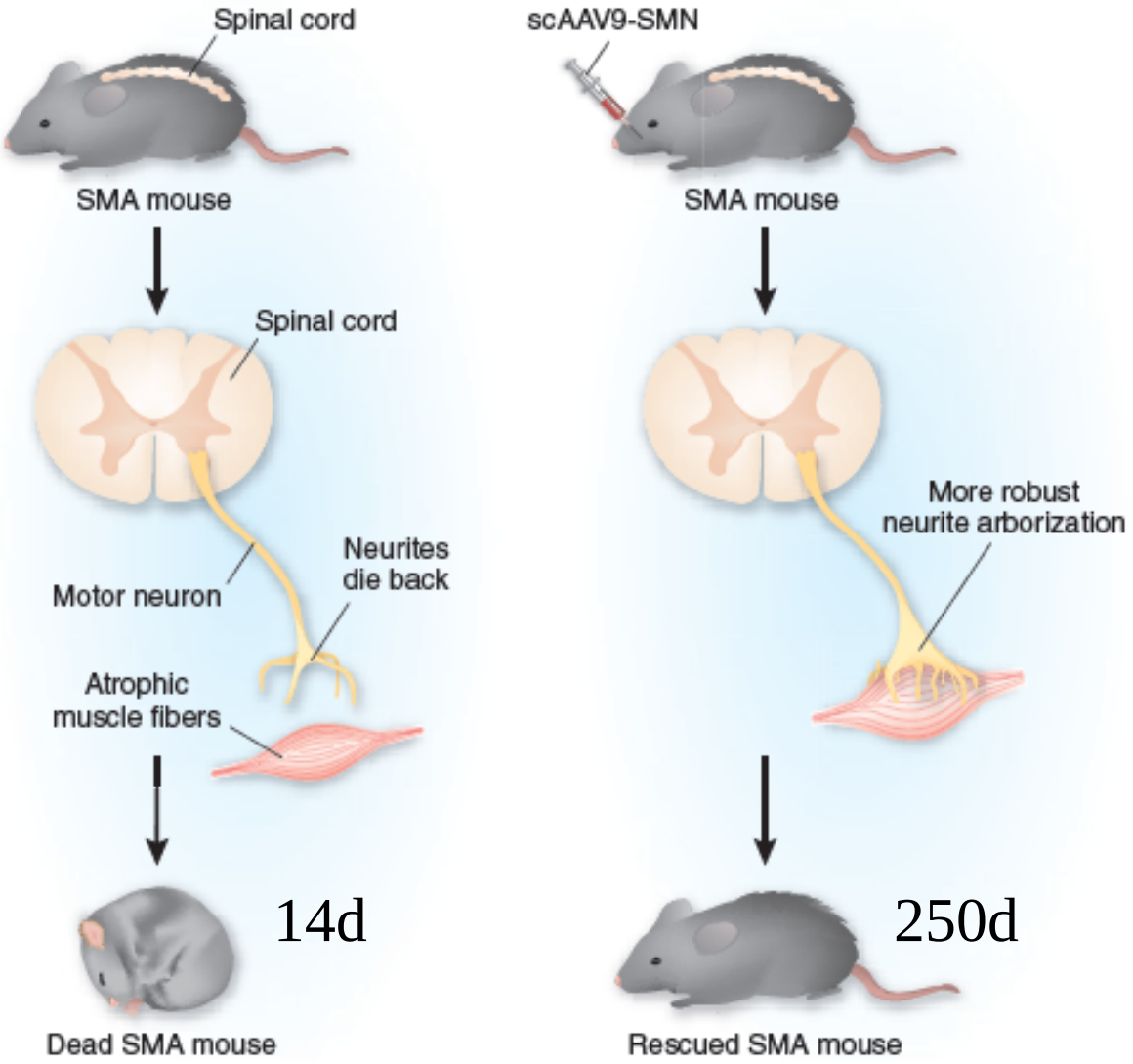


Figure 1 Gene therapy for mice with spinal muscular atrophy (SMA). SMA mice (null for the murine SMN gene and homozygous for variants of human SMN transgenes) are born with a normal motor neuron complement. However, the motor neurons undergo rapid attrition, likely a result of synaptic failure and denervation with attendant muscular atrophy. The mice become wasted and succumb at two weeks of age (left), analogous to an untreated mild human type I SMA. Injection of scAAV9-SMN into the facial vein of day-old SMA pups results in SMN expression in ~40% of motor neurons, normalization of synaptic electrophysiology and an extension of life span to >250 days, albeit at half the size of unaffected mice (right).

Gene therapy of SMA mice with scAAV9-SMN

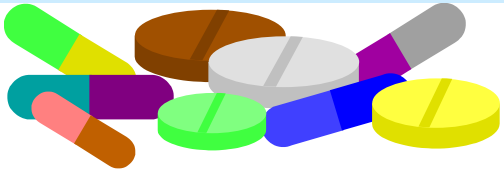
Foust et al., Nature Biotechnology, 2010

Estrategias terapéuticas AME

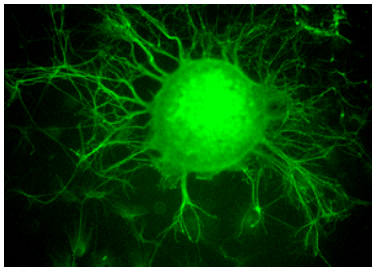
5. Sustituir las neuronas motoras por medio de la terapia celular (células madre).

   – La inyección de células madres de la estirpe neuronal es capaz de generar neuronas
   – Produce factores en el ambiente de las motoneuronas que puede ser de beneficio.
   – No migran a otros sectores de la médula (Wyatt et al., 2011)

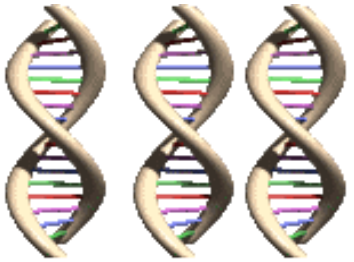
EL FUTURO: TERAPIA COMBINADA DE LA AME



Fármaco que active SMN2



¿TERAPIA CELULAR?



¿TERAPIA GENICA?

Neuroprotector

Rehabilitación

Fisioterapia

Nutrición

Respiratorio

Cirugía

Estimulante fuerza muscular

Es poco probable que una sola acción farmacológica suponga una completa solución terapéutica.

A tener en cuenta para diseñar un ensayo clinico

- Estratificación pacientes
- Historia natural
- Ventana terapéutica

Stratification

- Natural history varies among the different severity groups
- Spontaneous improvement even without treatment
- In the chronic forms the progression is relatively slow (plateau phase)
- Younger patients have more chance of improving following intervention
- Different cut off for different ages even within the same SMA type
- SMN2 copy number can be a criteria for stratification

(**SMA 1**; Rudnick-Schoneborn et al., 2009; SMA3; **Wirth** et al., 2006)

Natural history

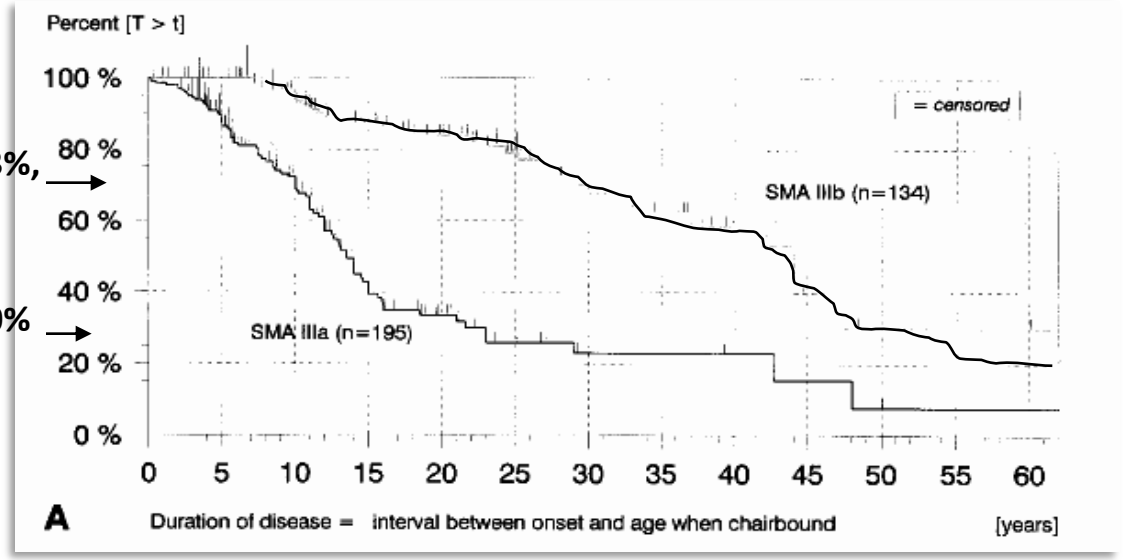
SMA type 1 has a varieble age of onset of symptoms, course and longevity						
SMA-1 subtype	Age at onset	Contractures	Neck control	Feeding	Respiratory function	Course
IA	Prenatal	+at birth	Poor	Poor	Poor	Days/ weeks Cardiac
IB	<3M	**-**	Poor	Fair	Fair	Linear decline
IC	>3M	**-**	Good/Fair	Good	Good	Plateau

Bertini et al., 2005

Natural history

70.3%,

22.0%

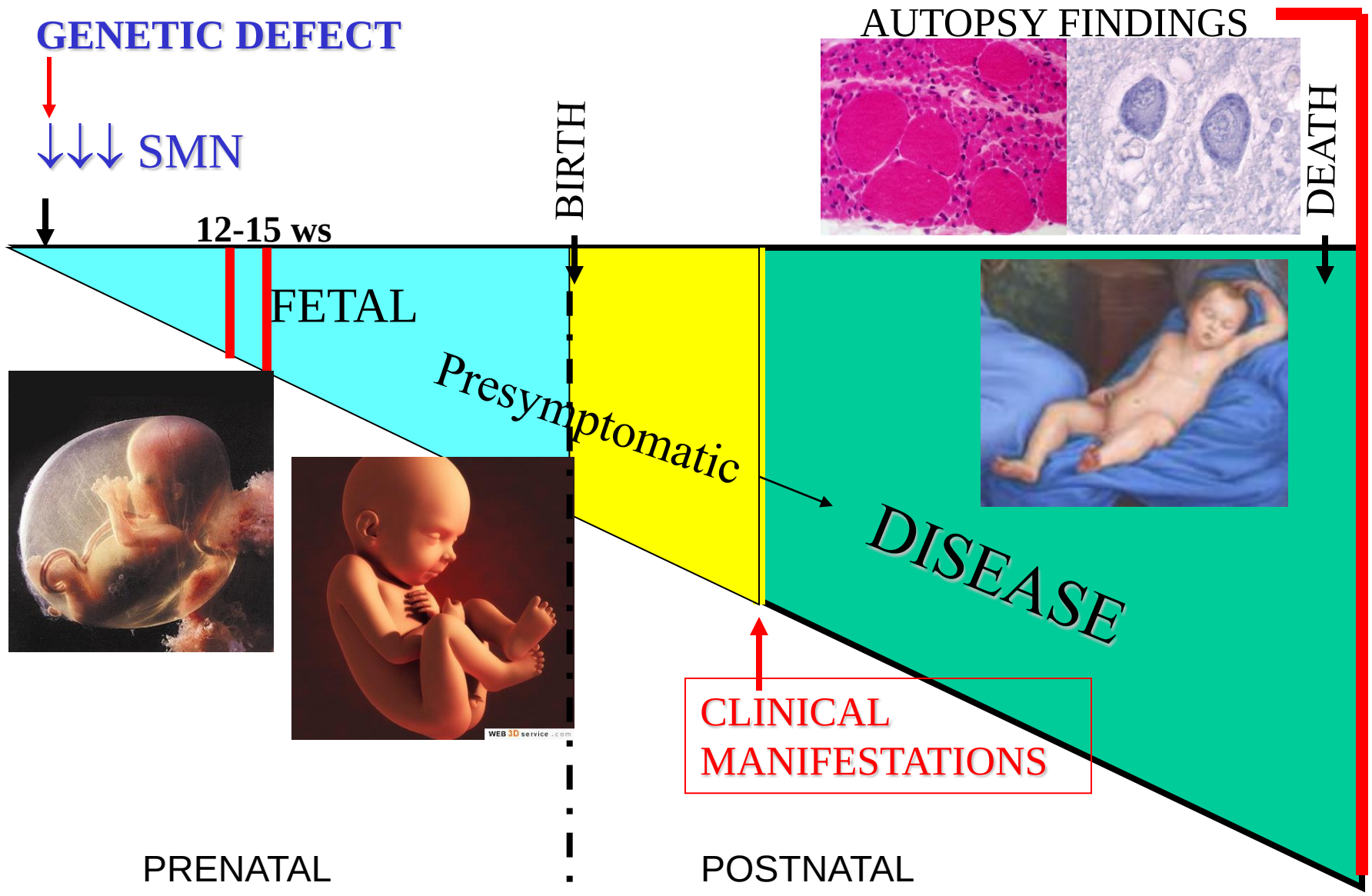


Zerres et al., J Neurol Sci. 1997;146: 67-72.

SMA type IIIa

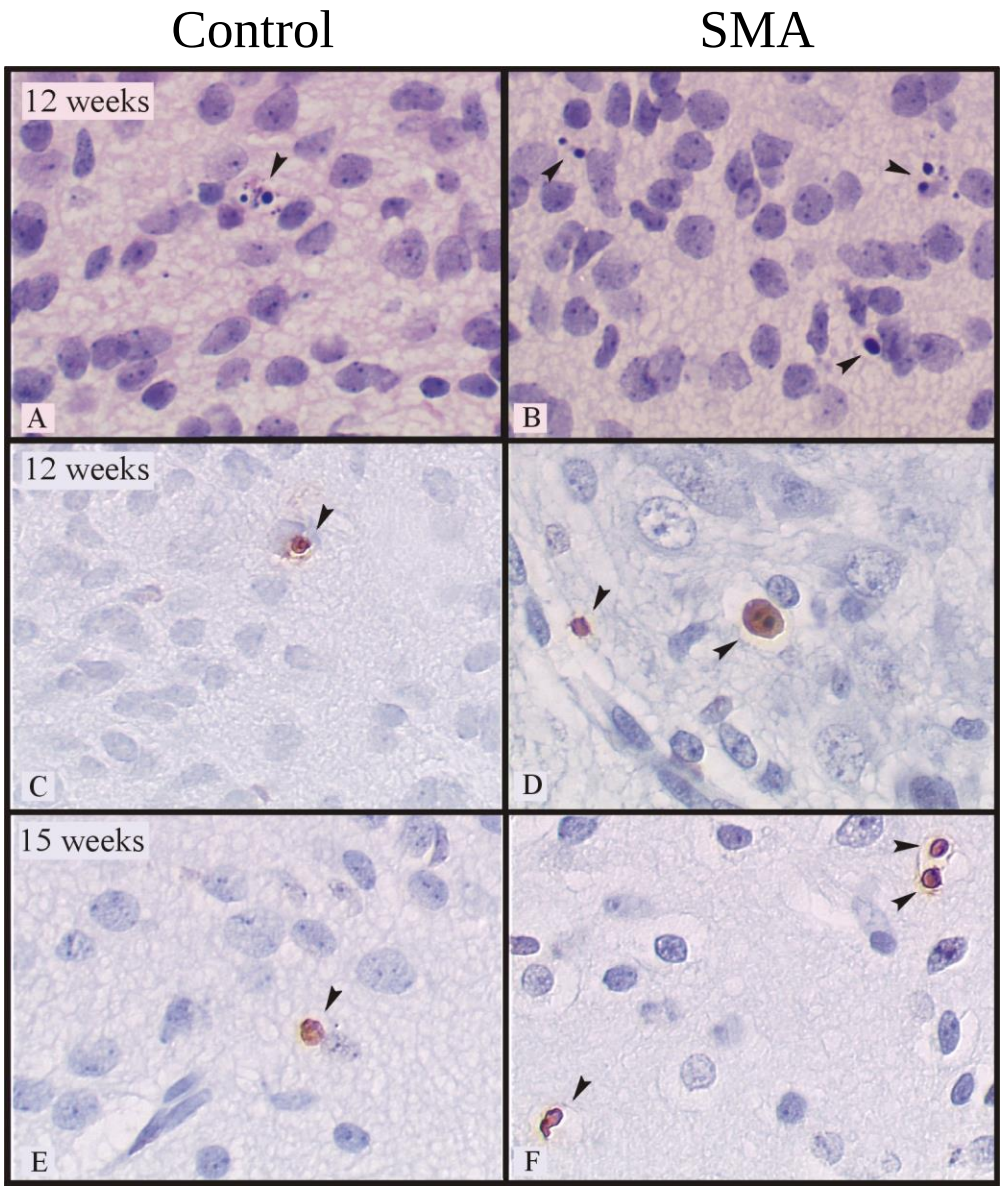
SMA type IIIb

Type I SMA stages



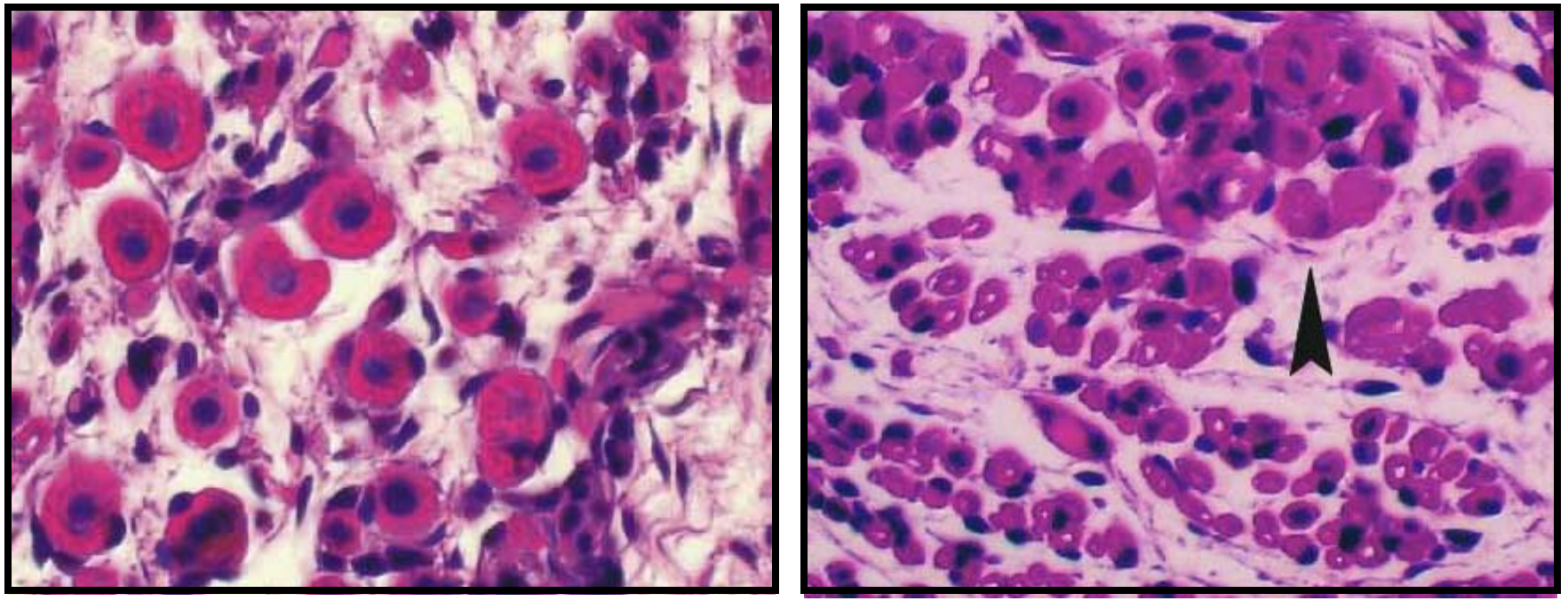
Supraphysiological neuronal death in AH

- A significant increase in cells with apoptotic morphology (intense chromatin condensation, apoptotic bodies) and DNA fragmentation was observed in the spinal cord of fetuses predicted to have type I SMA (Soler-Botija et al., *Brain* 2002).



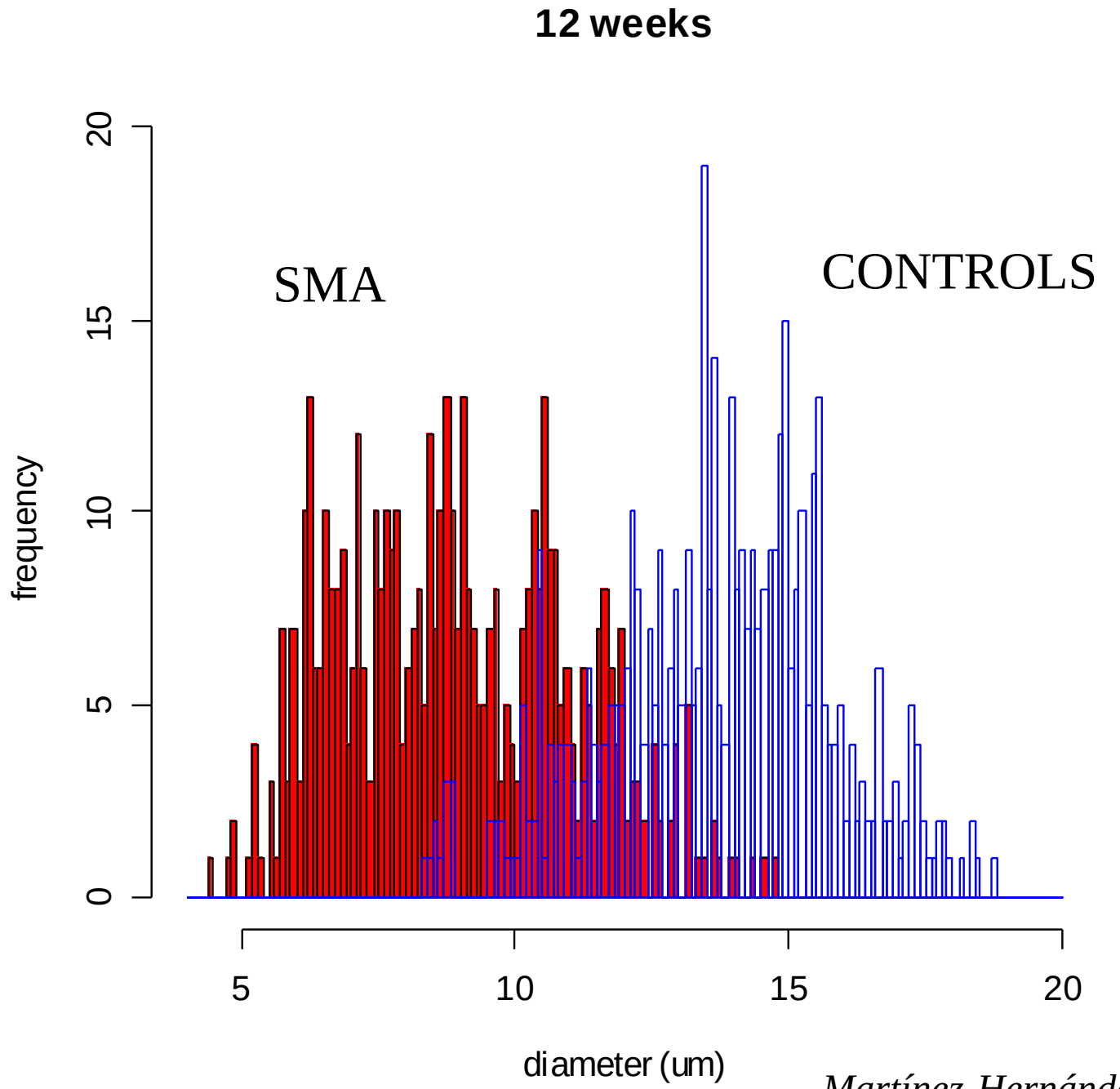
Smaller myotubes in SMA

CONTROL 12 weeks SMA

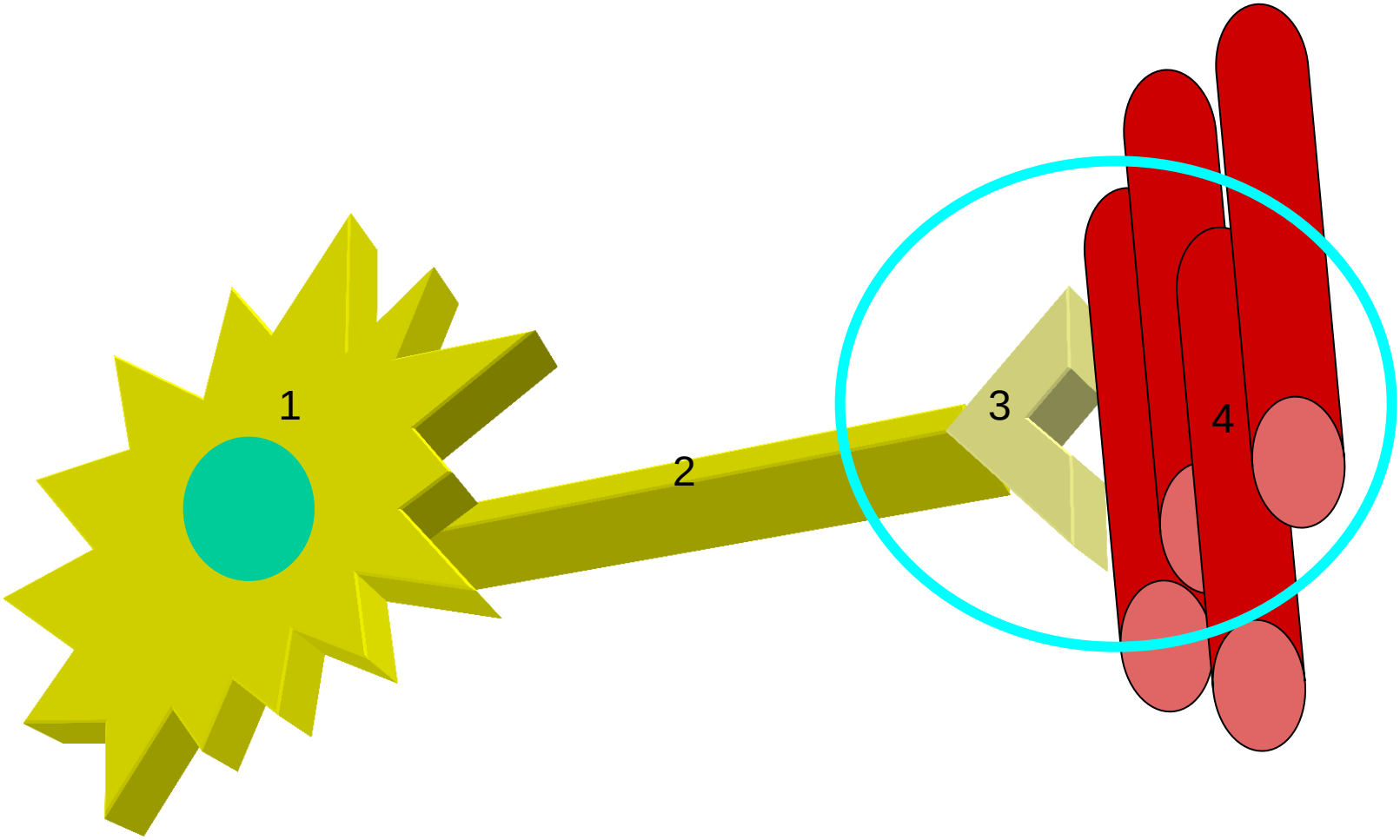


Clusters of smaller myotubes with isolated normal myotubes in SMA

Martínez-Hernández et al. (2009)



Martínez-Hernández et al. (2009)

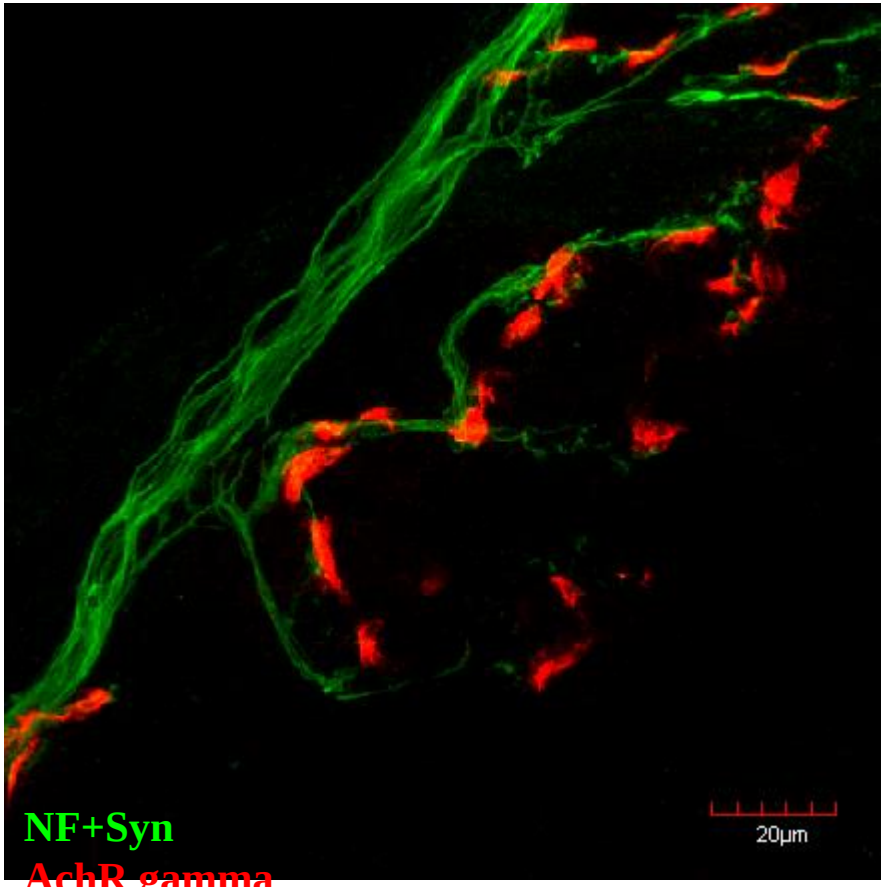


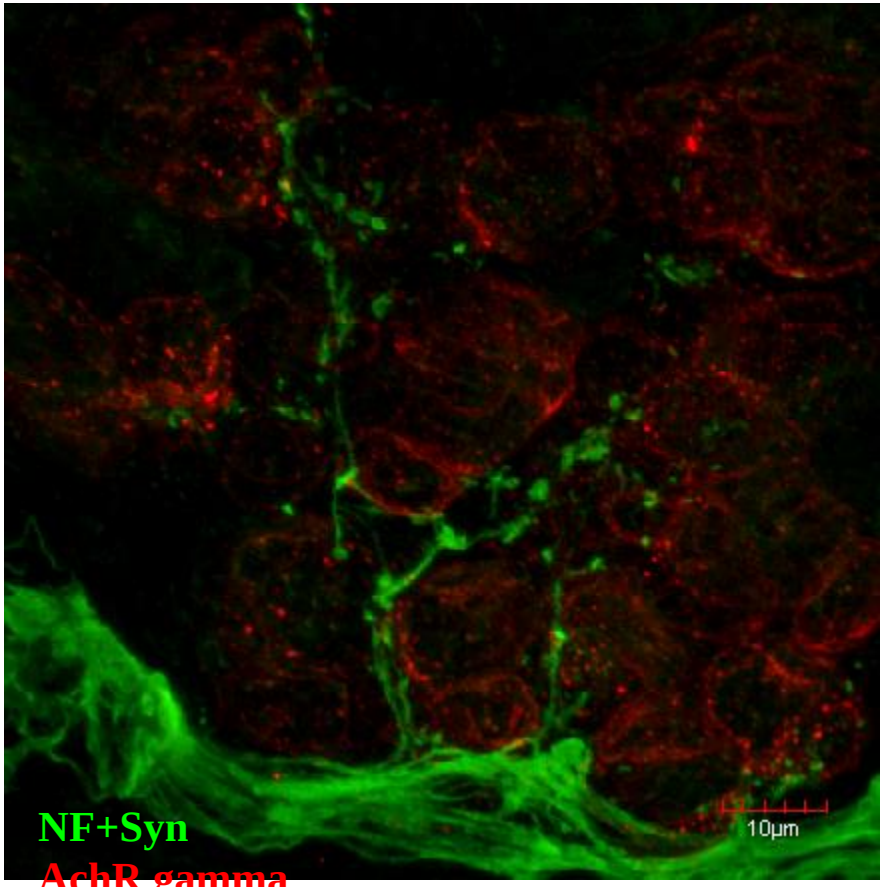
DEFECTOS EN LA UNION NEUROMUSCULAR EN LA AME

DEFECTOS EN LA MADURACION MUSCULAR EN LA AME

AXONS - ENDPLATES

Diaphragm 14 weeks Motor Endplates

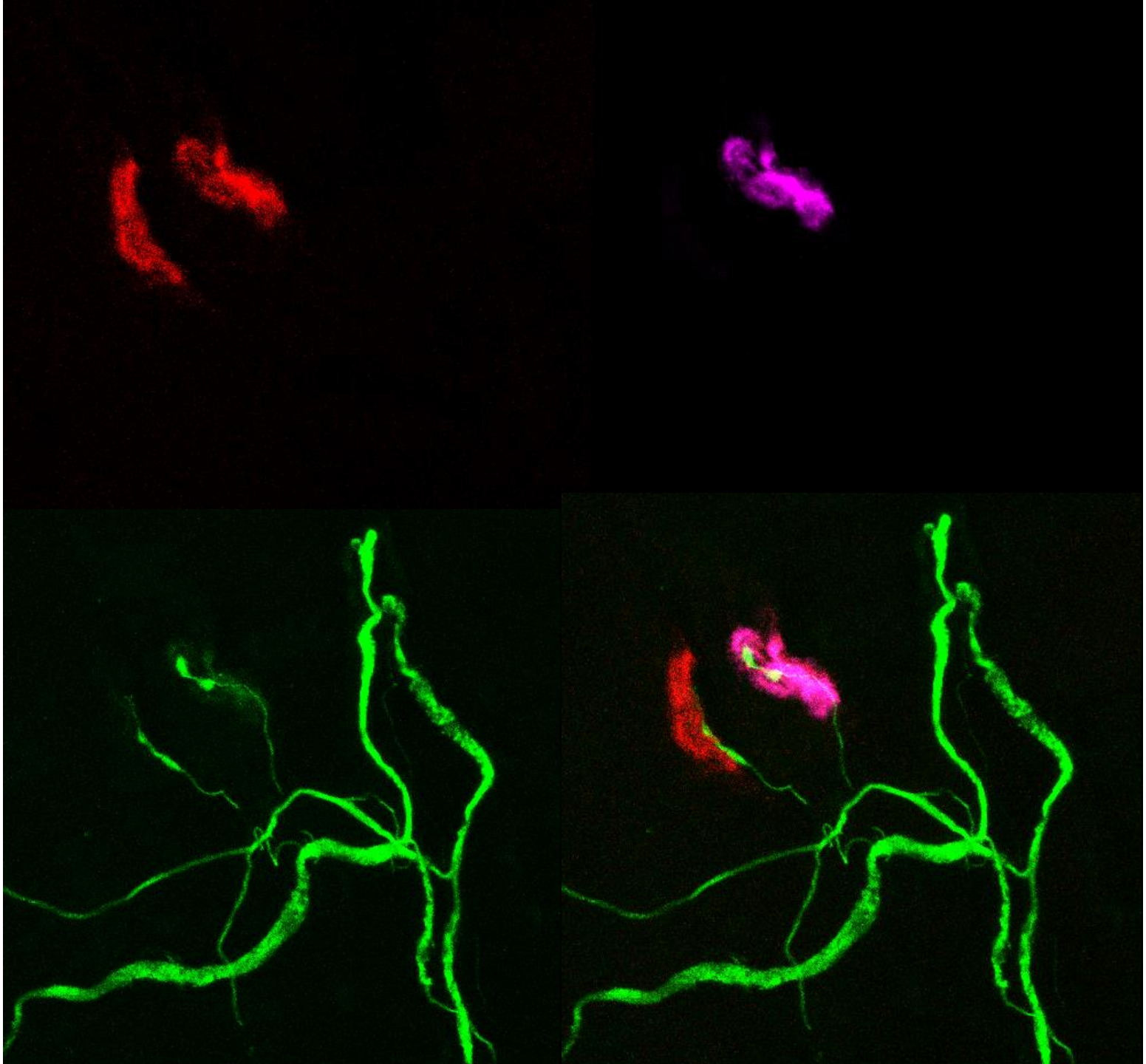


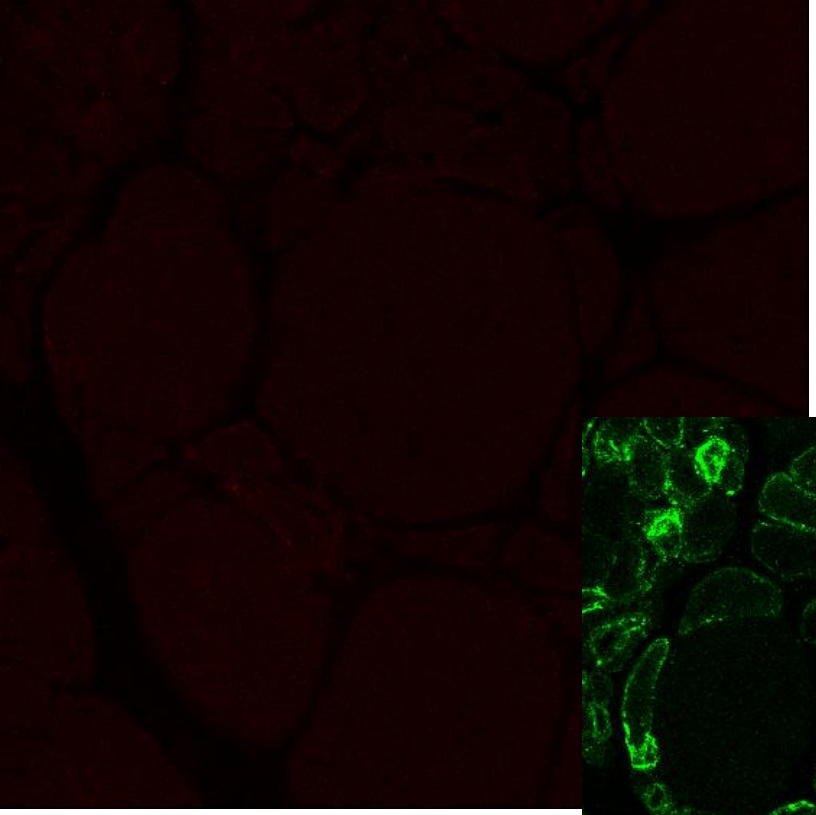


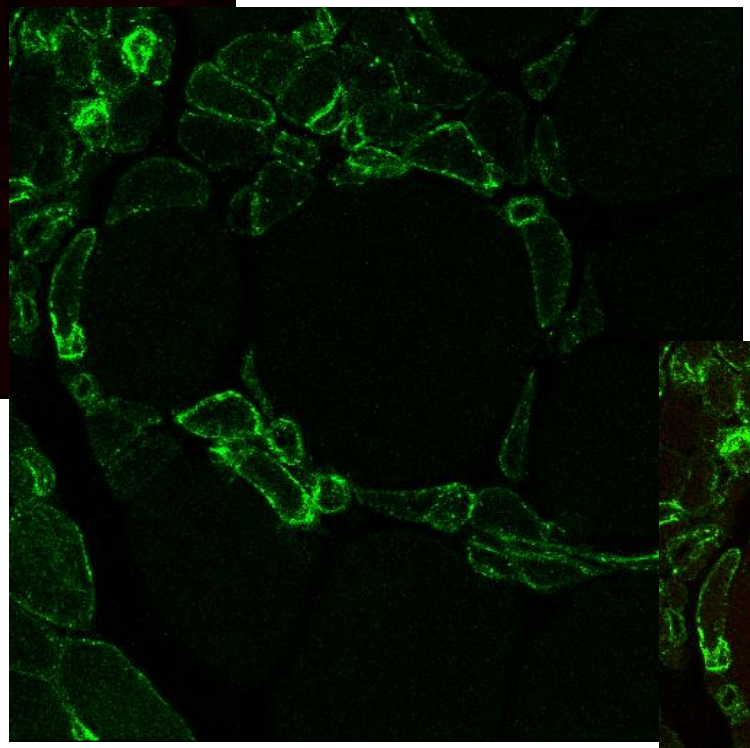
CONTROL

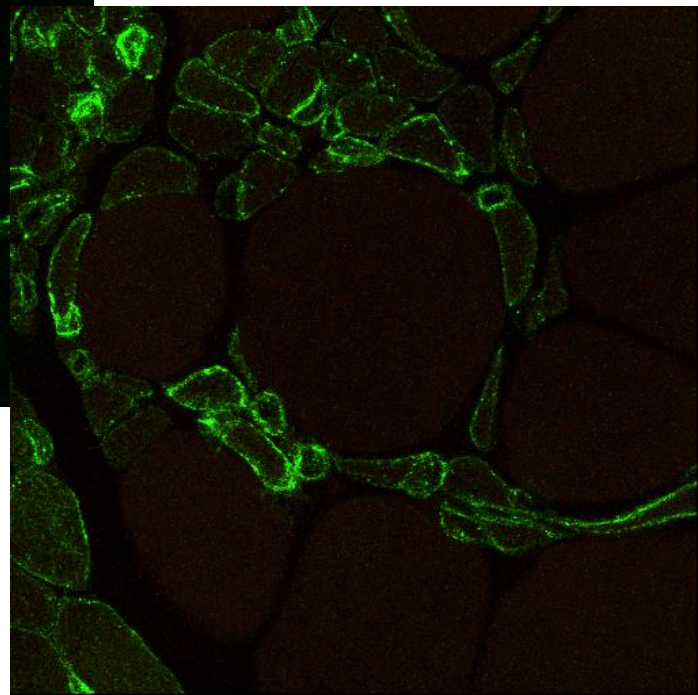
SMA

Martínez-Hernández et al. (en revisión)

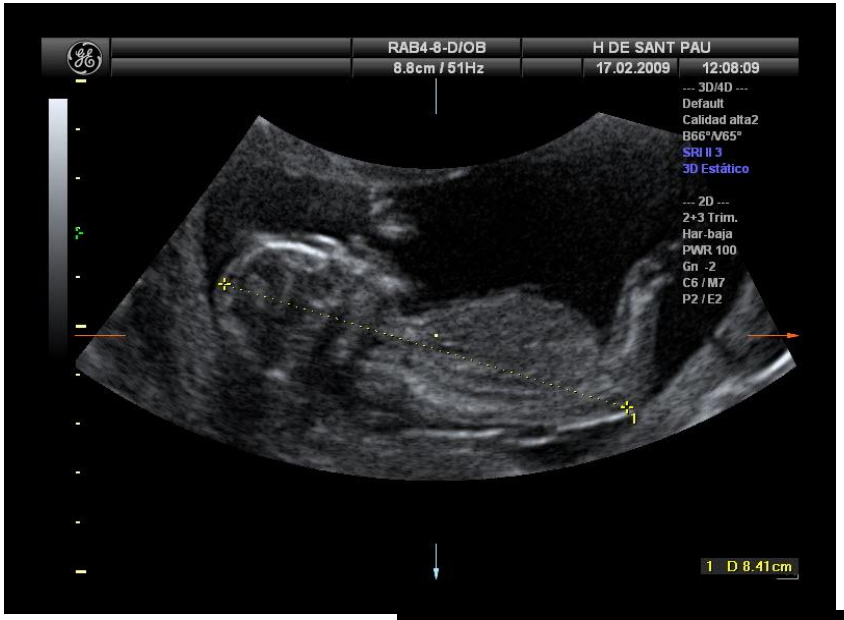


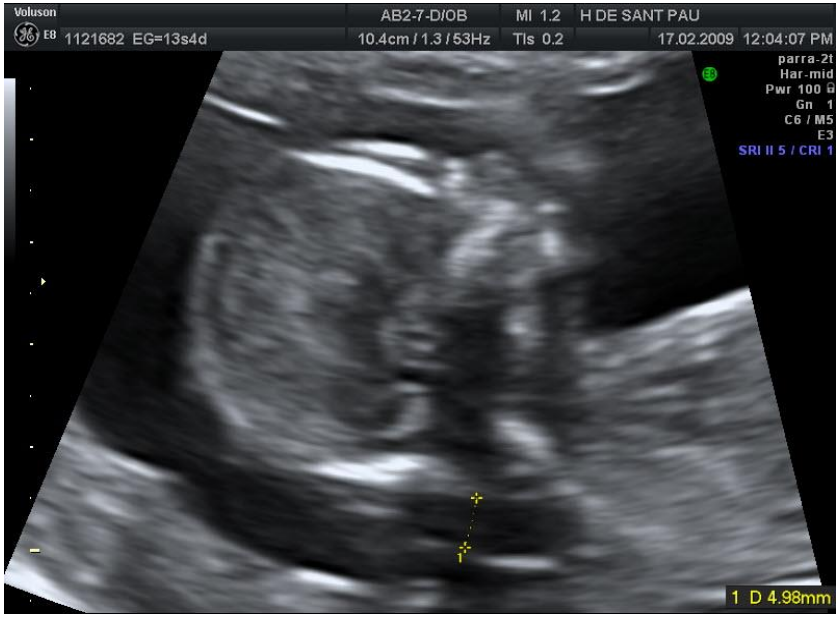


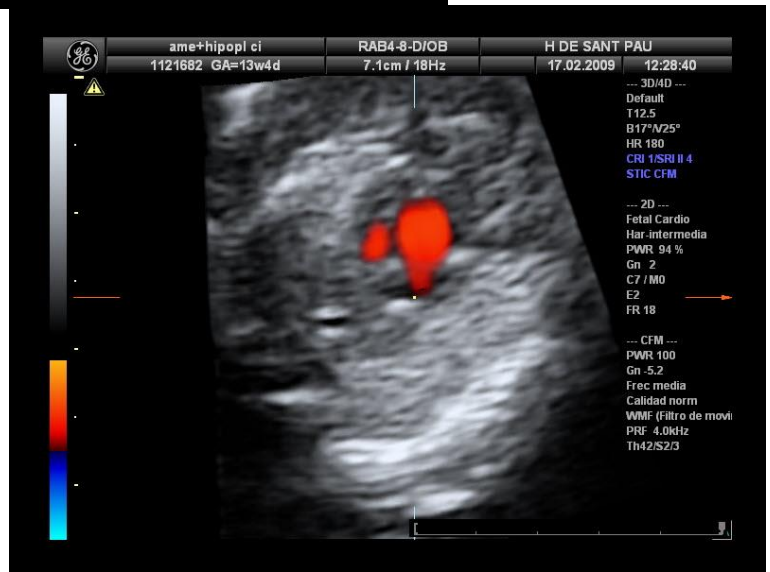




SMA case with 5mm NT, Hypoplasia of left heart and one SMN2 copy.







SMA
SMA overview
About SMA
SMA care standards
SMA patient registries
SMA research overview
Overview of therapeutic approaches for SMA
The problem
The splicing process
Therapeutic strategies for SMA
SMA clinical research and trials
SMA patient organizations
SMA meetings and events

Overview of therapeutic approaches for spinal muscular atrophy

Information on current best practice in care for people with spinal muscular atrophy (SMA) is provided in the **SMA care standards** section of this website. This research overview section aims to inform patients and their families about the approaches currently being investigated for the possible future treatment of SMA - therapies that are currently still at the laboratory or clinical trial stage. It discusses the strengths and limitations of each approach. These factors need to be carefully assessed before the alternatives described here can be implemented clinically.

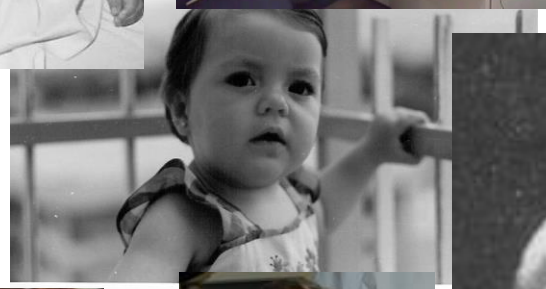
Many developments are taking place in the therapeutic approaches in SMA. This is a dynamic and promising field but as a detailed account of progress is outside the scope of this short review we provide a general overview of present investigation into SMA.

http://www.treat-nmd.eu/sma/research-overview/introduction/

GENAME Project (Genoma España- FUNDAME)
Intramural CIBERER U705.3
FIS 05-2416
FIS 08-0729





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Therapeutic strategies for SMA

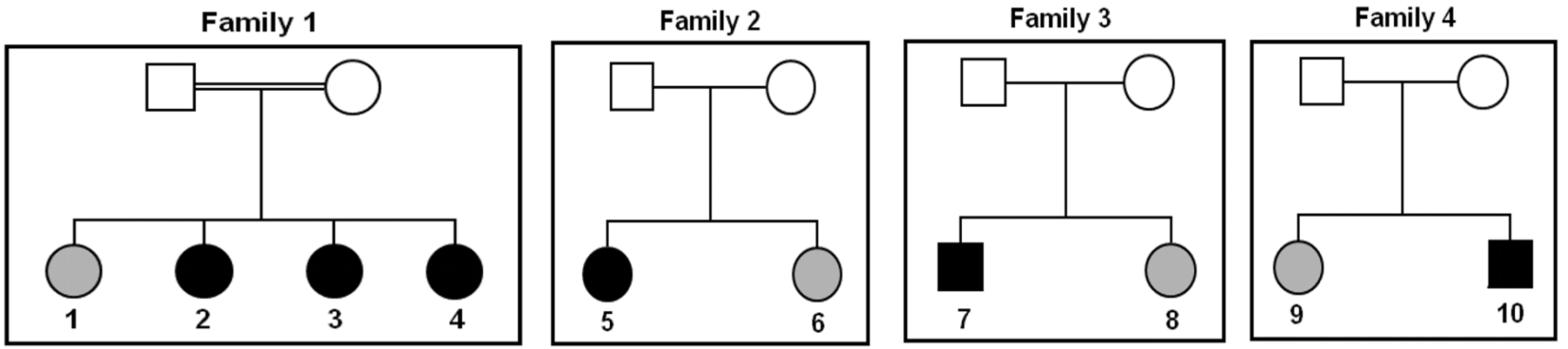
Two main strategies emerge as suitable in SMA. The first strategy directly addresses the genetic defect via SMN2 stimulation or via SMN1 replacement. The second strategy is an SMN-independent approach that aims to protect motor neurons and skeletal muscle. It seems likely that therapy would be more effective if a combination of these two strategies were used.

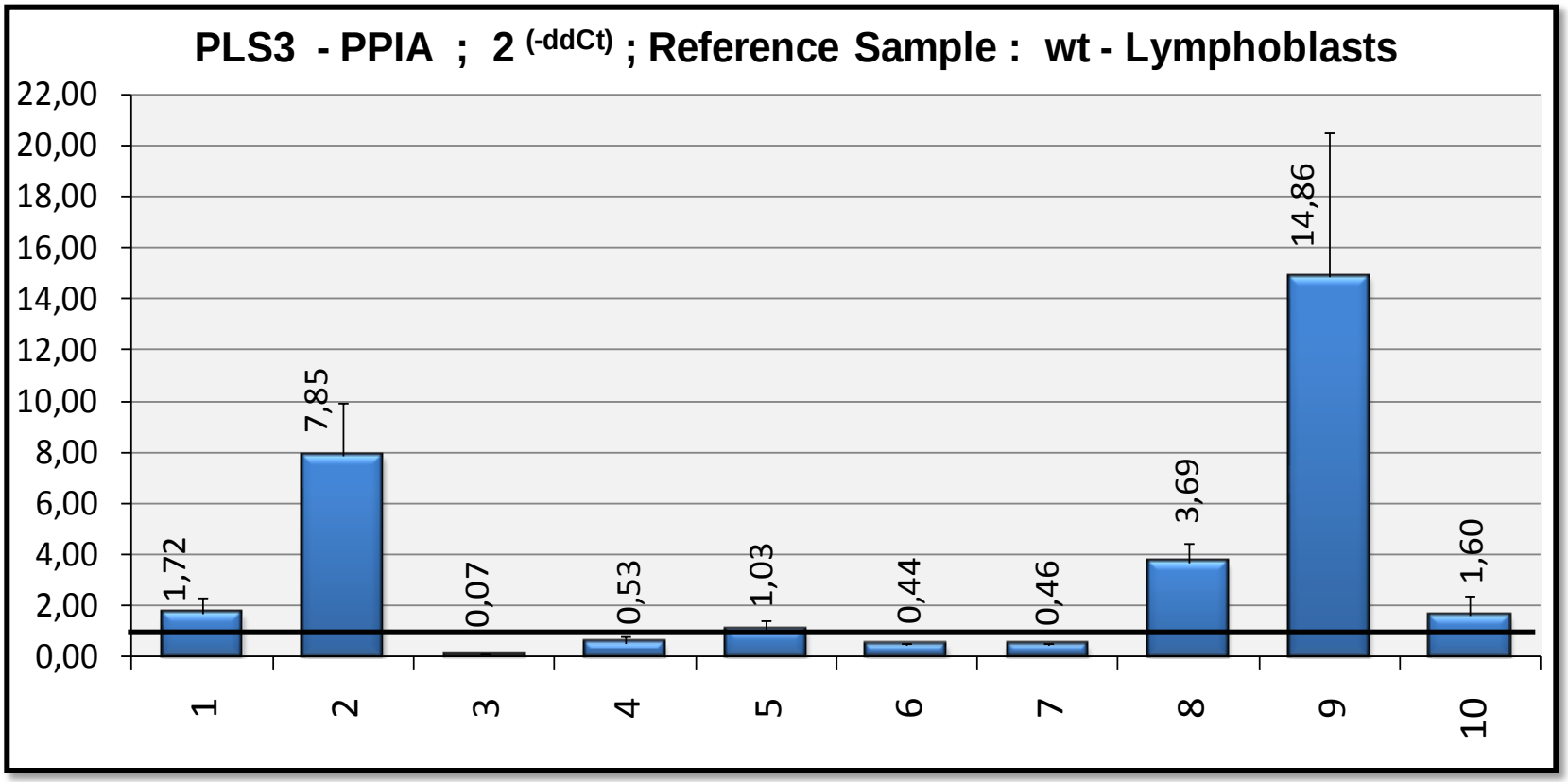
Aims of possible solutions

1. **To increase the amount of complete SMN protein produced by the SMN2 gene**
2. **To protect motor neurons from damage**
3. **To increase muscle strength and endurance**
4. **To deliver normal copies of SMN 1 by gene transfer (gene therapy)**
5. **To substitute motor neurons by cell therapy**

http://www.treat-nmd.eu/sma/research-overview/introduction/

Familias discordantes (limfoblastos inmortalizados)





Clinical Studies – Completed compilation by E. Bertini

DRUG	Proposed mechanism of action	Trial Phase	Study design	Outcome	Duration
Histone deacetylase (HDAC) inhibitors					
Sodium Phenylbutyrate II	SMN2 activation	2	RCT+Open label	HMFS, no benefit	13 weeks
Hydroxyurea II&III	SMN2 activation/splicing	2	Pilot	strenght, motor function, respiratory function no benefit	24 m
Other					
Gabapentin II	Neuroprotection	2	Open, randomized	Strength, function, resp. function, no benefit	12 m
Gabapentin III, IV	Neuroprotection	2	RCT	Strength, FVC, no benefit	12 m
Salbutamol II&III	SMN2 activation/splicing	2	Open label	FVC, strength MRC, resp. function, benefit	6 m
Salbutamol II	SMN2 activation/splicing	2	Open label	mRNA levels blood and motor function, HMFS, benefit	1 y
Riluzole I	Neuroprotection	1	RCT	Survival, safety, no benefit	9 m
Creatine II & III	Neuroprotection	2	RCT	GMFM, strenth, PEDsQL, PFT	6 m

Clinical Trials-Ongoing compilation by E. Bertini

DRUG	Proposed mechanism of action	Trial Phase	Study design	Outcome	Duration
Histone deacetylase (HDAC) inhibitors					
Valproate II-III	SMN2 activation/splicing	2	RCT	Strength	13 m
Valproate +cartinine I	SMN2 activation/splicing	1&2	Open label	Safety,	12 m
Sodium Phenylbutyrate I	SMN2 activation	1&2	Open label	mRNA and protein levels in blood	14 weeks
Sodium Phenylbutyrate Pres.	SMN2 activation	1&2	Open label	mRNA and protein levels in blood	24 m
Hydroxyurea I, II, III	SMN2 activation/splicing	1,2&3	RCT	mRNA and protein levels in blood, motor function	24 m
Other					
Salbutamol III, IV	SMN2 activation/splicing	2	RCT	mRNA levels in blood and motor function, CMAP, strength, function	1y
Riluzole II&III	Neuroprotection	2&3	RCT	Motor function	21 m
Somatotropin	Increase muscle strength	2	RCT	Strength	20 w

Discordancias fenotípicas

Hermanos haploidenticos pero con evolución diferente

Pacientes graves (tipo I) con 3 copias de SMN2

Pacientes moderados-leves (tipo II-III) con 2 copias de SMN2

J Neurol
DOI 10.1007/s00415-005-0912-y

ORIGINAL COMMUNICATION

I. Cuscó
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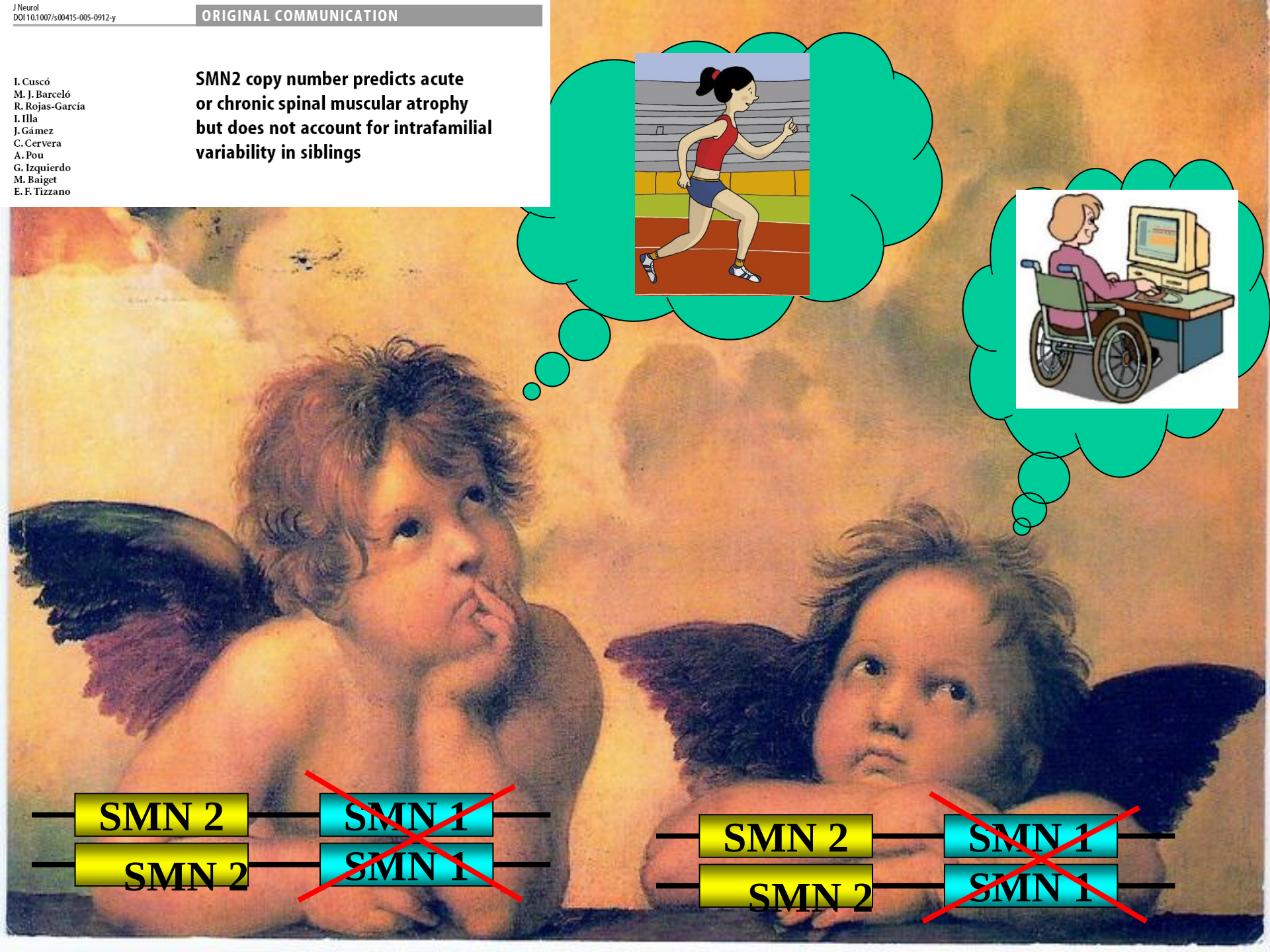
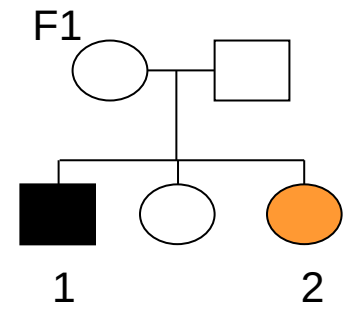
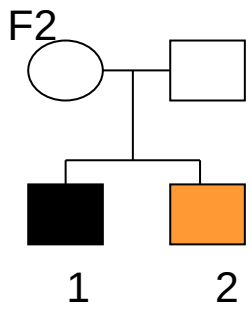
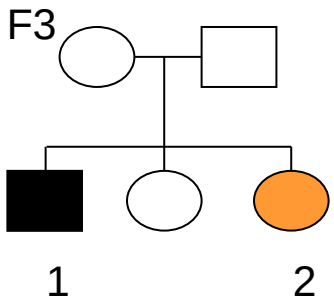
E. F. Tizzano

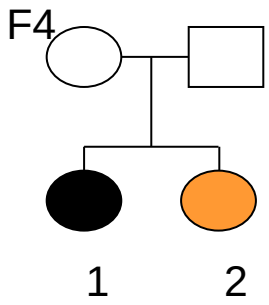
SMN2 copy number predicts acute or chronic spinal muscular atrophy but does not account for intrafamilial variability in siblings











Familias	Nº	Fenotipo	Edadt inicio	Edad de Silla de ruedaes	Manifestación de síntomas	EMG	NAIP	SMN2 còpies
F1	1	Tipo III	2	17	+++	D	+	4
	2	A	-	-	-	MUP	+	4
F2	1	Tipo III	8	12	+++	D	+	4
	2	Tipo IV	32	-	+	D	+	4
F3	1	Tipo III	2	12	+++	D	+	3
	2	Tipo III	2	-	+/++	D	+	3
F4	1	Tipo II	<1	2	++++	D	+	3
	2	Tipo III	12	20	++/+++	D	+	3

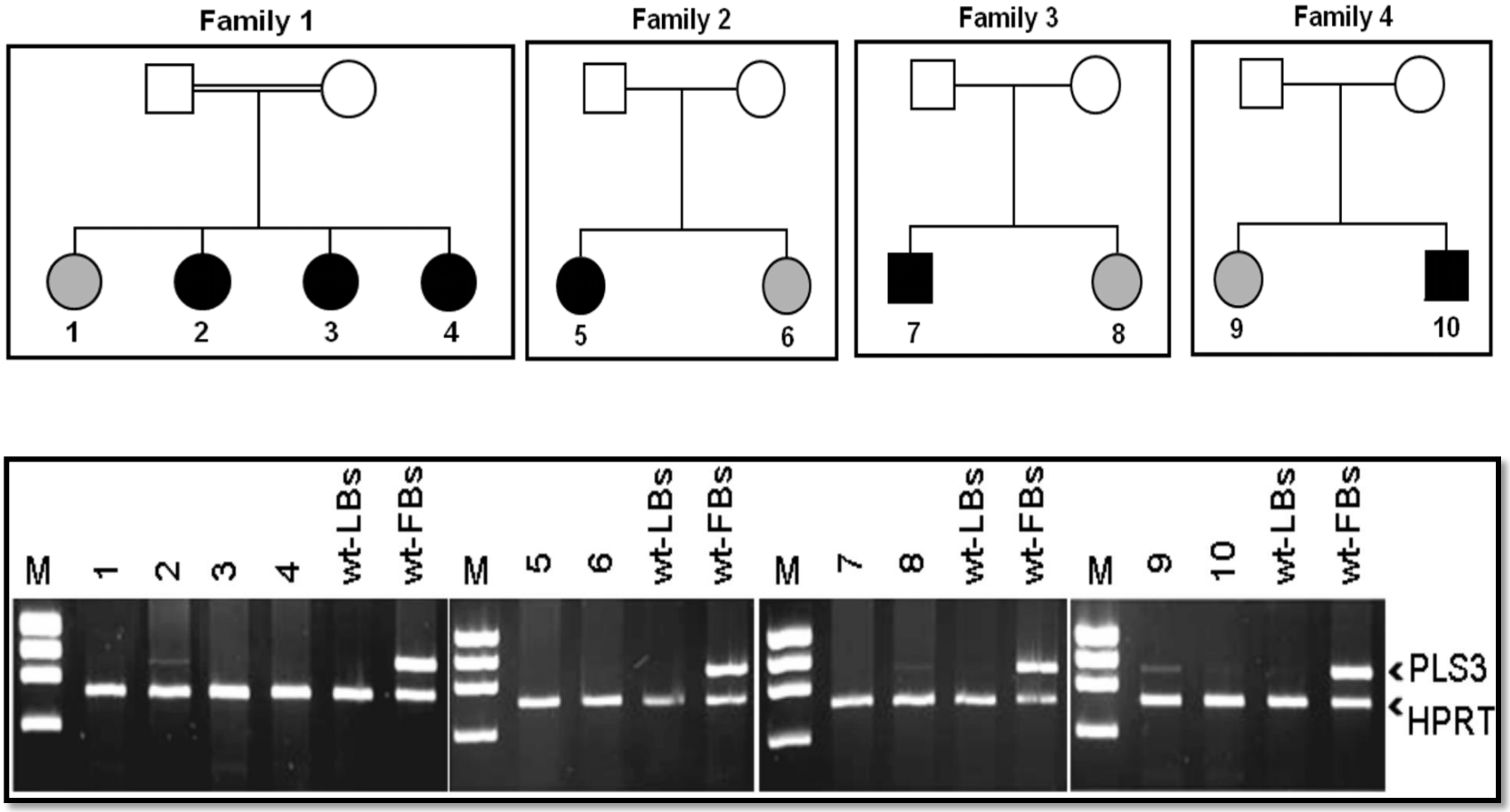
Plastin 3 Is a Protective Modifier of Autosomal Recessive Spinal Muscular Atrophy

Gabriela E. Oprea,[1,2,3] Sandra Kröber,[1,2] Michelle L. McWhorter,[4] Wilfried Rossoll,[5] Stefan Müller,[3] Michael Krawczak,[6] Gary J. Bassell,[5] Christine E. Beattie,[4] Brunhilde Wirth[1,2,3]*

Homozygous deletion of the survival motor neuron 1 gene (*SMN1*) causes spinal muscular atrophy (SMA), the most frequent genetic cause of early childhood lethality. In rare instances, however, individuals are asymptomatic despite carrying the same *SMN1* mutations as their affected siblings, thereby suggesting the influence of modifier genes. We discovered that unaffected *SMN1*-deleted females exhibit significantly higher expression of plastin 3 (*PLS3*) than their SMA-affected counterparts. We demonstrated that PLS3 is important for axonogenesis through increasing the F-actin level. Overexpression of PLS3 rescued the axon length and outgrowth defects associated with SMN down-regulation in motor neurons of SMA mouse embryos and in zebrafish. Our study suggests that defects in axonogenesis are the major cause of SMA, thereby opening new therapeutic options for SMA and similar neuromuscular diseases.

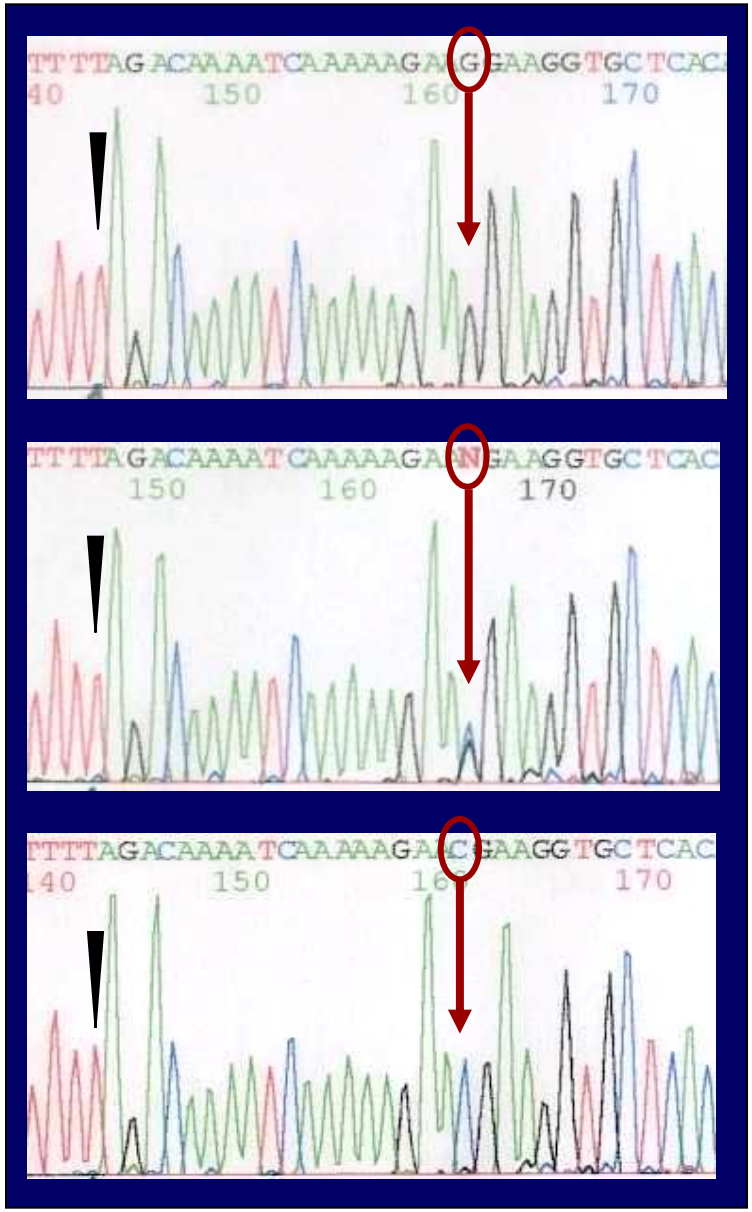
Science **320**, 524 (2008)

Familias discordantes (linfoblastos inmortalizados)



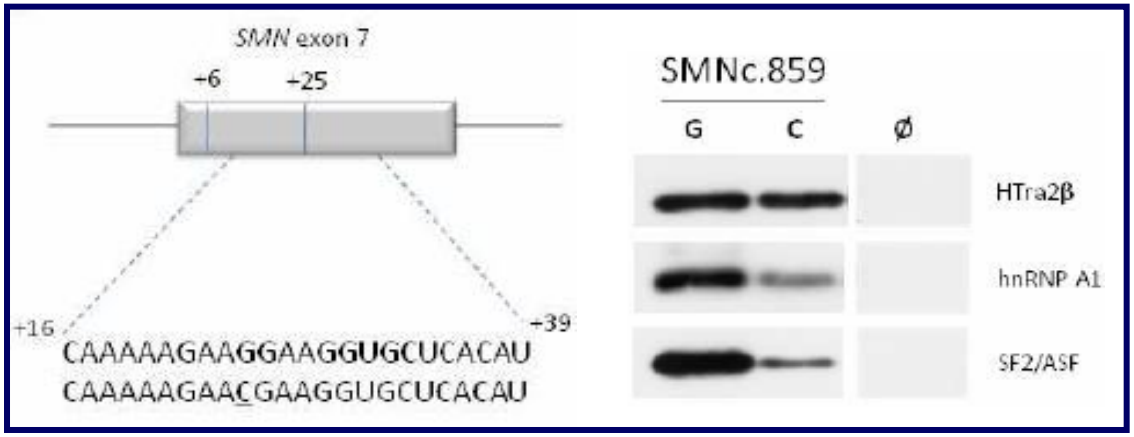
Bernal et al., 2011

Variante rara c.859 G>C en pacientes AME de origen Español

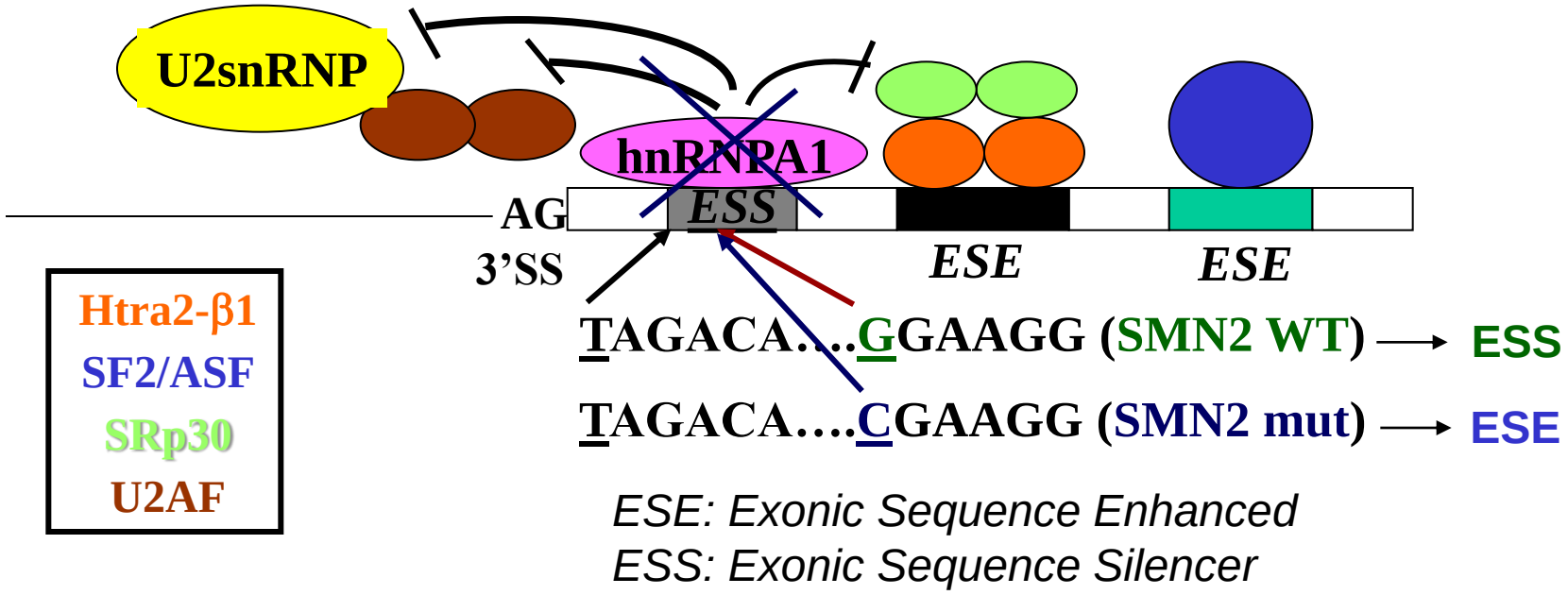


Metodología:

- Variante rara c.859 G>C
  - Posición +25 del inicio del exon 7
  - *Missense* (p.Gly287Arg)
- Secuenciación directa del exon 7 (no genera diana de restricción)
- Procedente de SMN2 debido al cambio:
  - Posición +6 del inicio del exon 7
  - Variante c.840 C>T
  - Sinónimo (p.Phe280Phe)



Inclusion exon 7 **(SMN2)** / exclusion exon 7 **(SMN2)**



ESE: Exonic Sequence Enhanced
ESS: Exonic Sequence Silencer

Table 1. Clinical and molecular data of 10 Spanish SMA patients with homozygous absence of the *SMN1* gene and with the c.859G>C variant in *SMN2*. Patients are listed according to SMA type. *NAIP* + indicates at least one copy of the *NAIP* gene. Alleles associated with the c.859G>C variant for C272 (Ag1-CA) and C212 markers are in bold. NA= parents were not available; M= Maternal; P=Paternal. *Used only to cover long distances. **Never walked. ***This patient is not yet using wheelchair because of her age although was never able to walk unaided.

Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Gender	Male	Male	Male	Male	Male	Male	Male	Male	Male	Female
Age (years)	65	36	22	59	34	18	30	12	5	3
SMA type	IIIb	IIIb	IIIb	IIIb	IIIb	IIIa	II	II	II	II
Age at onset of weakness (mths / yrs)	15 yrs	14 yrs	4 yrs	14 yrs	13 yrs	< 3 yrs	7 mths	8-9 mths	12 mths	14 mths
Walked unaided	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Wheelchair bound (age)	Yes (59 yrs)	No	No	Recently*	Recently*	Yes (6 yrs)	Yes**	Yes**	Yes**	-***
***SMN2* copies**	2	2	2	3	3	2	2	2	2	2
c.859G>C in *SMN2*	Homoz.	Homoz	Homoz	Hetz.	Hetz.	Hetz.	Hetz.	Hetz.	Hetz.	Hetz.
Telomeric *NAIP*	+	-	-	+	-	-	-	-	-	-
Parental inheritance	NA	Both	NA	NA	NA	M	P	NA	P	M
C272 alleles	**193**	**193**	**193**	189 191 **193**	181 189 **193**	181 **193**	183 **193**	181 **193**	183 **193**	181 **193**
C212 alleles	**225**	**227**	**227**	**225** **227** 233	219 221 **225**	217 **227**	217 **225**	215 **227**	217 **227**	215 **227**

- La variante c.859G>C es un modificador positivo del gen SMN2 que aumenta la cantidad de RNA completo y proteína SMN.
- Explica aproximadamente la mitad de los casos discordantes con fenotipo más benigno pero menos copias de SMN2.

Possible explanations for similar SMN2 copy number and different phenotypes

- Methylation

Human Molecular Genetics, 2009, Vol. 18, No. 2 **304–317**
doi:10.1093/hmg/ddn357
Advance Access published on October 29, 2008

Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal muscular atrophy disease severity and can be bypassed by histone deacetylase inhibition

Jan Hauke[1,2,3], Markus Riessland[1,2,3], Sebastian Lunke[4], Ilker Y. Eyüpoglu[5], Ingmar Blümcke[6], Assam El-Osta[4], Brunhilde Wirth[1,2,3] and Eric Hahnen[1,2,3,*]

SMN 2

SMN 2

I

SMN 2

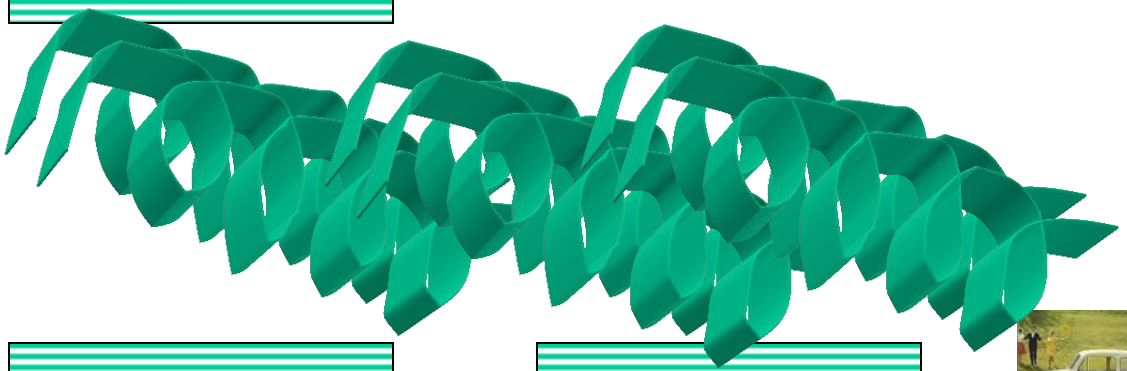
Ningún paciente se ha descrito con ausencia de los dos genes SMN

II / III

SMN 2

SMN 2

SMN 2



SMN 2

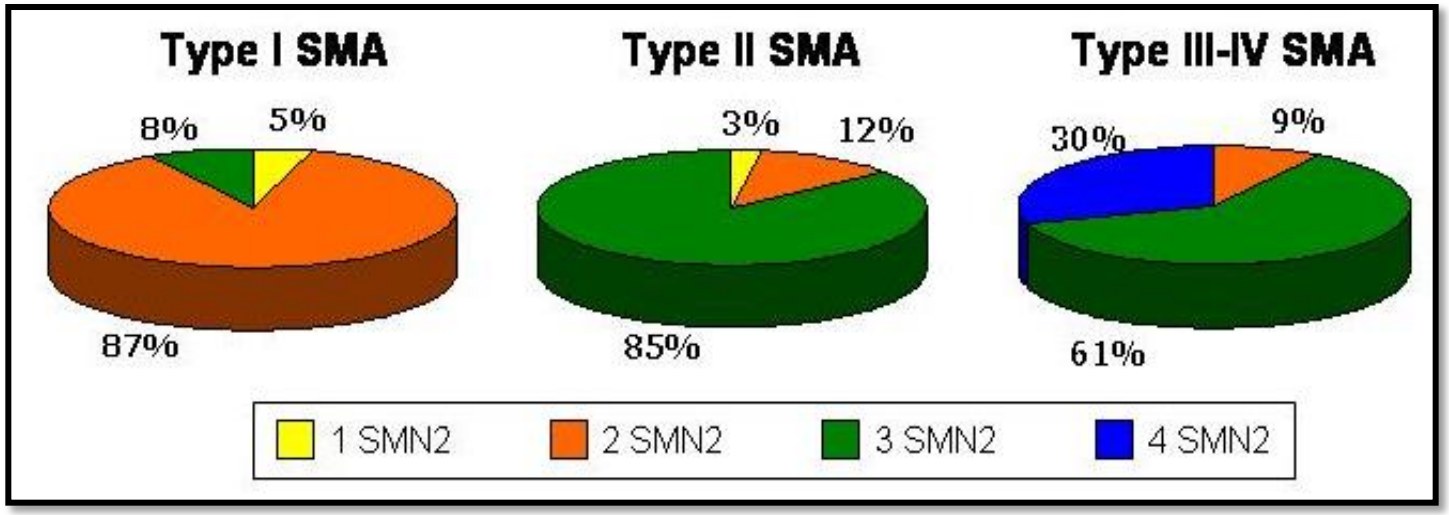
SMN 2

SMN 2

SMN 2

Clasificación : fenotipo y nº de copias SMN2

	1 SMN2	2 SMN2	3 SMN2	4 SMN2	
Type I SMA	12	220	19	0	251
Type II SMA	4	18	132	0	154
Type III-IV SMA	0	10	69	34	113
	16	248	220	34	518



- Existe una correlación inversa entre el número de copias SMN2 y la gravedad de la enfermedad, aunque no es absoluta.

Bernal et al., en preparación

Clasificación : fenotipo y nº de copias SMN2

	1 SMN2	2 SMN2	3 SMN2	4 SMN2	
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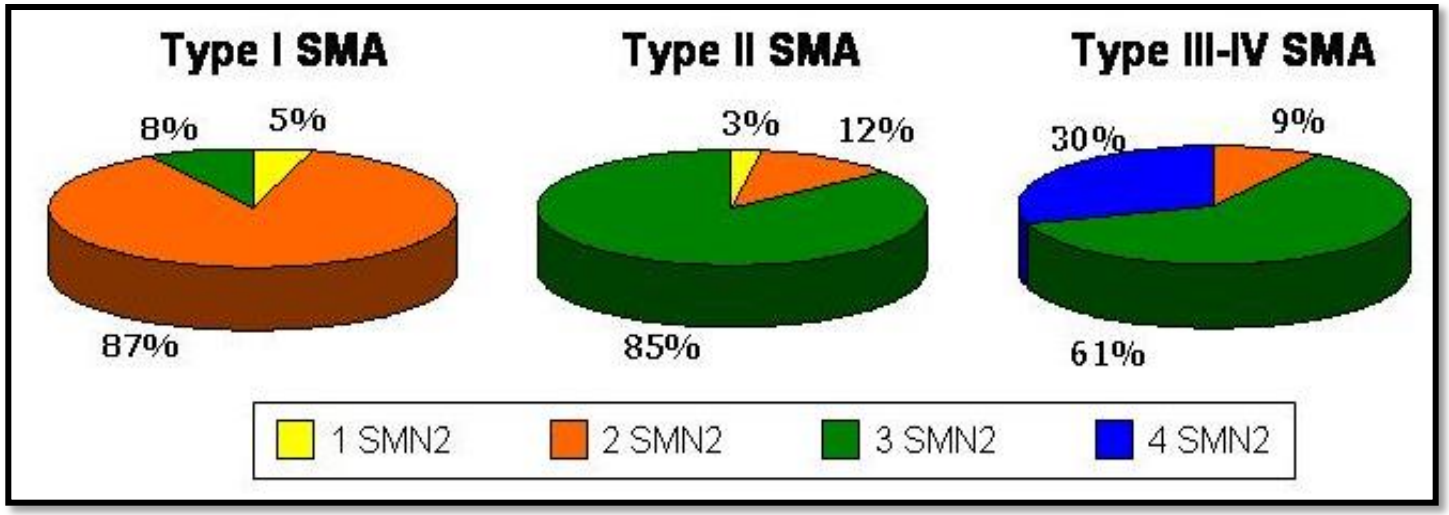

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Bernal et al., en preparación