UNIVERSITÀ DI TRENTO
Dipartimento di Biologia Cellulare, Computazionale e Integrata

Uso dei farmaci a RNA nelle demenze

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Uso dei farmaci a RNA nelle demenze …prospettive future

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Terapie a RNA (terapie su RNA)

British Medical Bulletin, 2023, 1–12
https://doi.org/10.1093/bmb/ldad010

OXFORD

Invited Review

RNA therapeutics for neurological diseases

Ilaria Brentari[1,†], Mariia Zadorozhna[2,†], Michela Alessandra Denti[1,*], and Elisa Giorgio[2,3]

www.osservatorioterapieavanzate.it

Table 1 Approved RNA therapeutics for the treatment of diseases affecting the NS

Drug name	Disease	Target	Administration Route/target organ	Approved	Company	Type of mechanism
ASO						
Nusinersen (Spinraza)	Spinal muscular dystrophy	Exon 7 of *SMN2*	IT/CNS (motoneurons)	FDA in 2016 EMA in 2017	Biogen	Splice switching: exon inclusion
Milasen	CLN7 Batten disease	Exon 6 of *MFSD8*	IT/CNS	FDA in 2019	Boston Children's Hospital	Splice switching: exon skipping
Eteplirsen (Exondys51)	Duchenne muscular dystrophy	Exon 51 of *DMD*	IV/skeletal muscle	FDA in 2016 refused by EMA	Sarepta Therapeutics	Splice switching: exon skipping
Golodirsen (Vyondys 53)	Duchenne muscular dystrophy	Exon 53 of *DMD*	IV/skeletal muscle	FDA in 2019	Sarepta Therapeutics	Splice switching: exon skipping
Viltolarsen (Viltepso)	Duchenne muscular dystrophy	Exon 53 of *DMD*	IV/skeletal muscle	FDA in 2020	NS Pharma	Splice switching: exon skipping
Carimersen (Amondys 45)	Duchenne muscular dystrophy	Exon 45 of *DMD*	IV/skeletal muscle	FDA in 2021	Sarepta Therapeutics	Splice switching: exon skipping
Fomivirsen (Vitravene)	Cytomegalovirus retinitis (immunocompromised patients)	*UL123*	IVT/eye	FDA in 1998 EMA in 1999 Withdrawn from market	Ionis Pharmaceuticals	Translation inhibition
Inotersen (Tegsedi)	Hereditary transthyretin amyloidosis (polyneuropathy)	*TTR*	SC/liver	FDA in 2018 EMA in 2018	Ionis Pharmaceuticals	mRNA degradation
Valeriasen	Developmental and epileptic encephalopathy-14	*KCNT1*	IT/CNS	FDA in 2020	Boston Children's Hospital	mRNA degradation
Tofersen (Qualsody)	Amyotrophic lateral sclerosis	*SOD1*	IT/CNS (motoneurons)	FDA in 2023	Biogen	mRNA degradation
siRNA						
Patisiran (Onpattro)	Hereditary transthyretin amyloidosis (polyneuropathy)	*TTR*	IV/liver	FDA in 2018 EMA in 2018	Alnylam Pharmaceuticals	mRNA degradation
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Givosiran (Givlaari)	Acute hepatic porphyria	*ALAS1*	SC/liver	FDA in 2019 EMA in 2020	Alnylam Pharmaceuticals	mRNA degradation
RNA aptamer						
Pegaptanib (Macugen)	Age-related macular degeneration	*VEGF(165)*	IVT/eye	FDA in 2004 EMA in 2006	OSI pharmaceuticals	Protein inhibition

ASO: antisense oligonucleotide; siRNA: short interfering RNA; IT: intrathecal; CNS: central nervous system; IV: intravenous; IVT: intravitreal; SC: subcutaneous.

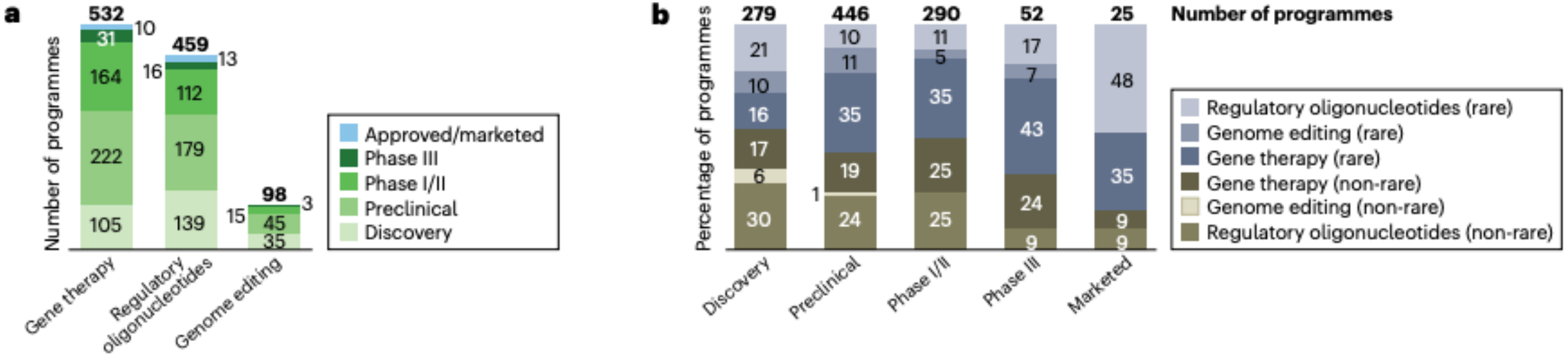
Spice switching:
- Exon inclusion
- Exon skipping

mRNA degradation:
- Gapmers
- siRNAs

From the analyst's couch

https://doi.org/10.1038/d41573-023-00098-6

Genomic medicines: the coming waves?

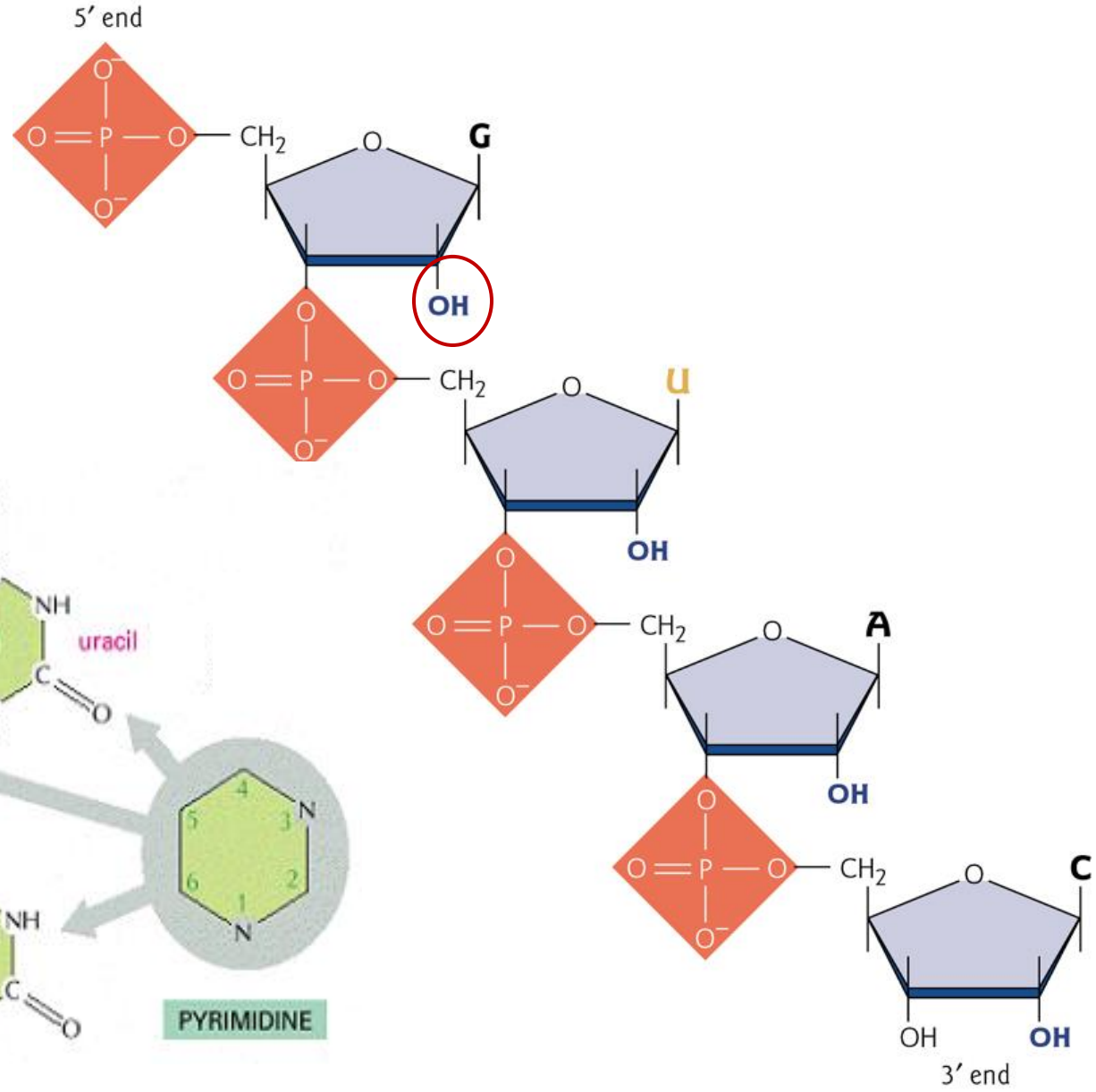


Oligonucleotidi regolatori

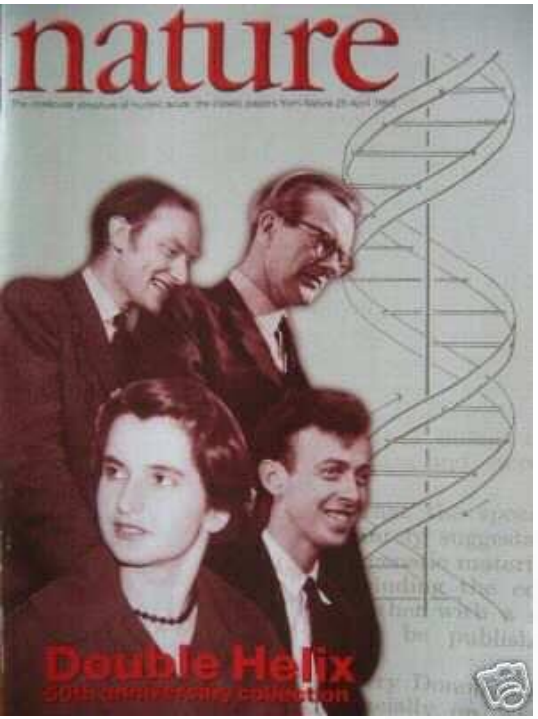
Gli acidi nucleici: RNA

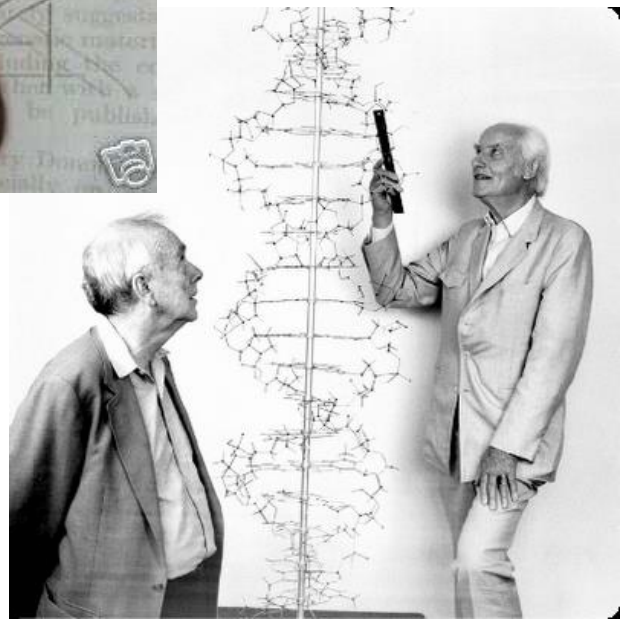
Tre differenze tra DNA ed RNA:

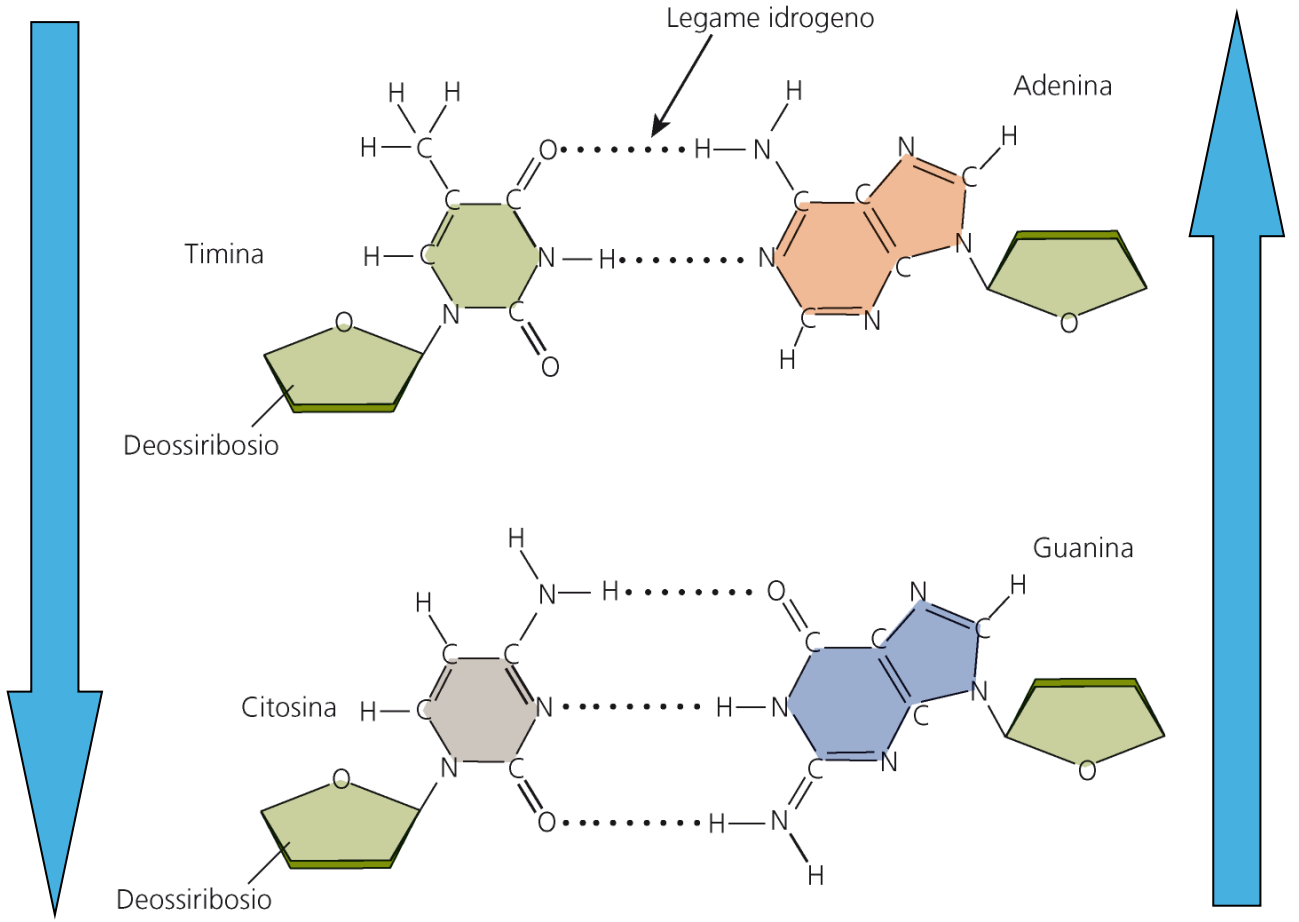
1. **Uracile** invece di timina. L'uracile ha una struttura simile alla timina ma non ha un gruppo metile in posizione 5
2. E' normalmente presente come **singolo filamento**
3. **Ribosio** invece che deossiribosio. Il ribosio ha un ossidrile in posizione 2'.



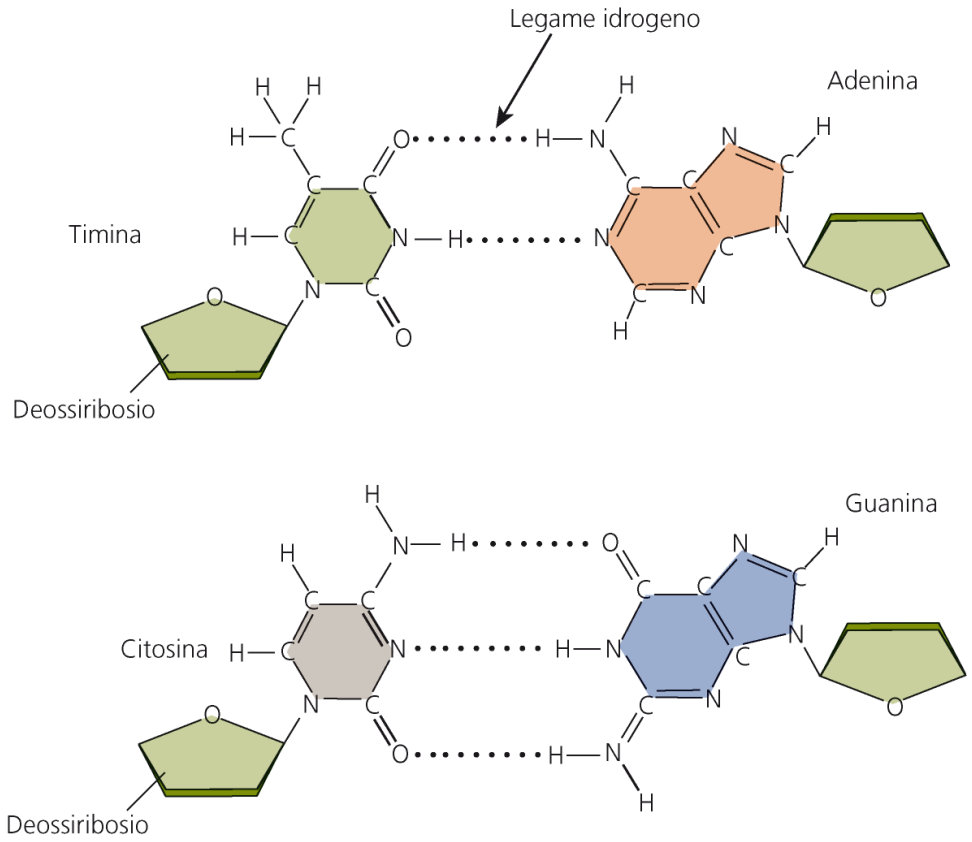
La complementarietà delle basi azotate

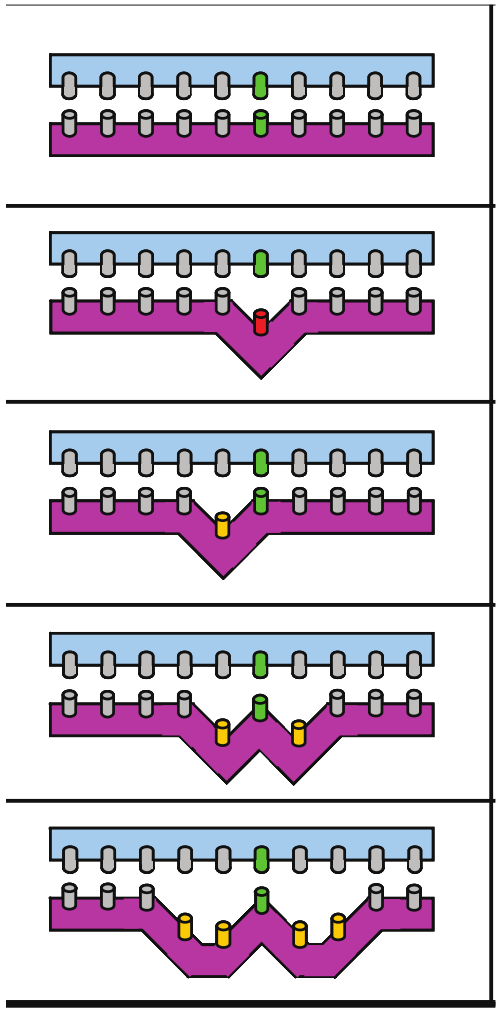






La complementarietà del legame tra basi azotate è alla base della **specificità** del legame di DNA ed RNA

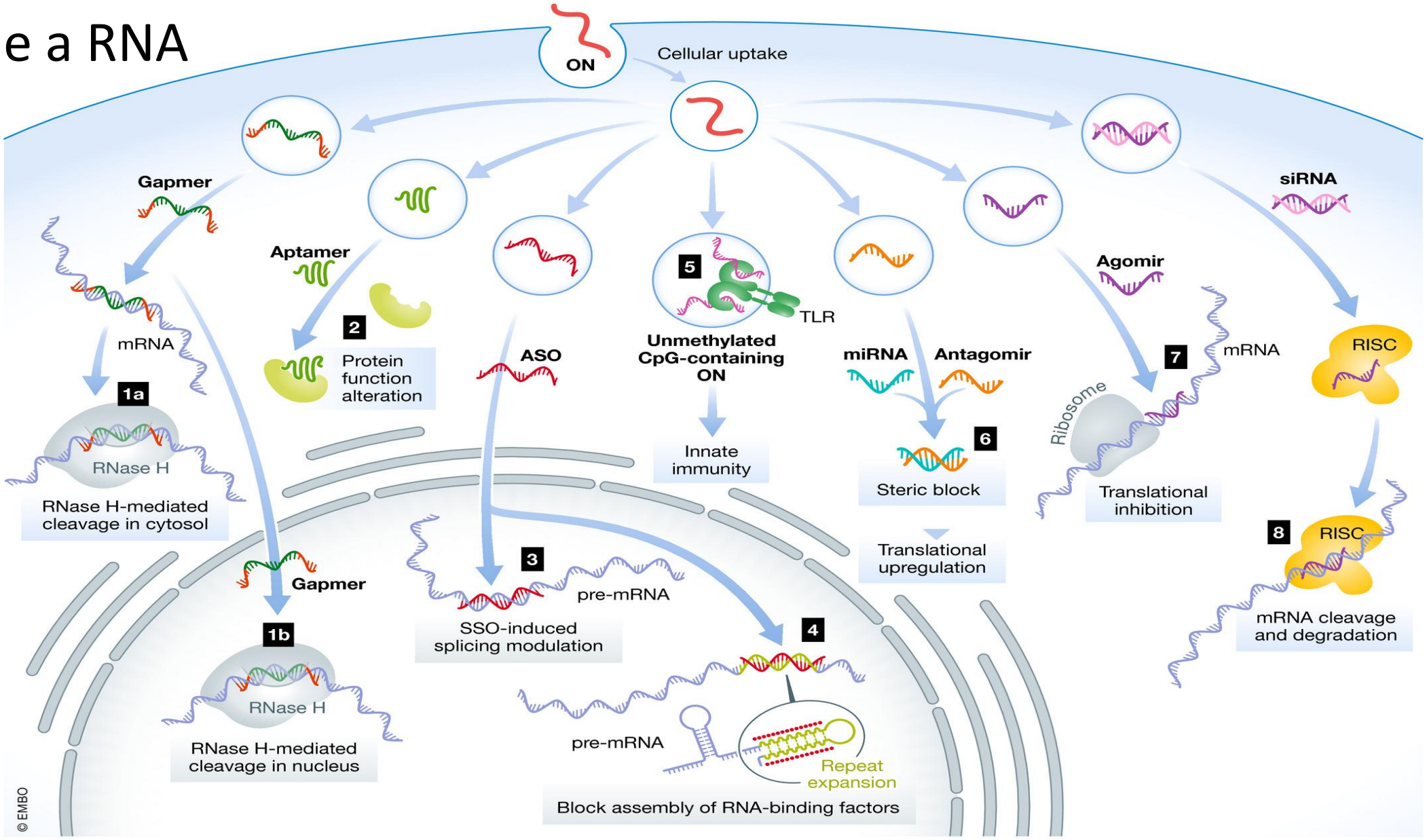




Appaiamento perfetto (match)

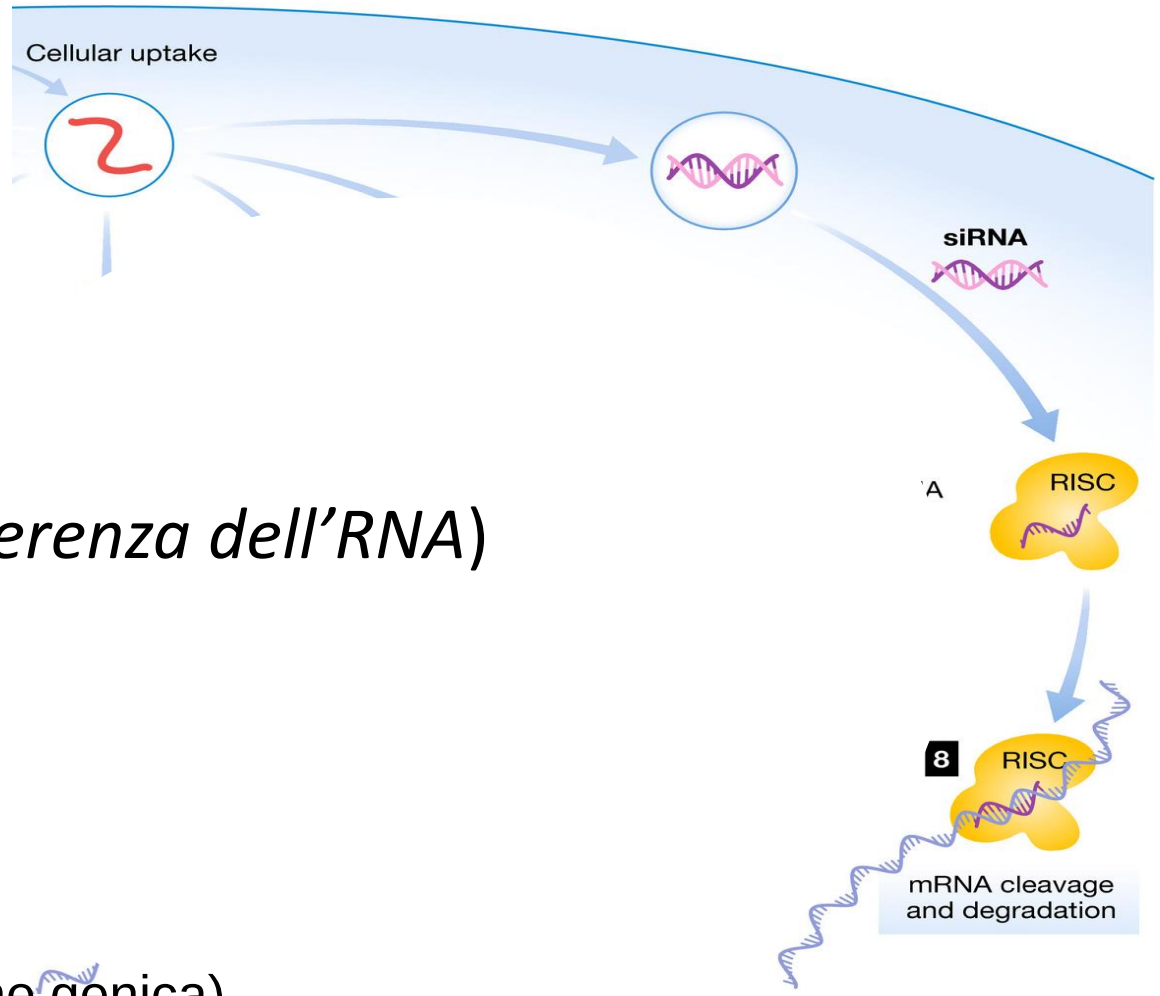
Appaiamento scarso (mismatch)

Terapie a RNA



Delivery of oligonucleotide-based therapeutics: challenges and opportunities

EMBO Mol Med, Volume: 13, Issue: 4, First published: 06 April 2021, DOI: (10.15252/emmm.202013243)



RNAi = RNA interference (*interferenza dell'RNA*)

siRNA= short interfering RNAs

Knock-down (inattivazione della funzione genica)

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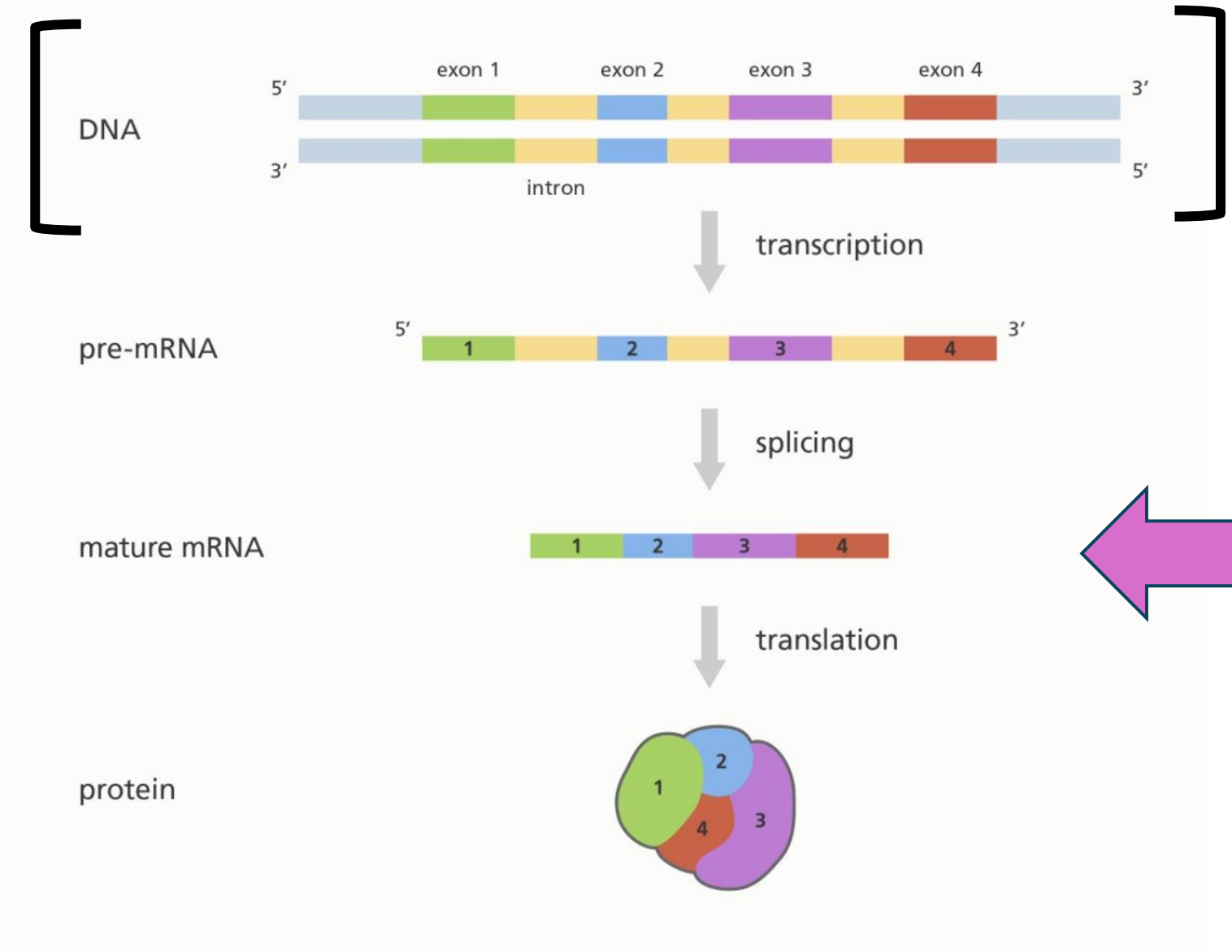
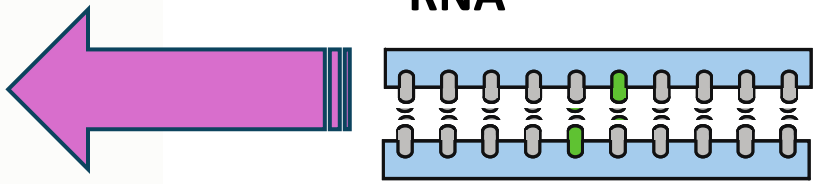
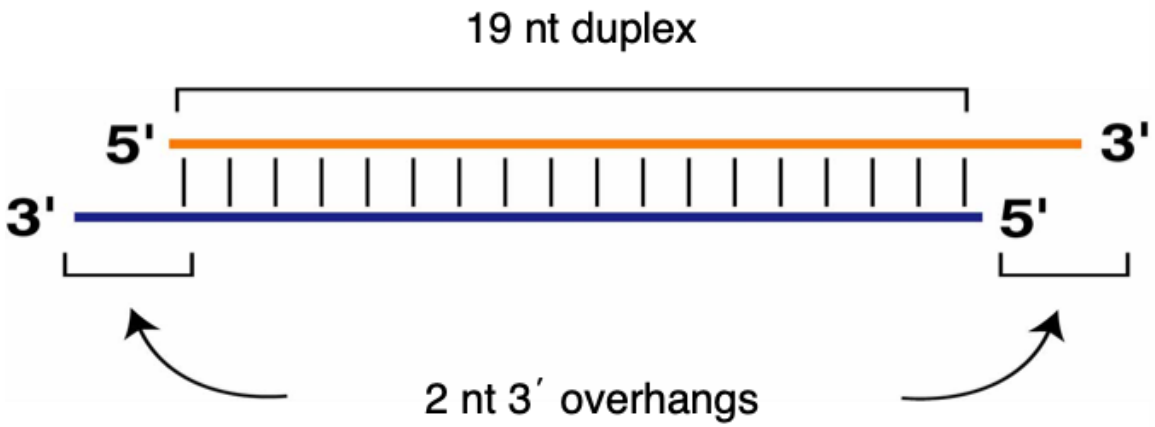


Short interfering RNA

= siRNA



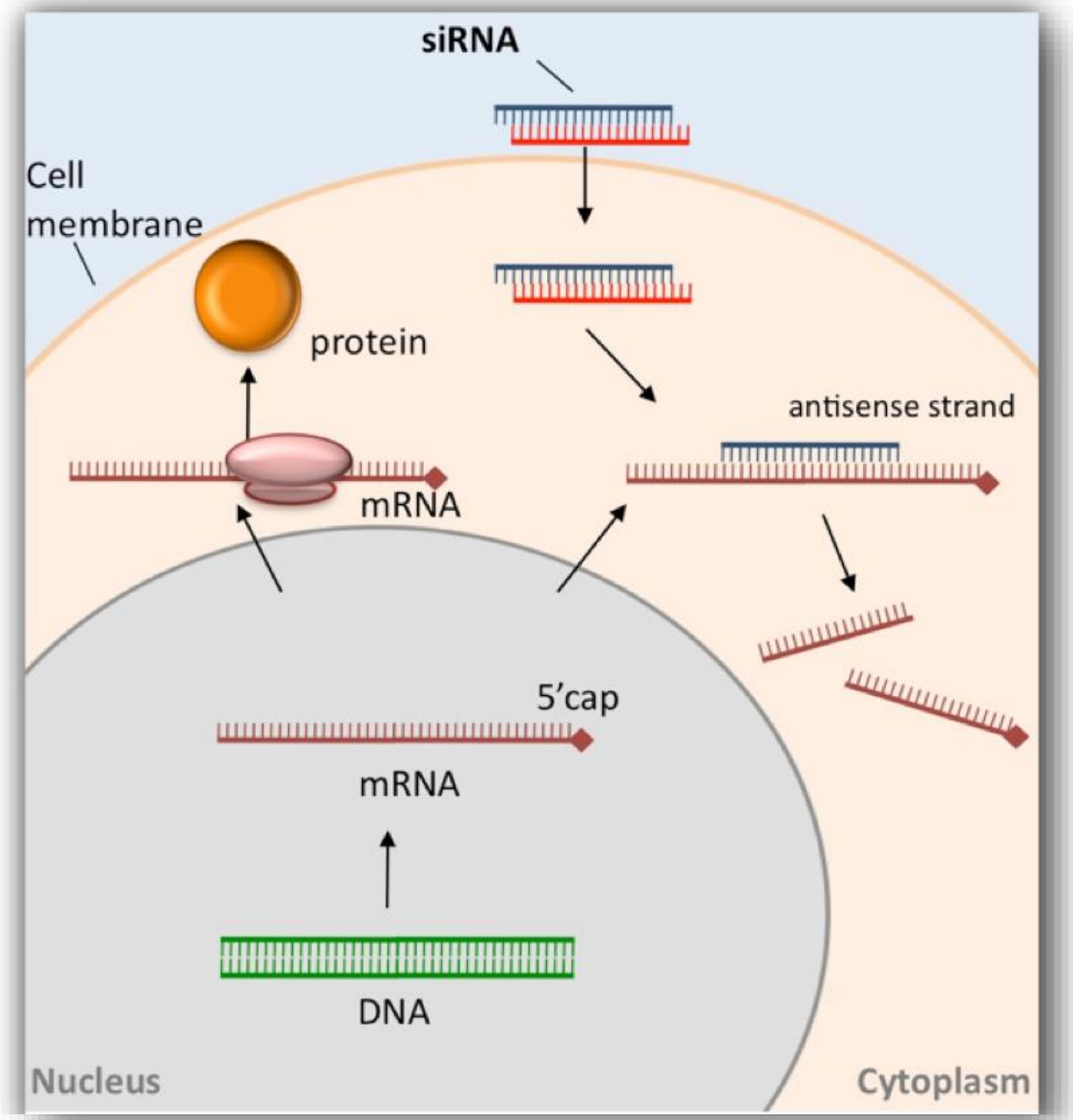
RNA interference



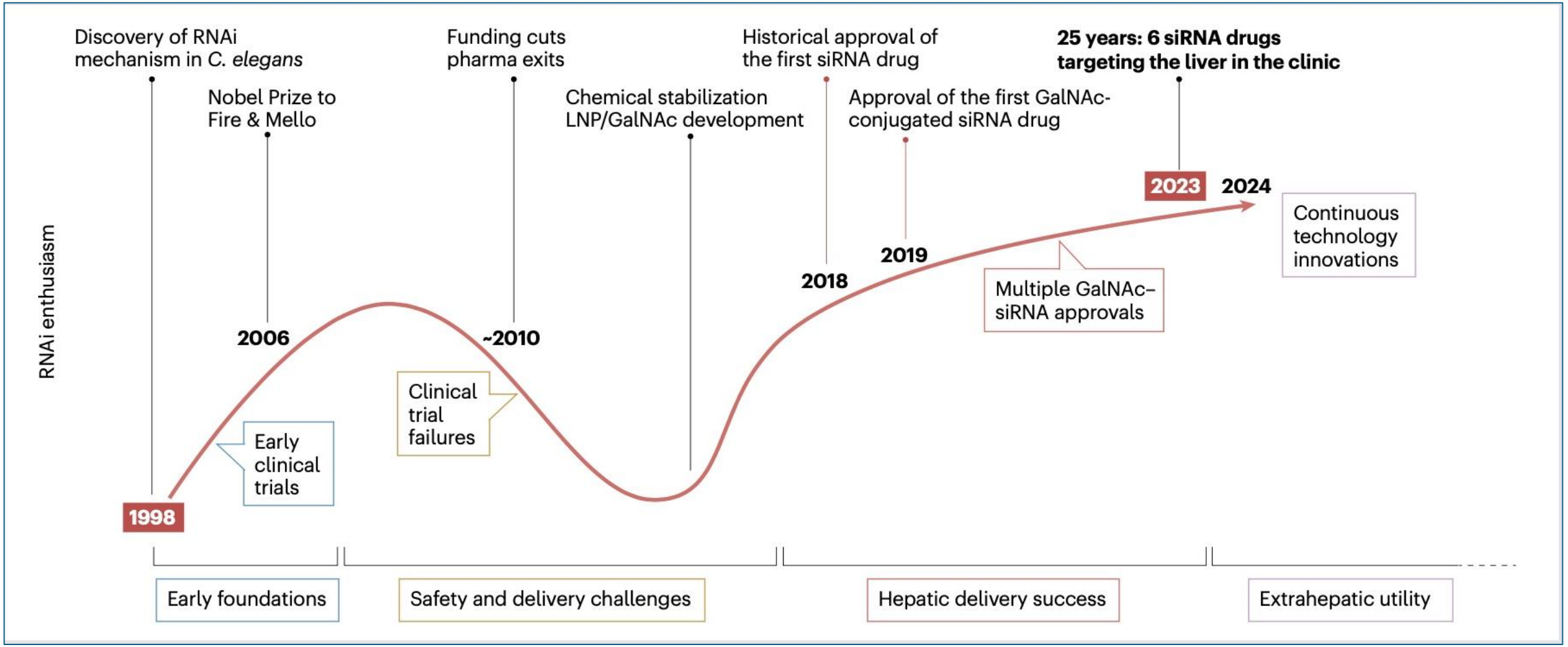
The Nobel Prize in Physiology or Medicine 2006

Andrew Z. Fire and Craig C. Mello

for their discovery of "RNA interference – gene silencing by double-stranded RNA"



25 anni di RNAi dalla scoperta alla clinica



Tang and Khvorova. (2024) *Nat Rev Drug Discovery*

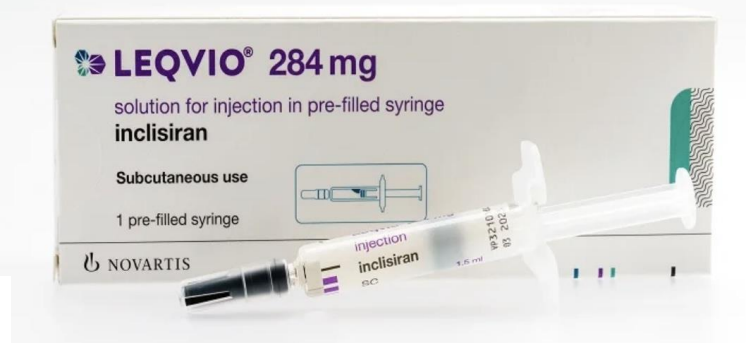
I **siRNA** (interferenza ad RNA): Patisiran e altri siRNA per malattie genetiche.

ONPATTRO® (patisiran)[3]	*hATTR Amyloidosis-PN*
GIVLAARI® (givosiran)[4]	*Acute Hepatic Porphyria*
OXLUMO® (lumasiran)[5]	*Primary Hyperoxaluria Type 1*
Leqvio® (inclisiran)[6]	*Hypercholesterolemia*
Vutrisiran	*hATTR Amyloidosis-PN*
Rivfloza (nedosiran)	*Primary hyperoxaluria*

2018

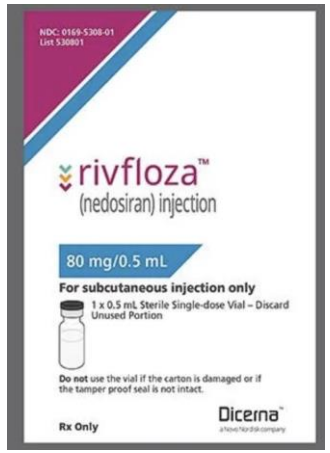
2019

2020



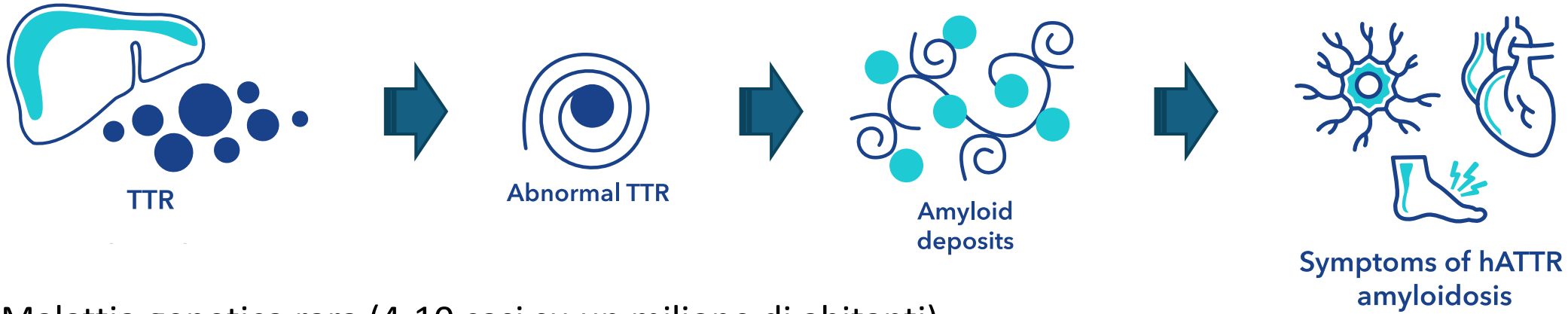
2021

2022



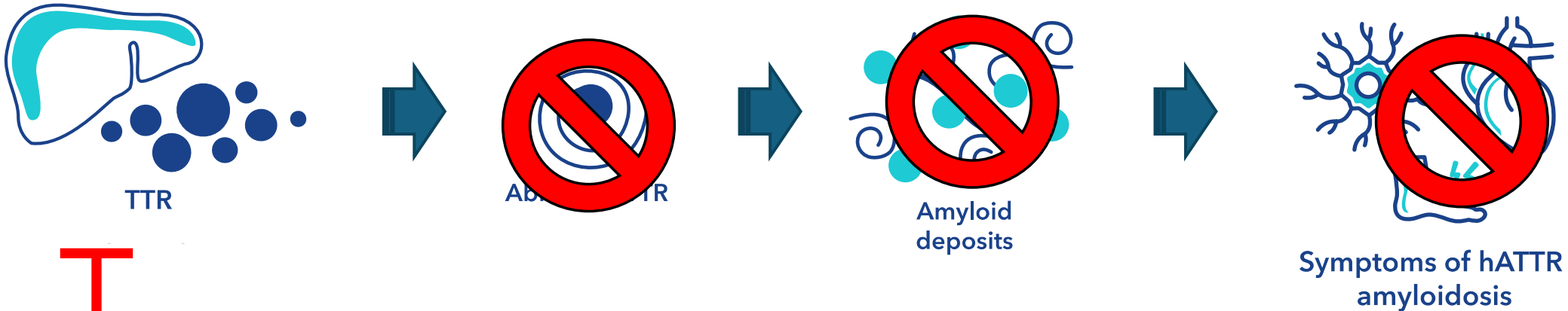
2023

hATTR: Amiloidosi ereditaria mediata da Transtiretina con polineuropatia



Malattia genetica rara (4-10 casi su un milione di abitanti)
Sola possibilità terapeutica: trapianto di fegato

hATTR: Amiloidosi ereditaria mediata da Transtiretina con polineuropatia

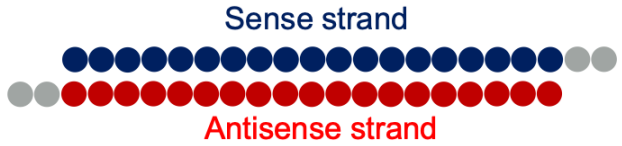


In Italia patisiran e' approvato dal 2020

Setten et al.,
Nat Rev Drug Discov 2019

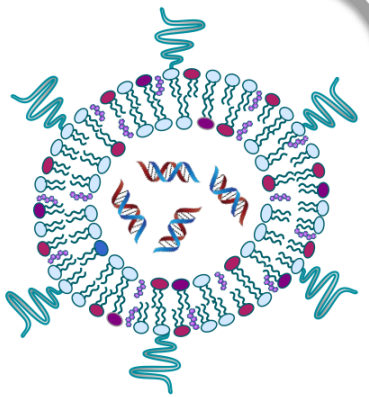
Addressing Delivery Challenge

Alnylam Platforms for Functional siRNA Delivery to Target Tissue

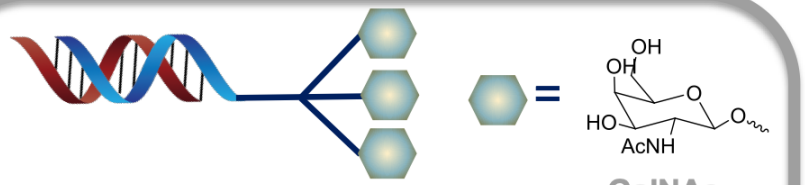


Lipid Nanoparticles (LNPs)

- Multi-component lipid formulation (~100 nm in size)
- Encapsulated siRNA
- Highly efficient for targeted delivery to liver
- Administered intravenously (IV)
- Clinically validated



Patisiran



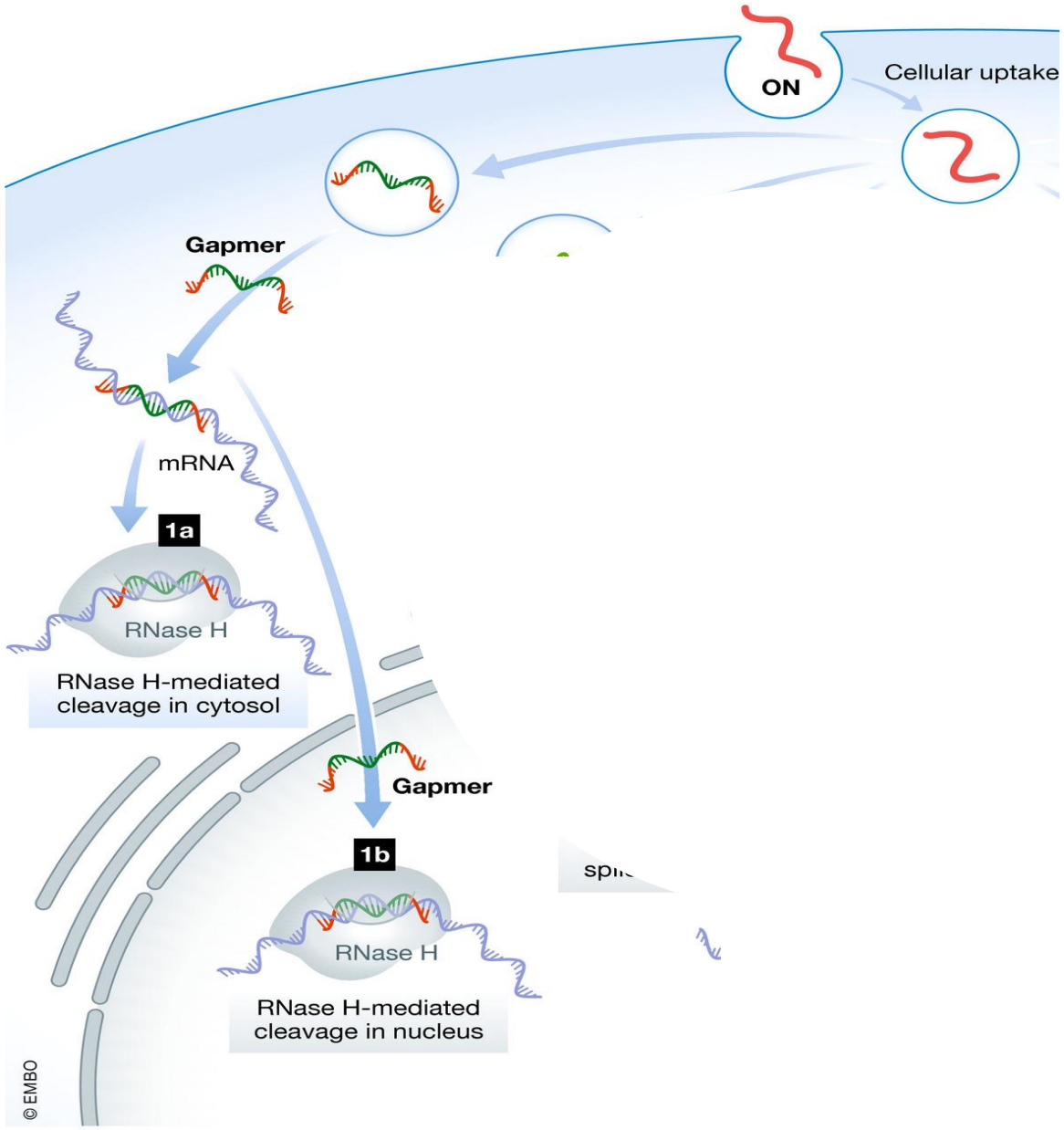
GalNAc

GalNAc-siRNA Conjugates

- Single chemical entity
- GalNAc ligand conjugated to extensively modified siRNA
- Targeted delivery to liver
- Administered subcutaneously (SC)
- Clinically validated

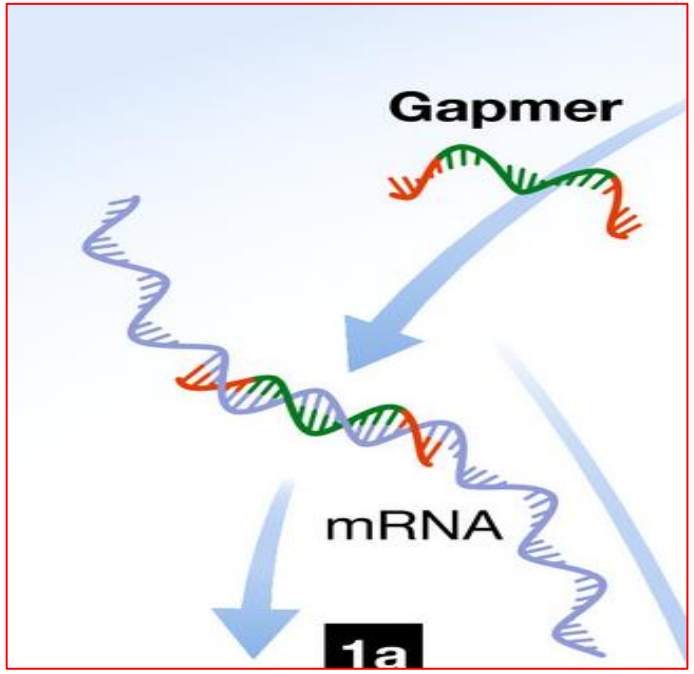
Complementary Approaches for Efficient siRNA Delivery to Liver

Alnylam PHARMACEUTICALS



Gapmer

RNAsiH



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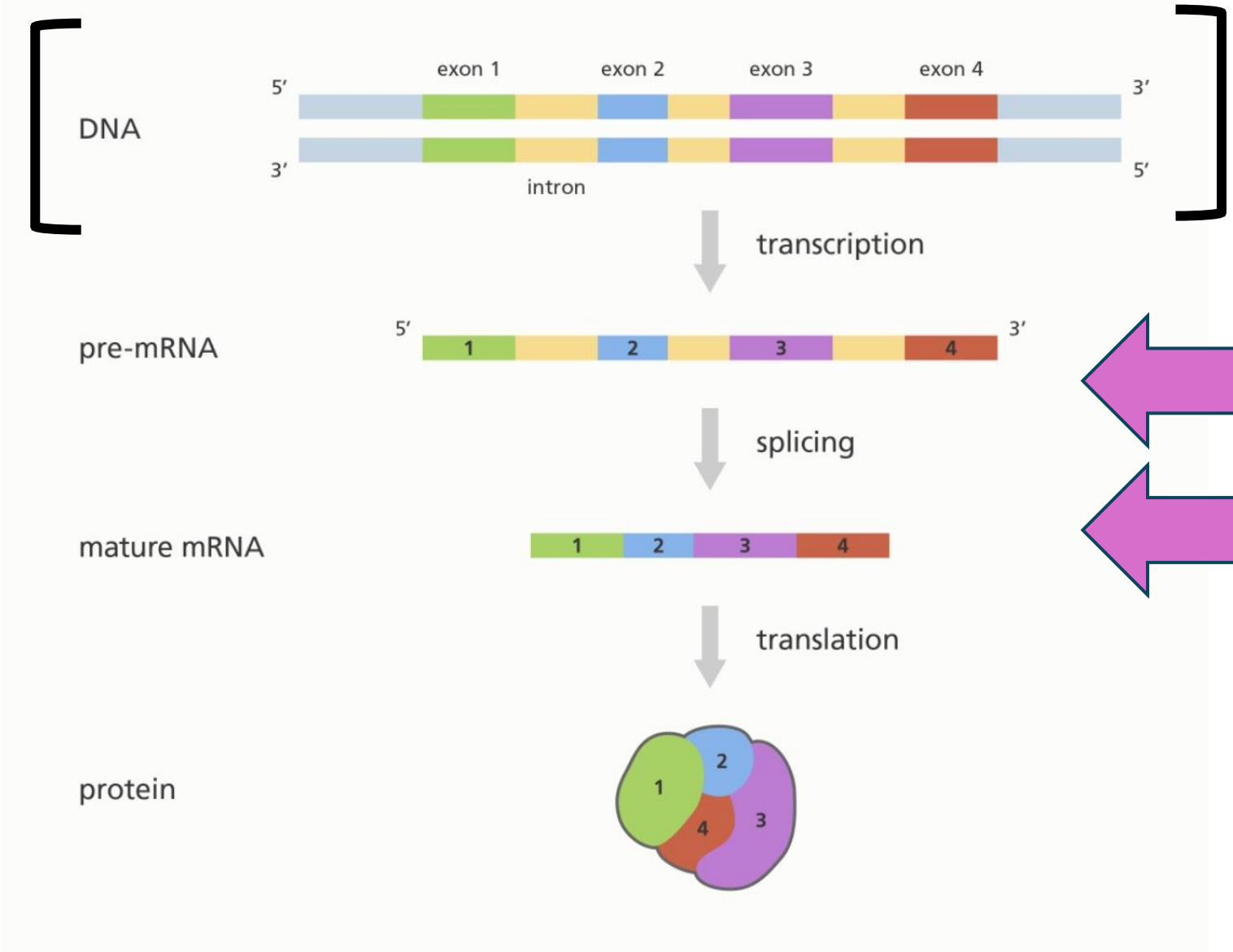

Oligonucleotidi antisenso **= ASO**

Gapmers (RNasiH)

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- Exon inclusion
- Exon skipping

mRNA degradation:
- **Gapmers**
- siRNAs

Corea di Huntington: TOMINERSEN

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Targeting Huntingtin Expression in Patients with Huntington's Disease

Sarah J. Tabrizi, M.B., Ch.B., Ph.D., Blair R. Leavitt, M.D., C.M., G. Bernhard Landwehrmeyer, M.D., Edward J. Wild, M.B., B.Chir., Ph.D., Carsten Saft, M.D., Roger A. Barker, M.R.C.P., Ph.D., Nick F. Blair, M.B., B.S.,* David Craufurd, M.B., B.S., Josef Priller, M.D., Hugh Rickards, M.D., Anne Rosser, M.B., B.Chir., Ph.D., Holly B. Kordasiewicz, Ph.D., Christian Czech, Ph.D., Eric E. Swayze, Ph.D., Daniel A. Norris, Ph.D., Tiffany Baumann, B.S., Irene Gerlach, Ph.D., Scott A. Schobel, M.D., Erika Paz, B.S., Anne V. Smith, Ph.D., C. Frank Bennett, Ph.D., and Roger M. Lane, M.D., for the Phase 1–2a IONIS-HTT$_{Rx}$ Study Site Teams†

ABSTRACT

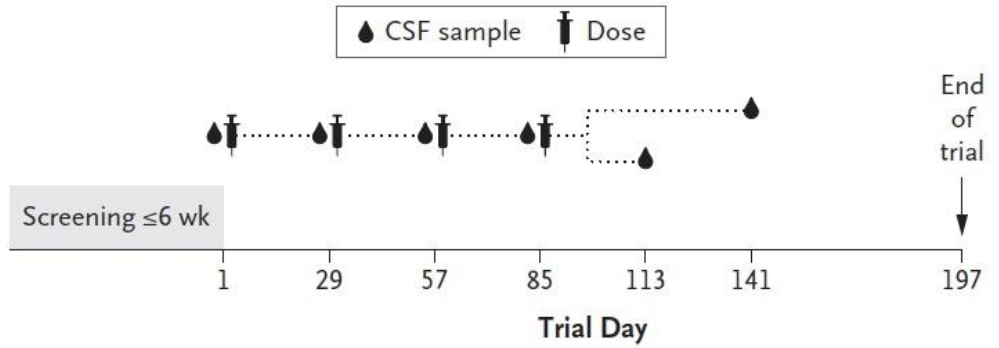


Figure 1. Trial Design.

At the conclusion of the screening period, eligible patients were randomly assigned in a 3:1 ratio to receive the antisense oligonucleotide drug HTT$_{Rx}$ or placebo. Cerebrospinal fluid (CSF) samples were obtained before the administration of the trial agent on days 1, 29, 57, and 85. The CSF sample on day 1 served as the baseline sample, and the CSF samples on days 29, 57, and 85 served as 28-day post-dose trough samples. One sample was obtained from each patient after the completion of the regimen, either on day 113 or day 141 according to randomized assignment. The CSF sample that was obtained on day 113 served as a 28-day post–last dose sample; the sample obtained on day 141 served as a 56-day post–last dose sample. Dotted lines indicate the relationship between each dose and the subsequent CSF sample.

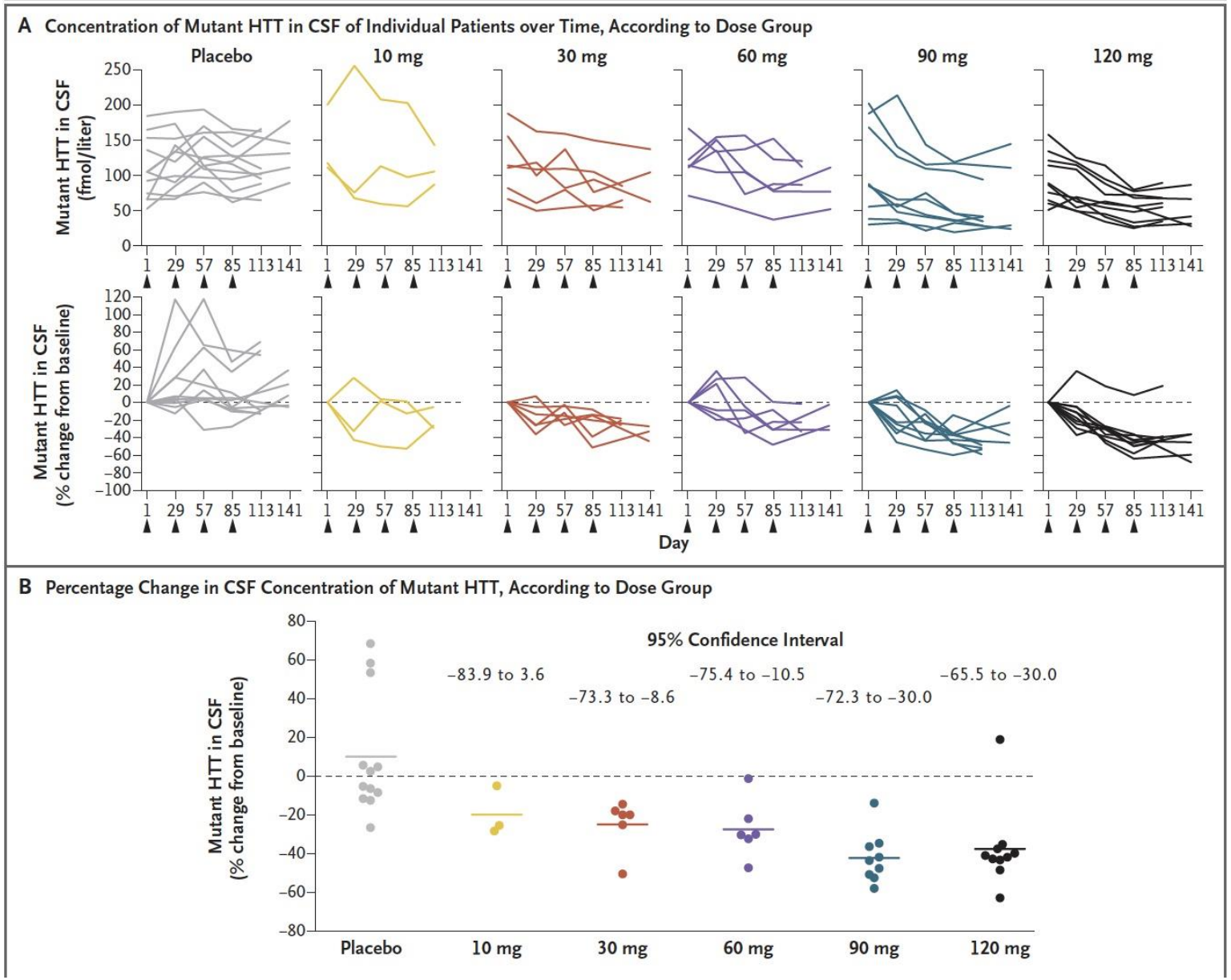
TRIAL DRUG

HTT_{Rx} is a chemically modified synthetic oligomer that is perfectly complementary to a 20-nucleotide stretch of *HTT* mRNA. HTT_{Rx} binds to *HTT* mRNA by means of Watson–Crick base pairing, with hybridization resulting in endogenous RNase H1-mediated degradation of the *HTT* mRNA, thus inhibiting translation of the huntingtin protein. The sequence of HTT_{Rx} is (5′ to 3′) $ct_oc_oa_ogTAACATTGACa_oc_oc_oac$, in which capital letters represent 2′-deoxyribose nucleosides, and small letters 2′-(2-methoxyethyl)ribose nucleosides. Nucleoside linkages that are represented with a subscripted "o" are phosphodiester, and all others are phosphorothioate. Letters "a" and "A" represent adenine, "c" and "C" 5-methylcytosine, "g" and "G" guanine, and "t" and "T" thymine nucleobases.

TRIAL DESIGN AND END POINTS

HTT_{Rx}-CS1 was a randomized, double-blind, placebo-controlled, multicenter, phase 1–2a trial. The trial was performed at nine centers in the United Kingdom, Germany, and Canada from August 2015 through November 2017. A centralized automated randomization system was used to assign patients in a 3:1 ratio to receive HTT_{Rx} or placebo within each of five dose cohorts in an ascending-dose design (10 mg, 30 mg, 60 mg, 90 mg, or 120 mg).

Each patient received four bolus intrathecal injections of HTT_{Rx} or placebo (artificial cerebrospinal fluid) at 4-week intervals; subsequently, there was a 4-month follow-up period during which no trial agent was administered. A cerebrospinal fluid (CSF) sample was obtained before each administration of HTT_{Rx} or placebo and either 4 or 8 weeks after the last dose was administered (Fig. 1). Investigators, patients, the sponsor (Ionis Pharmaceuticals), and its collaborator (F. Hoffmann–La Roche) were unaware of the trial-group assignments for the duration of the trial.


A Concentration of Mutant HTT in CSF of Individual Patients over Time, According to Dose Group
Placebo
10 mg
30 mg
60 mg
90 mg
120 mg
Mutant HTT in CSF (fmol/liter)
Mutant HTT in CSF (% change from baseline)
Day
B Percentage Change in CSF Concentration of Mutant HTT, According to Dose Group
95% Confidence Interval
−83.9 to 3.6
−73.3 to −8.6
−75.4 to −10.5
−72.3 to −30.0
−65.5 to −30.0
Mutant HTT in CSF (% change from baseline)
Placebo
10 mg
30 mg
60 mg
90 mg
120 mg

GENETIC THERAPIES FOR HUNTINGTON'S DISEASE FAIL IN CLINICAL TRIALS

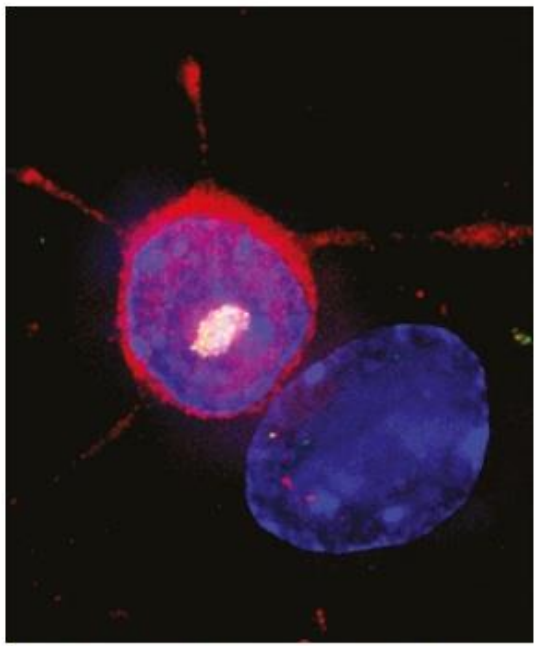
Hopes were high for drugs designed to lower levels of a mutant protein, but development has stalled.

By Diana Kwon

Two pharmaceutical companies have halted clinical trials of gene-targeting therapies for Huntington's disease (HD), following the drugs' disappointing performance.

Researchers had hoped that the treatments – known as antisense oligonucleotides (ASOs) – would be a game changer for HD, an incurable genetic condition that affects cognition, behaviour and movement. But back-to-back announcements from Roche, headquartered in Basel, Switzerland, and Wave Life Sciences, in Cambridge, Massachusetts, have dealt a crushing blow to those affected by the disease.

"I was really shocked, really tearful," says Marion, a woman in London with HD, who was part of one of the trials. "We didn't see it coming at all. I felt really frightened and worried about my future." Marion requested that her last name be withheld to protect her privacy.



A mutant form of the huntingtin protein accumulates in nerve cells.

180 | Nature | Vol 593 | 13 May 2021

'The saddest possible result'

The phase III tominersen trial tested 2 dosing regimens: 120 milligrams of the drug – the highest safe dose, based on earlier trials – given either every 8 weeks or every 16 weeks.

Roche reported that after 69 weeks, people on the 8-week regimen experienced a more marked decline than did those in the placebo group, with worsened outcomes in areas such as motor function and cognition. Participants in the 16-week treatment group had better outcomes than did those in the 8-week arm, but experienced no overall benefit compared with those given a placebo. Those in the treatment group also showed larger increases in the size of fluid-filled cavities in the brain known as

Several factors could have contributed to tominersen's failure, says Sarah Tabrizi, a neurologist at University College London and one of the investigators in the Roche trial. The drug suppresses production of the healthy, as well as the mutant, form of huntingtin, and this could have caused problems. Other possibilities are that the ASO did not reach the right parts of the brain, or that the disease had simply progressed too far for the drug to be beneficial. It will take several months of further analysis to pinpoint what went wrong, Tabrizi adds. Roche's results were preliminary, and important data are still being assessed.

https://www.osservatoriomalattierare.it/malattie-rare/malattia-di-huntington/17226-malattia-di-huntington-interrotta-la-sperimentazione-clinica-del-farmaco-tominersen

https://www.osservatoriomalattierare.it/malattie-rare/malattia-di-huntington/18202-malattia-di-huntington-nuova-speranza-dalla-sperimentazione-di-tominersen

Sclerosi Laterale Amiotrofica SOD1: TOFERSEN

BACKGROUND

Tofersen is an antisense oligonucleotide that mediates the degradation of superoxide dismutase 1 (SOD1) messenger RNA to reduce SOD1 protein synthesis. Intrathecal administration of tofersen is being studied for the treatment of amyotrophic lateral sclerosis (ALS) due to *SOD1* mutations.

METHODS

We conducted a phase 1–2 ascending-dose trial evaluating tofersen in adults with ALS due to *SOD1* mutations. In each dose cohort (20, 40, 60, or 100 mg), participants were randomly assigned in a 3:1 ratio to receive five doses of tofersen or placebo, administered intrathecally for 12 weeks. The primary outcomes were safety and pharmacokinetics. The secondary outcome was the change from baseline in the cerebrospinal fluid (CSF) SOD1 concentration at day 85. Clinical function and vital capacity were measured.

RESULTS

A total of 50 participants underwent randomization and were included in the analyses; 48 participants received all five planned doses. Lumbar puncture–related adverse events were observed in most participants. Elevations in CSF white-cell count and protein were reported as adverse events in 4 and 5 participants, respectively, who received tofersen. Among participants who received tofersen, one died from pulmonary embolus on day 137, and one from respiratory failure on day 152; one participant in the placebo group died from respiratory failure on day 52. The difference at day 85 in the change from baseline in the CSF SOD1 concentration between the tofersen groups and the placebo group was 2 percentage points (95% confidence interval [CI], –18 to 27) for the 20-mg dose, –25 percentage points (95% CI, –40 to –5) for the 40-mg dose, –19 percentage points (95% CI, –35 to 2) for the 60-mg dose, and –33 percentage points (95% CI, –47 to –16) for the 100-mg dose.

CONCLUSIONS

In adults with ALS due to *SOD1* mutations, CSF SOD1 concentrations decreased at the highest concentration of tofersen administered intrathecally over a period of 12 weeks. CSF pleocytosis occurred in some participants receiving tofersen. Lumbar puncture–related adverse events were observed in most participants. (Funded by Biogen; ClinicalTrials.gov number, NCT02623699; EudraCT number, 2015-004098-33.)

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 9, 2020 VOL. 383 NO. 2

Phase 1–2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS

T. Miller, M. Cudkowicz, P.J. Shaw, P.M. Andersen, N. Atassi, R.C. Bucelli, A. Genge, J. Glass, S. Ladha, A.L. Ludolph, N.J. Maragakis, C.J. McDermott, A. Pestronk, J. Ravits, F. Salachas, R. Trudell, P. Van Damme, L. Zinman, C.F. Bennett, R. Lane, A. Sandrock, H. Runz, D. Graham, H. Houshyar, A. McCampbell, I. Nestorov, I. Chang, M. McNeill, L. Fanning, S. Fradette, and T.A. Ferguson

ABSTRACT

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS

T.M. Miller, M.E. Cudkowicz, A. Genge, P.J. Shaw, G. Sobue, R.C. Bucelli, A. Chiò, P. Van Damme, A.C. Ludolph, J.D. Glass, J.A. Andrews, S. Babu, M. Benatar, C.J. McDermott, T. Cochrane, S. Chary, S. Chew, H. Zhu, F. Wu, I. Nestorov, D. Graham, P. Sun, M. McNeill, L. Fanning, T.A. Ferguson, and S. Fradette, for the VALOR and OLE Working Group*

ABSTRACT

N ENGL J MED 387;12 NEJM.ORG SEPTEMBER 22, 2022

Neurotherapeutics (2022) 19:1248–1258
https://doi.org/10.1007/s13311-022-01237-4

ORIGINAL ARTICLE

Design of a Randomized, Placebo-Controlled, Phase 3 Trial of Tofersen Initiated in Clinically Presymptomatic *SOD1* Variant Carriers: the ATLAS Study

Michael Benatar[1] · Joanne Wuu[1] · Peter M. Andersen[2] · Robert C. Bucelli[3] · Jinsy A. Andrews[4] · Markus Otto[5] · Nita A. Farahany[6] · Elizabeth A. Harrington[7] · Weiping Chen[8] · Adele A. Mitchell[8] · Toby Ferguson[8] · Sheena Chew[8] · Liz Gedney[8] · Sue Oakley[8] · Jeong Heo[8] · Sowmya Chary[8] · Laura Fanning[8] · Danielle Graham[8] · Peng Sun[8] · Yingying Liu[8] · Janice Wong[8] · Stephanie Fradette[8]

Accepted: 10 April 2022 / Published online: 18 May 2022

Sclerosi Laterale Amiotrofica: Tofersen

https://www.osservatoriomalattierare.it/malattie-rare/sla/19754-sclerosi-laterale-amiotrofica-il-farmaco-tofersen-approvato-negli-stati-uniti

FDA 25 Aprile 2023
EMA 30 Maggio 2024

nature medicine

Article

https://doi.org/10.1038/s41591-023-02326-3

Tau-targeting antisense oligonucleotide $MAPT_{Rx}$ in mild Alzheimer's disease: a phase 1b, randomized, placebo-controlled trial

Received: 1 September 2022

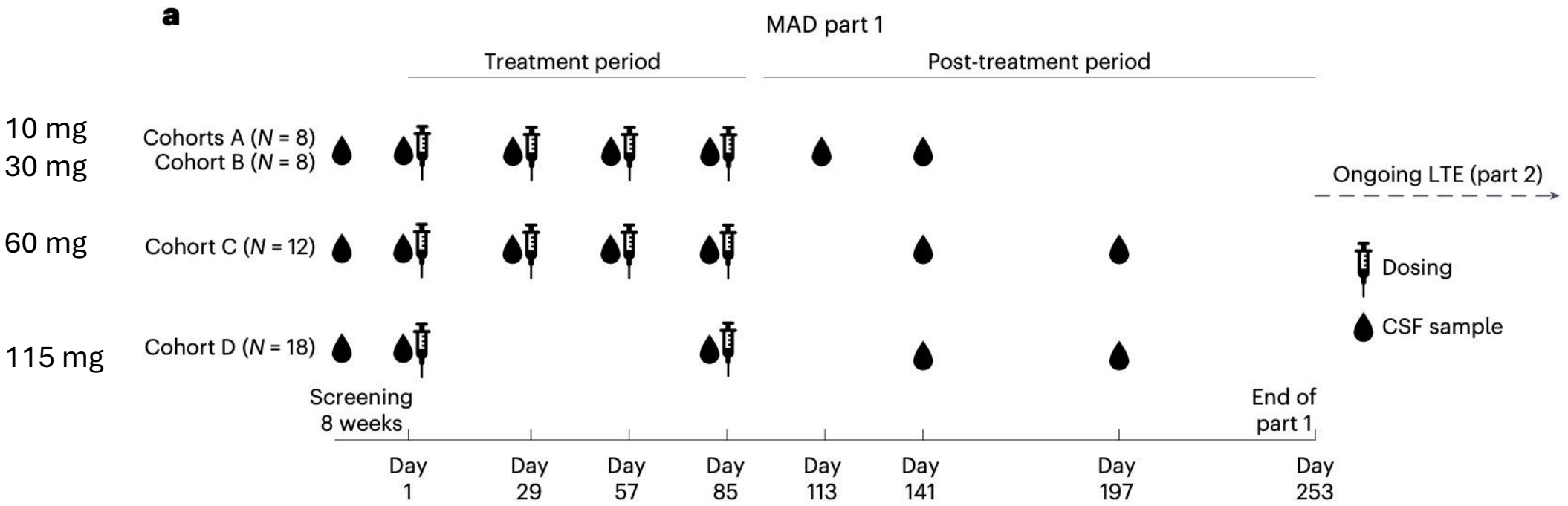
Accepted: 29 March 2023

Published online: 24 April 2023

Check for updates

Catherine J. Mummery[1,14] ✉, Anne Börjesson-Hanson[2,14], Daniel J. Blackburn[3,14], Everard G. B. Vijverberg[4,14], Peter Paul De Deyn[5,14], Simon Ducharme[6,14], Michael Jonsson[7,14], Anja Schneider[8,14], Juha O. Rinne[9,14], Albert C. Ludolph[10,14], Ralf Bodenschatz[11,14], Holly Kordasiewicz[12,15], Eric E. Swayze[12,15], Bethany Fitzsimmons[12,15], Laurence Mignon[12,14], Katrina M. Moore[12,15], Chris Yun[12,15], Tiffany Baumann[12,15], Dan Li[12,15], Daniel A. Norris[12,15], Rebecca Crean[12,15], Danielle L. Graham[13,15], Ellen Huang[13,15], Elena Ratti[13,15], C. Frank Bennett[12,15], Candice Junge[12,14] & Roger M. Lane[12,14]

From August 2017 through February 2020



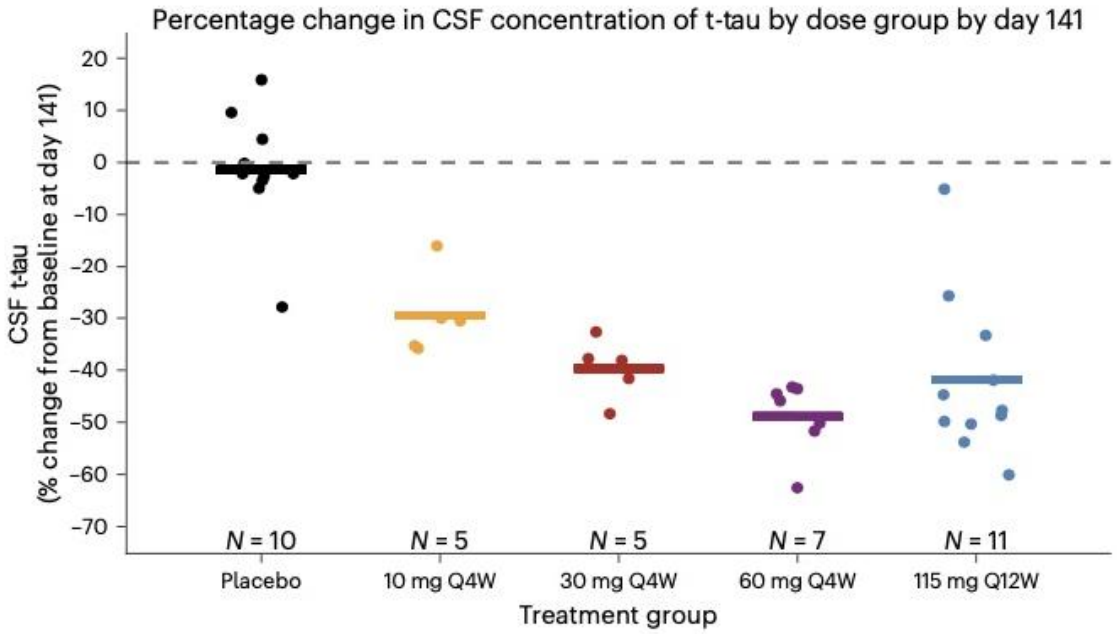
Four ascending dose cohorts were enrolled sequentially and randomized 3:1 to intrathecal bolus administrations of MAPT$_{Rx}$ or placebo every 4 or 12 weeks during the 13-week treatment period, followed by a 23 week post-treatment period.
The primary endpoint was safety.
The secondary endpoint was MAPT$_{Rx}$ pharmacokinetics in cerebrospinal fluid (CSF).
The prespecified key exploratory outcome was CSF total-tau protein concentration.

Table 2 | AEs reported in at least three patients receiving $MAPT_{Rx}$ according to severity[a]

Event	Mild (grade 1)		Moderate (grade 2)		Severe (grade 3)	
	$MAPT_{Rx}$ groups (*N*=34)	Placebo group (*N*=12)	$MAPT_{Rx}$ groups (*N*=34)	Placebo group (*N*=12)	$MAPT_{Rx}$ groups (*N*=34)	Placebo group (*N*=12)
Number of patients with event (%)						
Any AE (%)	21 (62)	5 (42)	11 (32)	4 (33)	0	0
Any serious AE	0	0	0	2 (16.7)	0	0
Post-LP headache[b]	13 (38)	1 (8)	2 (6)	3 (25)	0	0
Procedural pain	4 (12)	1 (8)	3 (9)	0	0	0
Musculoskeletal pain	3 (9)	0	1 (3)	0	0	0
Vomiting	4 (12)	0	0	0	0	0
Back pain	2 (6)	1 (8)	1 (3)	0	0	0
Confusional state	2 (6)	0	1 (3)	0	0	0
Contusion	1 (3)	0	2 (6)	0	0	0
Diarrhea	2 (6)	0	1 (3)	0	0	0
Dizziness	3 (9)	1 (8)	0	0	0	0
Fatigue	3 (9)	0	0	0	0	0
Myalgia	2 (6)	1 (8)	1 (3)	0	0	0
Nasopharyngitis	3 (9)	2 (17)	0	0	0	0
Nausea	3 (9)	0	0	0	0	0
Tinnitus	3 (9)	0	0	0	0	0

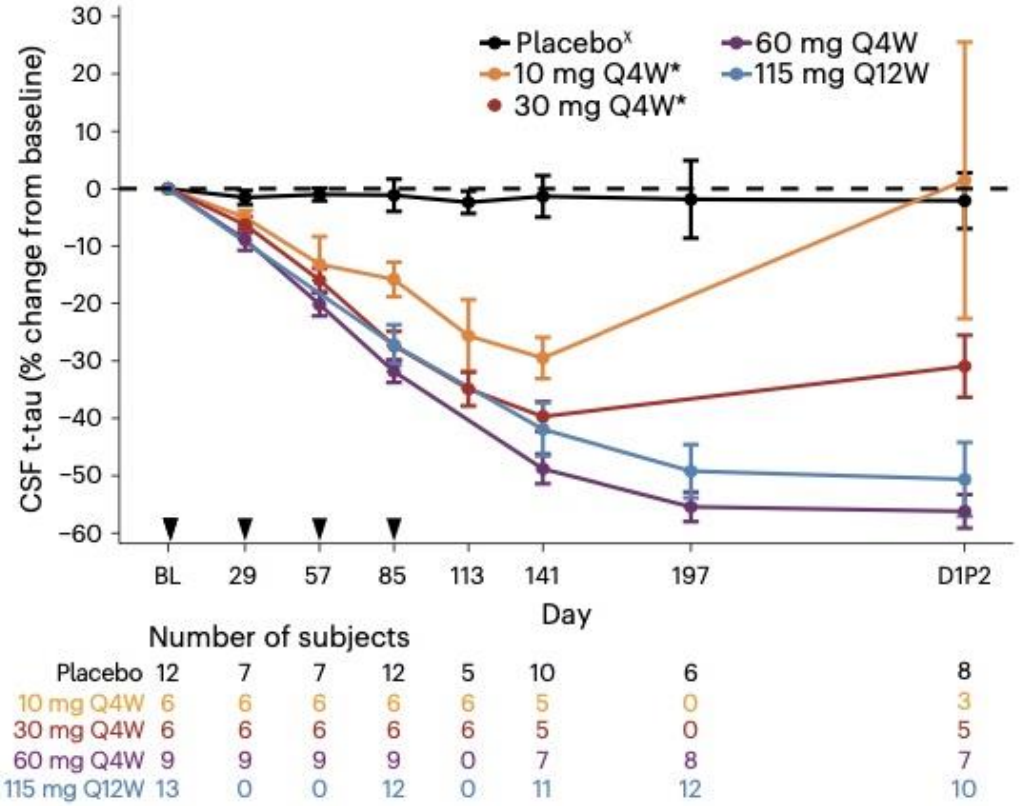
[a]Shown are AEs that occurred from the first dose of study drug through the end of MAD part 1 (treatment and post-treatment periods). Each AE was rated as mild, moderate or severe, corresponding to grades of 1, 2 and 3, respectively. In addition, serious AEs were rated as life-threatening (grade 4) or not life-threatening. At each level of summation (overall and according to system organ class or preferred term), patients for whom more than one AE was reported were counted only once for the incidence according to the most severe grade, and if there was a missing severity for the same subject, then the non-missing severity, if available, was chosen for the same subject. [b]Post-LP headache indicates both post-LP syndrome and headache that were potentially related to study LP procedure. Related was defined as 'related', 'possible' or missing relationship to LP procedure.

Tau totale



nent group

ıtage change from baseline by dose group



Number of subjects

Placebo	12	7	7	12	5	10	6	8
10 mg Q4W	6	6	6	6	6	5	0	3
30 mg Q4W	6	6	6	6	6	5	0	5
60 mg Q4W	9	9	9	9	0	7	8	7
115 mg Q12W	13	0	0	12	0	11	12	10

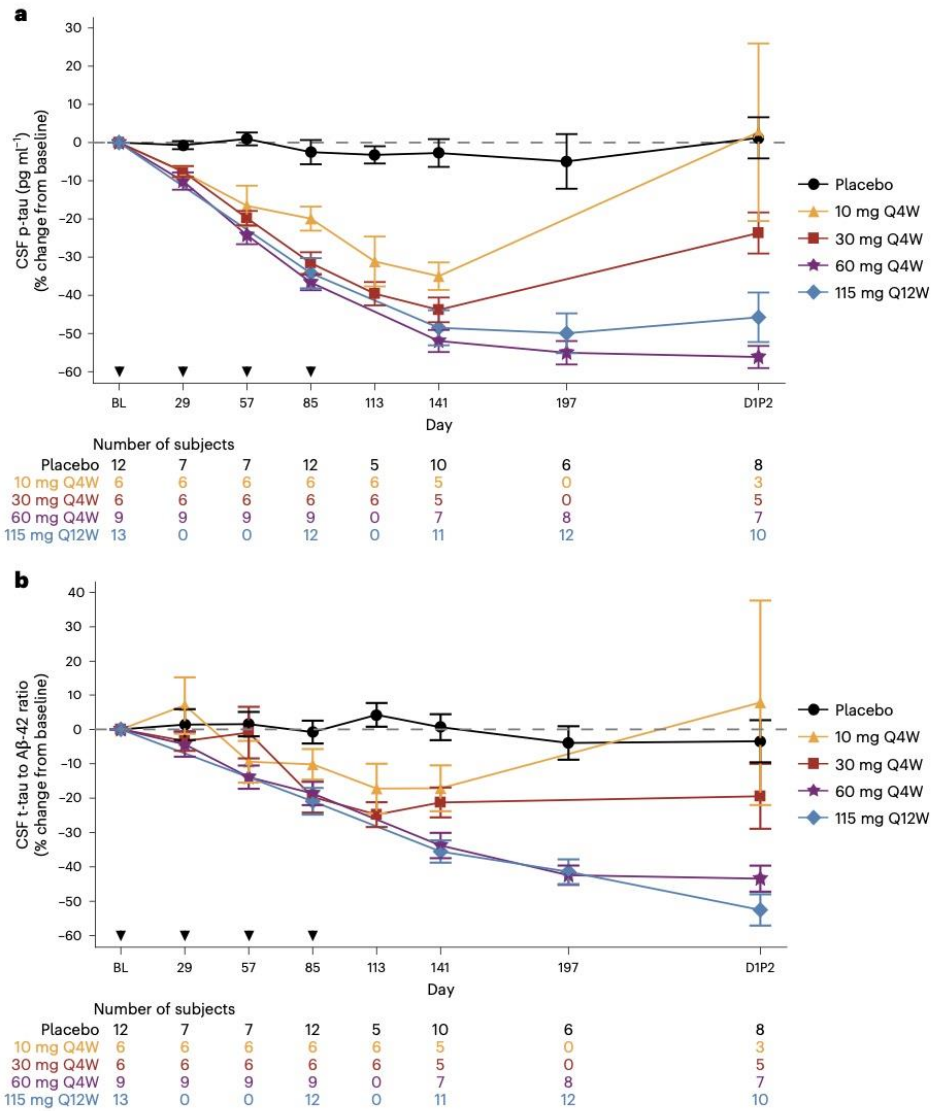
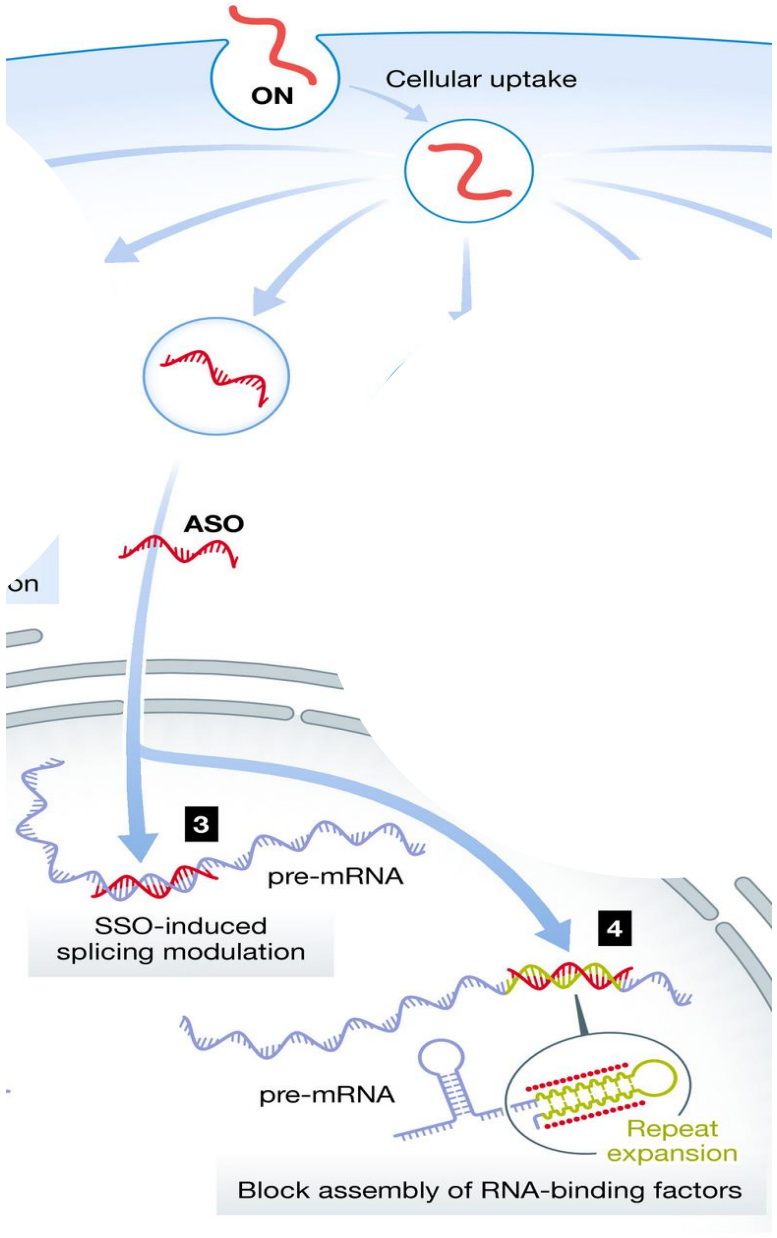


Fig. 4 | Effect of MAPT$_{Rx}$ on CSF concentrations of p-tau protein and tau/Aβ42. a, The mean percentage change from baseline in p-tau over time according to dose group. **b**, The mean percentage change from baseline in the ratio of t-tau to Aβ42 over time according to dose group. Error bars indicate the standard error of the mean. Q4W and Q12W indicates dosing every 4 or 12 weeks, respectively.

*Participants assigned to cohort A or B did not seamlessly transition to LTE part 2 and experienced a variable gap ranging from 5 to 19 months between completion of MAD part 1 at day 253 and start of LTE part 2 (D1P2). ᵡPlacebo group was pooled. Subjects assigned to cohorts A or B and randomized to placebo had a variable gap between completion of MAD part 1 and start of LTE part 2 (D1P2).



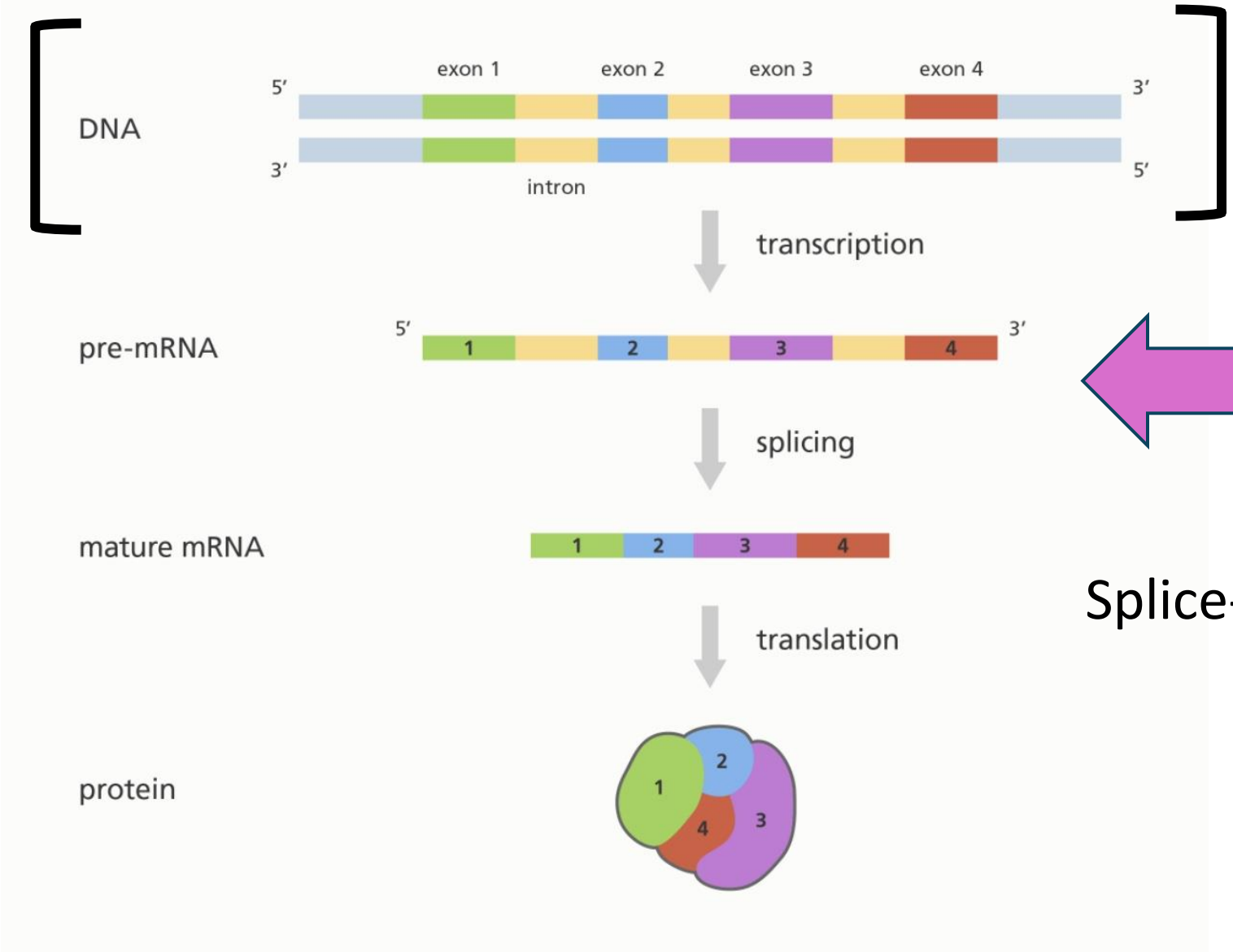
Oligonucleotidi antisenso **= ASO**

SSO=
Splice-Switching
Oligonucleotides

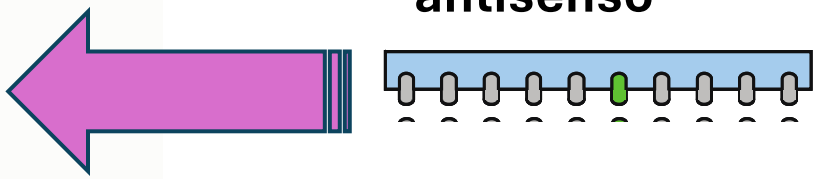
Delivery of oligonucleotide-based therapeutics: challenges and opportunities

EMBO Mol Med, Volume: 13, Issue: 4, First published: 06 April 2021, DOI: (10.15252/emmm.202013243)

Le Terapie su RNA: usare gli acidi nucleici per **eliminare, rimpiazzare o correggere l'RNA** messaggero dei geni.



Oligonucleotidi antisenso **= ASO**



SSO=
Splice-Switching Oligonucleotides

Table 1 Approved RNA therapeutics for the treatment of diseases affecting the NS

Drug name	Disease	Target	Administration Route/target organ	Approved	Company	Type of mechanism
ASO						
Nusinersen (Spinraza)	Spinal muscular dystrophy	Exon 7 of *SMN2*	IT/CNS (motoneurons)	FDA in 2016 EMA in 2017	Biogen	Splice switching: exon inclusion
Milasen	CLN7 Batten disease	Exon 6 of *MFSD8*	IT/CNS	FDA in 2019	Boston Children's Hospital	Splice switching: exon skipping
Eteplirsen (Exondys51)	Duchenne muscular dystrophy	Exon 51 of *DMD*	IV/skeletal muscle	FDA in 2016 refused by EMA	Sarepta Therapeutics	Splice switching: exon skipping
Golodirsen (Vyondys 53)	Duchenne muscular dystrophy	Exon 53 of *DMD*	IV/skeletal muscle	FDA in 2019	Sarepta Therapeutics	Splice switching: exon skipping
Viltolarsen (Viltepso)	Duchenne muscular dystrophy	Exon 53 of *DMD*	IV/skeletal muscle	FDA in 2020	NS Pharma	Splice switching: exon skipping
Carimersen (Amondys 45)	Duchenne muscular dystrophy	Exon 45 of *DMD*	IV/skeletal muscle	FDA in 2021	Sarepta Therapeutics	Splice switching: exon skipping
Fomivirsen (Vitravene)	Cytomegalovirus retinitis (immunocompromised patients)	*UL123*	IVT/eye	FDA in 1998 EMA in 1999 Withdrawn from market	Ionis Pharmaceuticals	Translation inhibition
Inotersen (Tegsedi)	Hereditary transthyretin amyloidosis (polyneuropathy)	*TTR*	SC/liver	FDA in 2018 EMA in 2018	Ionis Pharmaceuticals	mRNA degradation
Valeriasen	Developmental and epileptic encephalopathy-14	*KCNT1*	IT/CNS	FDA in 2020	Boston Children's Hospital	mRNA degradation
Tofersen (Qualsody)	Amyotrophic lateral sclerosis	*SOD1*	IT/CNS (motoneurons)	FDA in 2023	Biogen	mRNA degradation
siRNA						
Patisiran (Onpattro)	Hereditary transthyretin amyloidosis (polyneuropathy)	*TTR*	IV/liver	FDA in 2018 EMA in 2018	Alnylam Pharmaceuticals	mRNA degradation
Vutrisiran (Amvuttra)	Hereditary transthyretin amyloidosis (polyneuropathy)	*TTR*	SC/liver	FDA in 2022 EMA in 2022	Alnylam Pharmaceuticals	mRNA degradation
Givosiran (Givlaari)	Acute hepatic porphyria	*ALAS1*	SC/liver	FDA in 2019 EMA in 2020	Alnylam Pharmaceuticals	mRNA degradation
RNA aptamer						
Pegaptanib (Macugen)	Age-related macular degeneration	*VEGF(165)*	IVT/eye	FDA in 2004 EMA in 2006	OSI pharmaceuticals	Protein inhibition

ASO: antisense oligonucleotide; siRNA: short interfering RNA; IT: intrathecal; CNS: central nervous system; IV: intravenous; IVT: intravitreal; SC: subcutaneous.

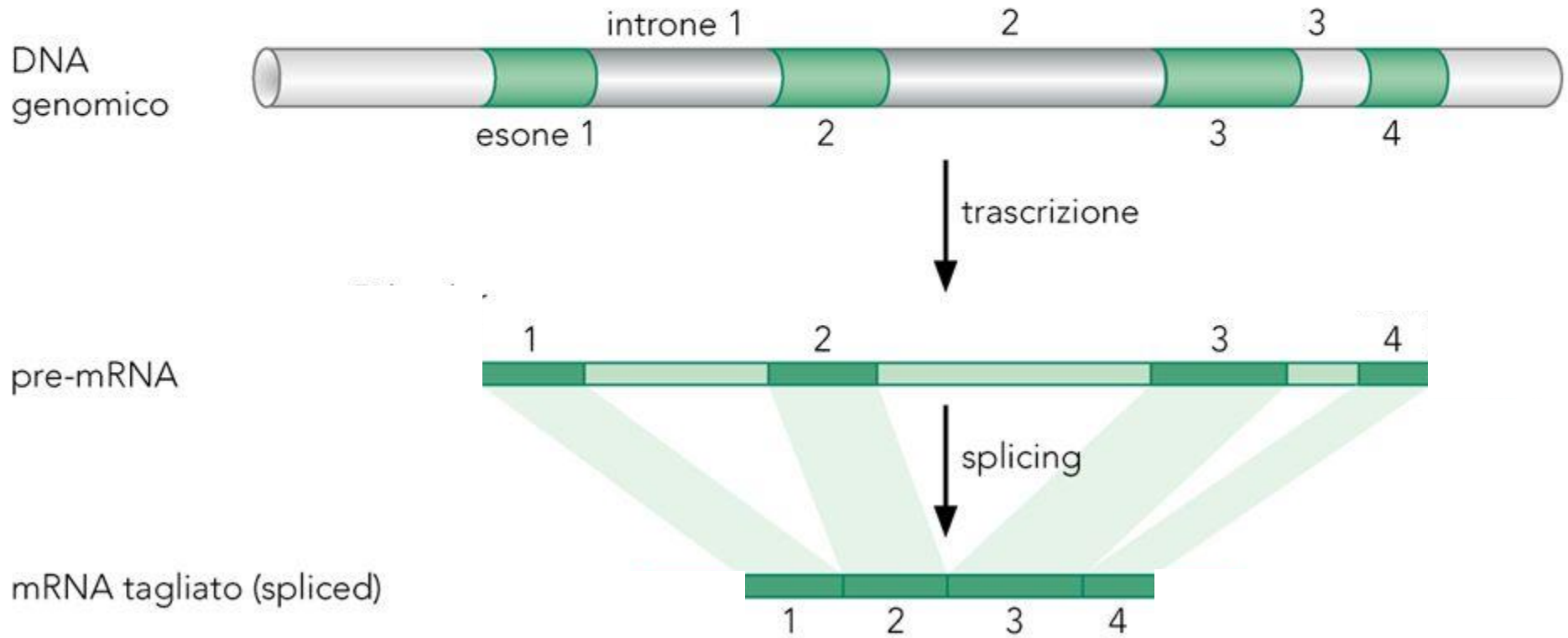
Spice switching:

- Exon inclusion
- Exon skipping

mRNA degradation:

- Gapmers
- siRNAs

Lo splicing


DNA genomico
introne 1
2
3
esone 1
2
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4
trascrizione
pre-mRNA
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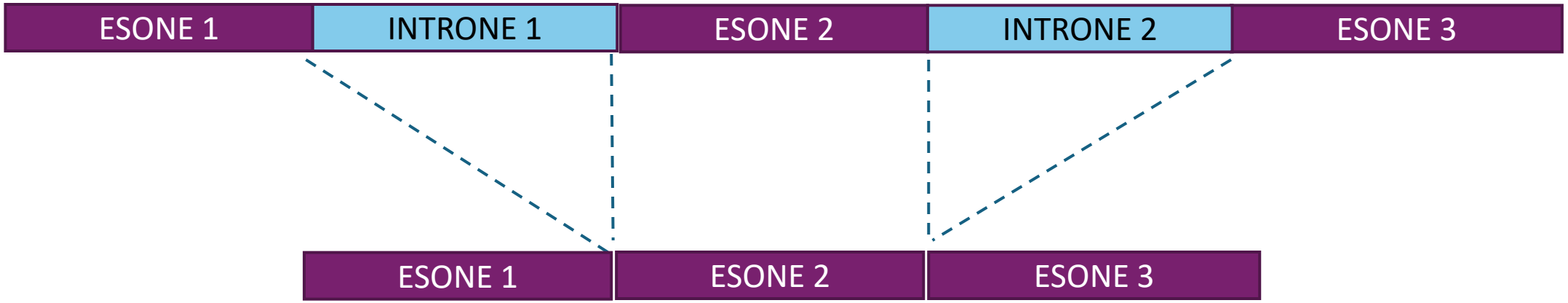
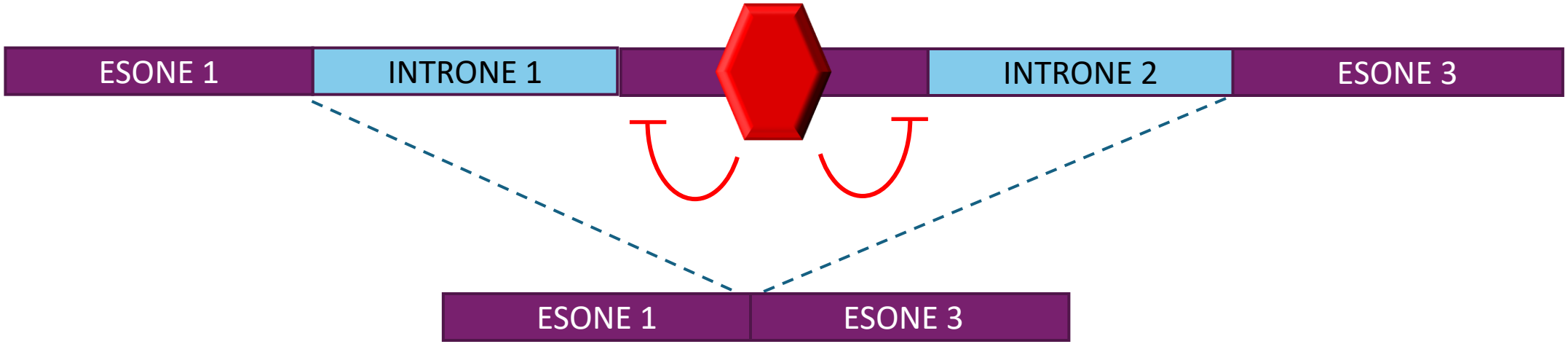
2
3
4
splicing
mRNA tagliato (spliced)
1
2
3
4



REPRESSORI

ATTIVATORI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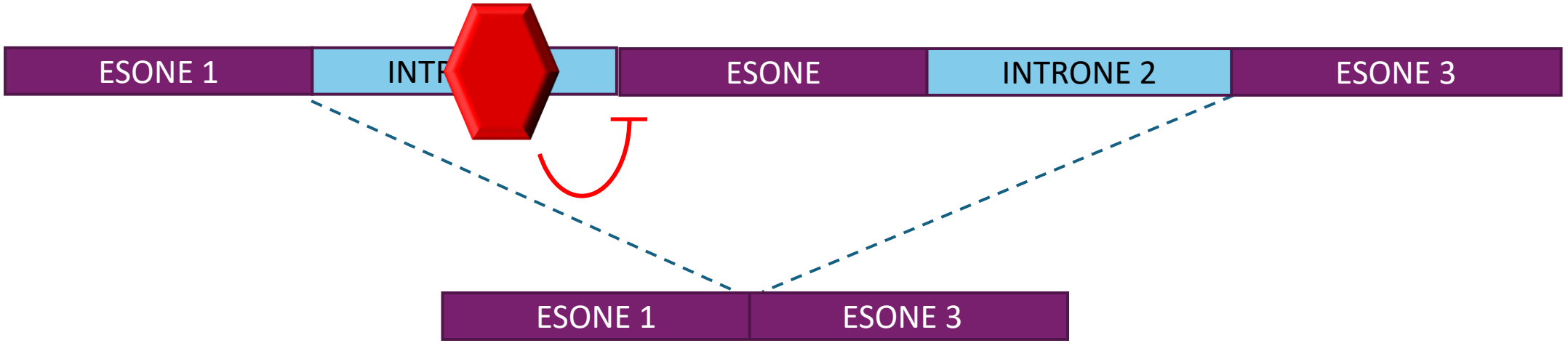
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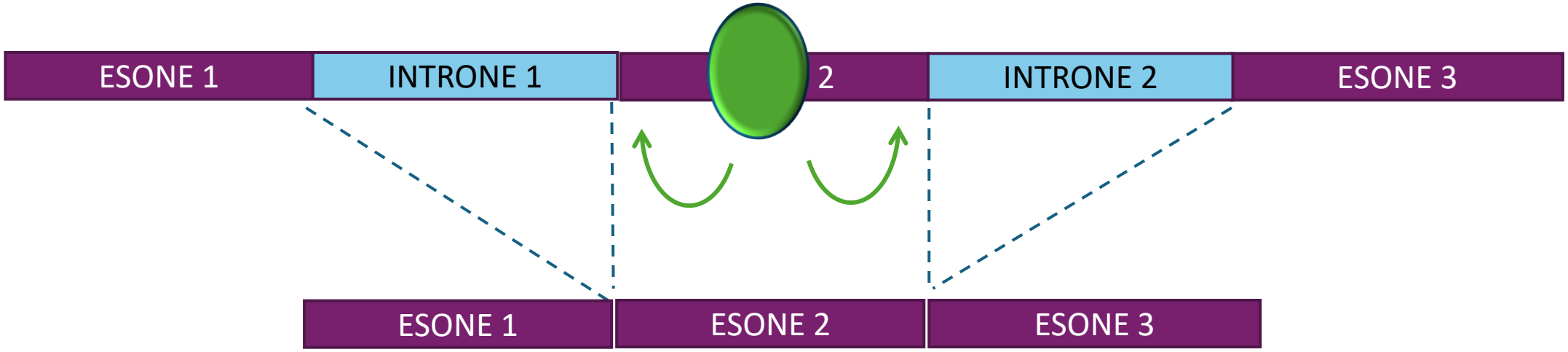
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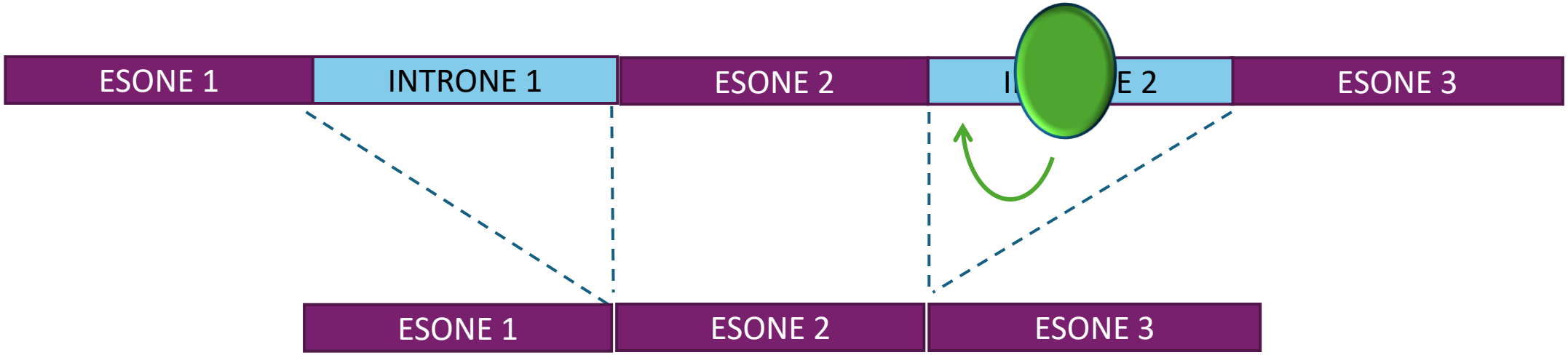
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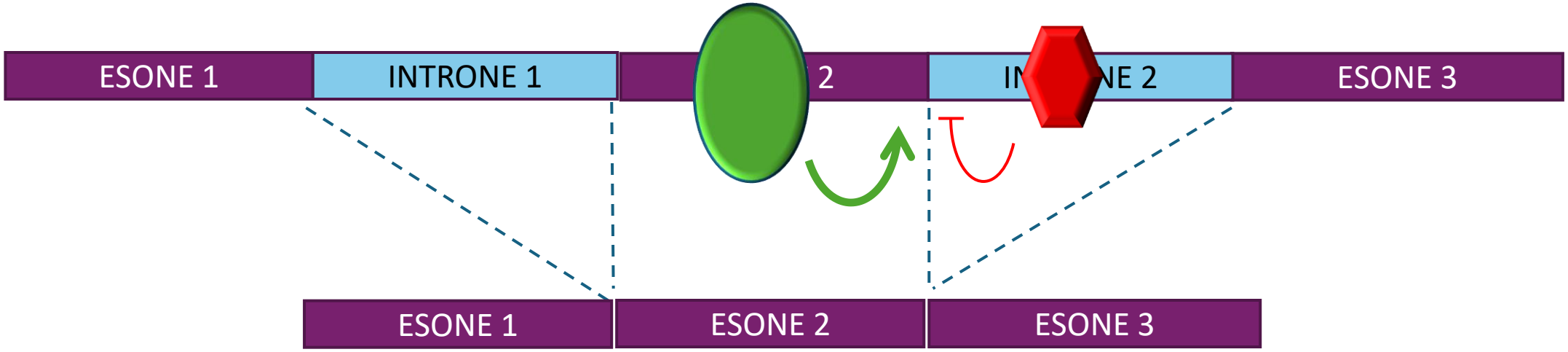
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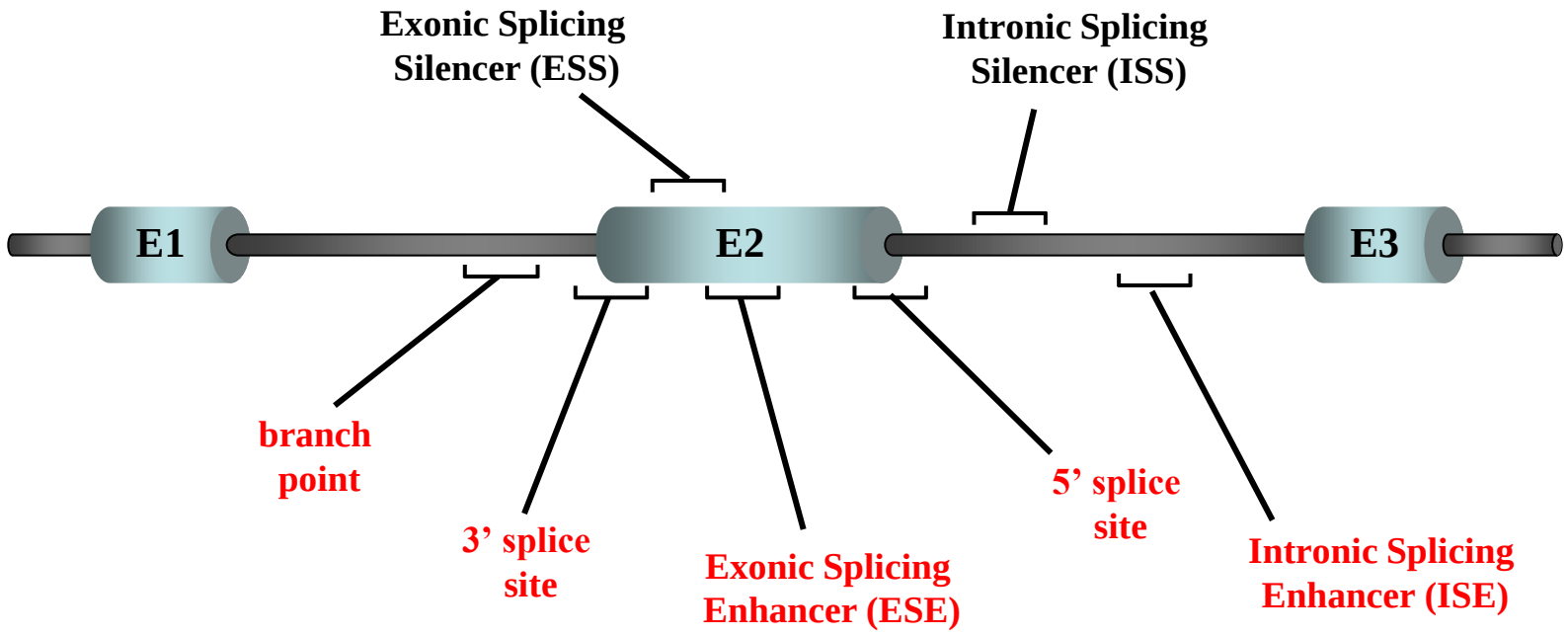
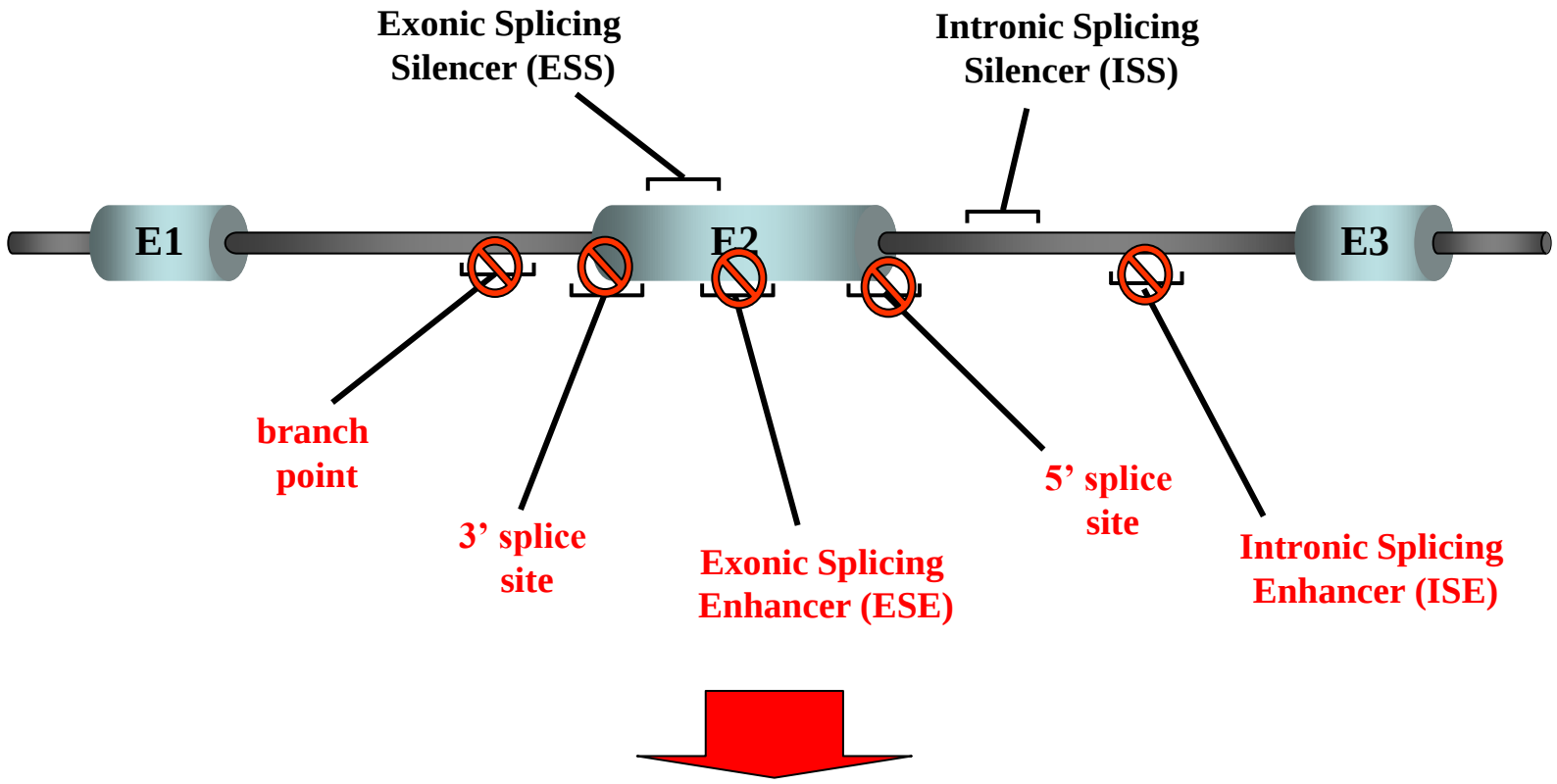
Cis-acting splicing-regulating elements



Antisense-based modulation of splicing

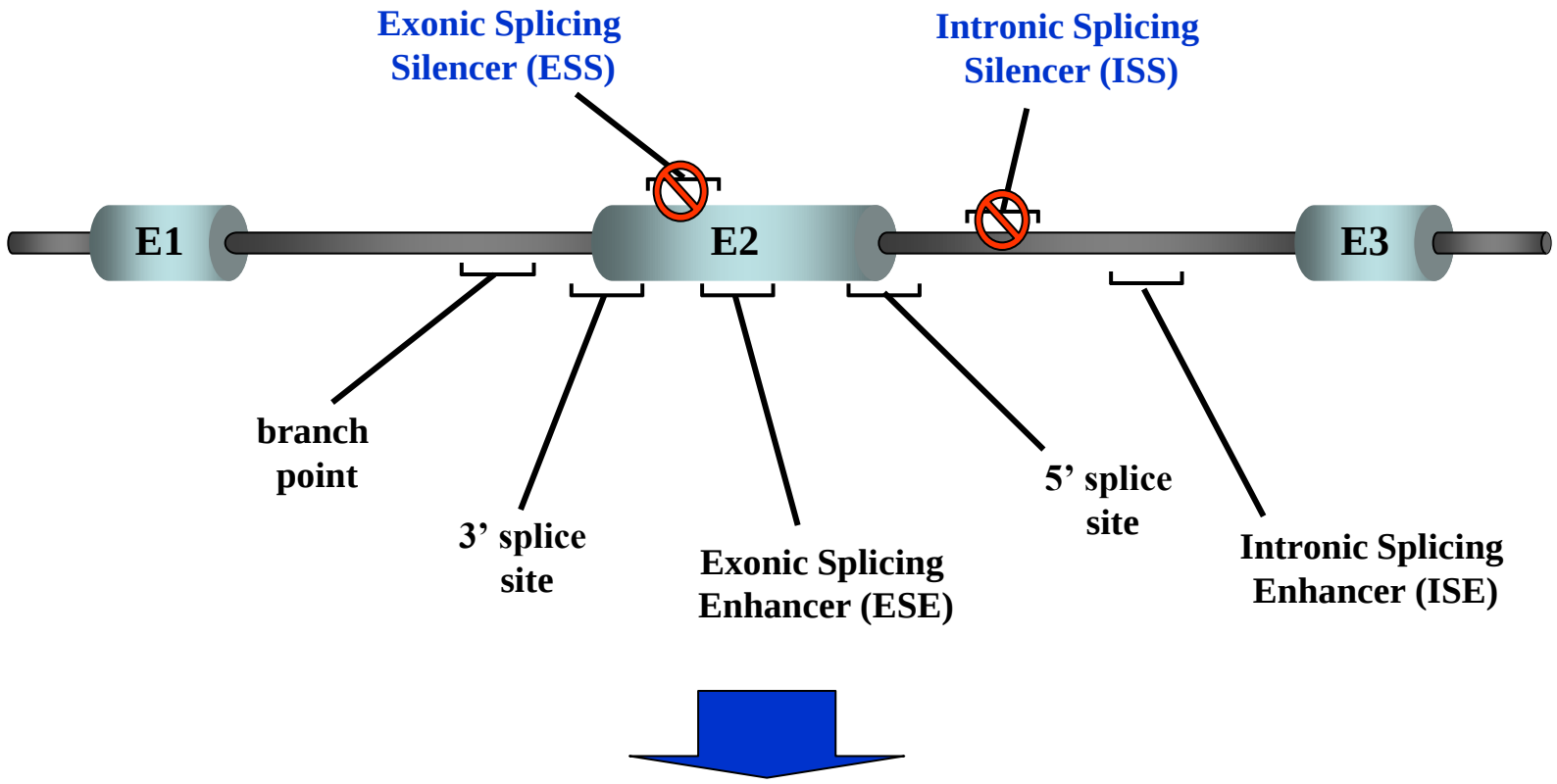
Masking splicing *cis*-elements to the binding of *trans*-factors



EXON SKIPPING

Antisense-based modulation of splicing

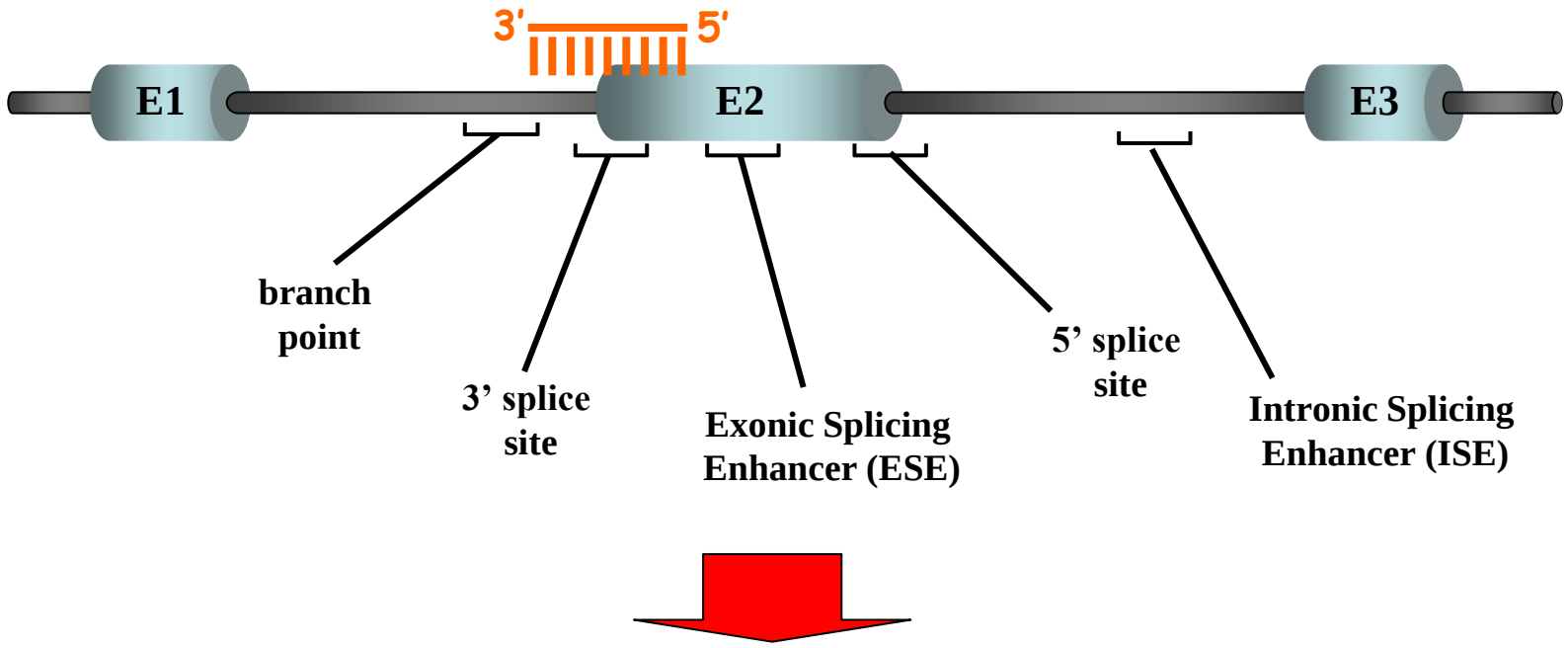
Masking splicing *cis*-elements to the binding of *trans*-factors



EXON INCLUSION

Antisense oligonucleotides for the modulation of splicing

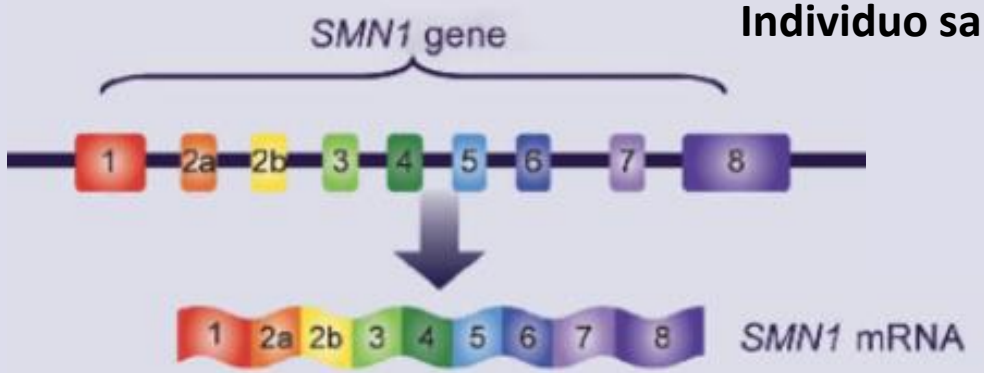
High sequence-specificity, obtained with molecules of low complexity



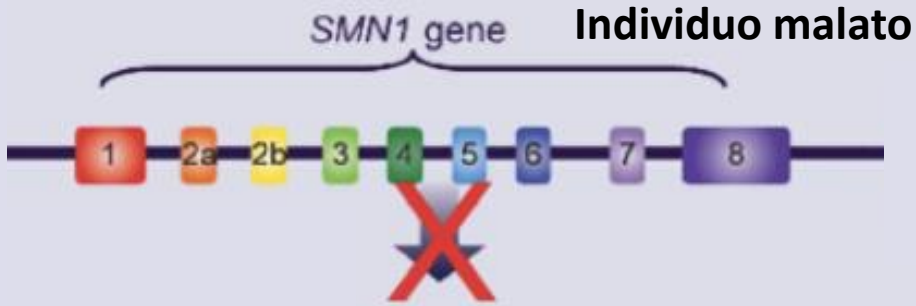
EXON SKIPPING

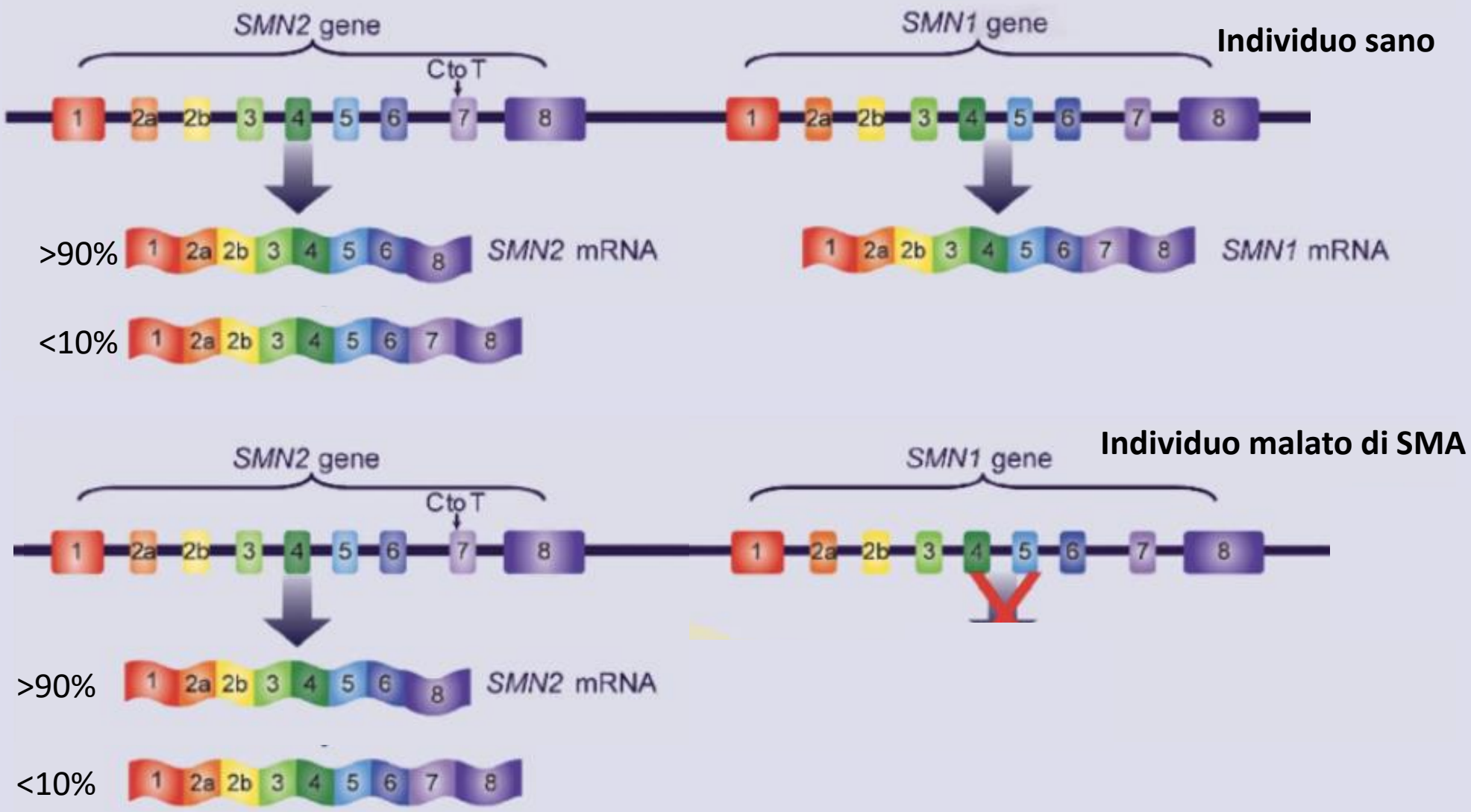
Exon inclusion: Nusinersen apre nuove strade

Individuo sano



Individuo malato di SMA

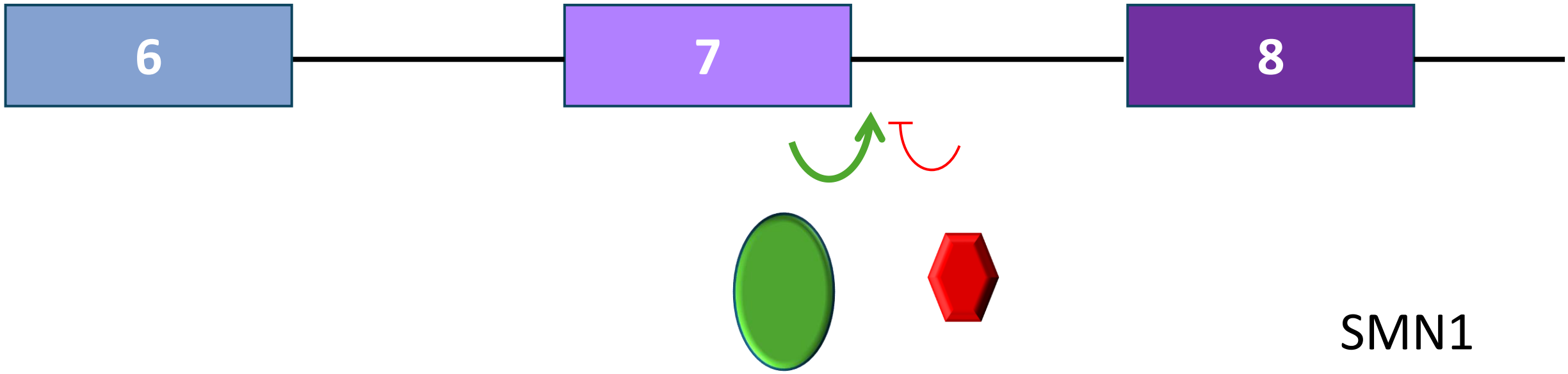



SMN2 gene
C to T
SMN1 gene
Individuo sano
1 2a 2b 3 4 5 6 7 8
>90%
SMN2 mRNA
SMN1 mRNA
<10%
Individuo malato di SMA

Cosa causa la differenza nello splicing dell'esone 7 tra SMN1 e SMN2?

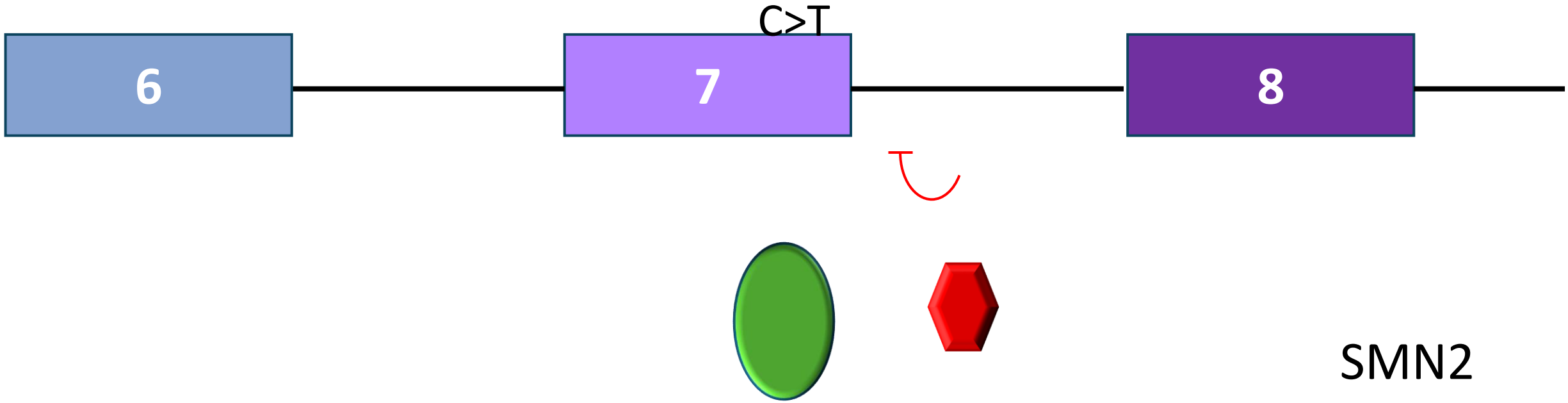
Adrian Krainer

Cosa causa la differenza nello splicing dell'esone 7 tra SMN1 e SMN2?



SMN1

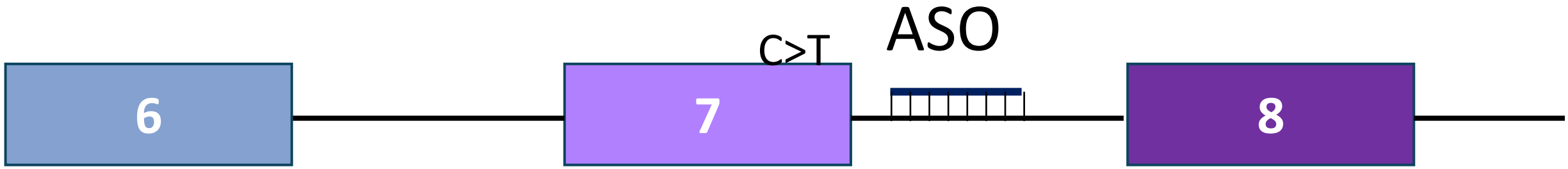
Cosa causa la differenza nello splicing dell'esone 7 tra SMN1 e SMN2?



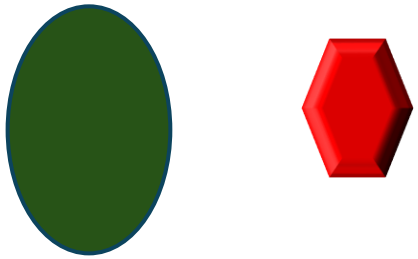
Possiamo regolare lo splicing di SMN2 in modo da fargli fare una proteina Smn funzionale?

Possiamo usare l’RNA come medicina?

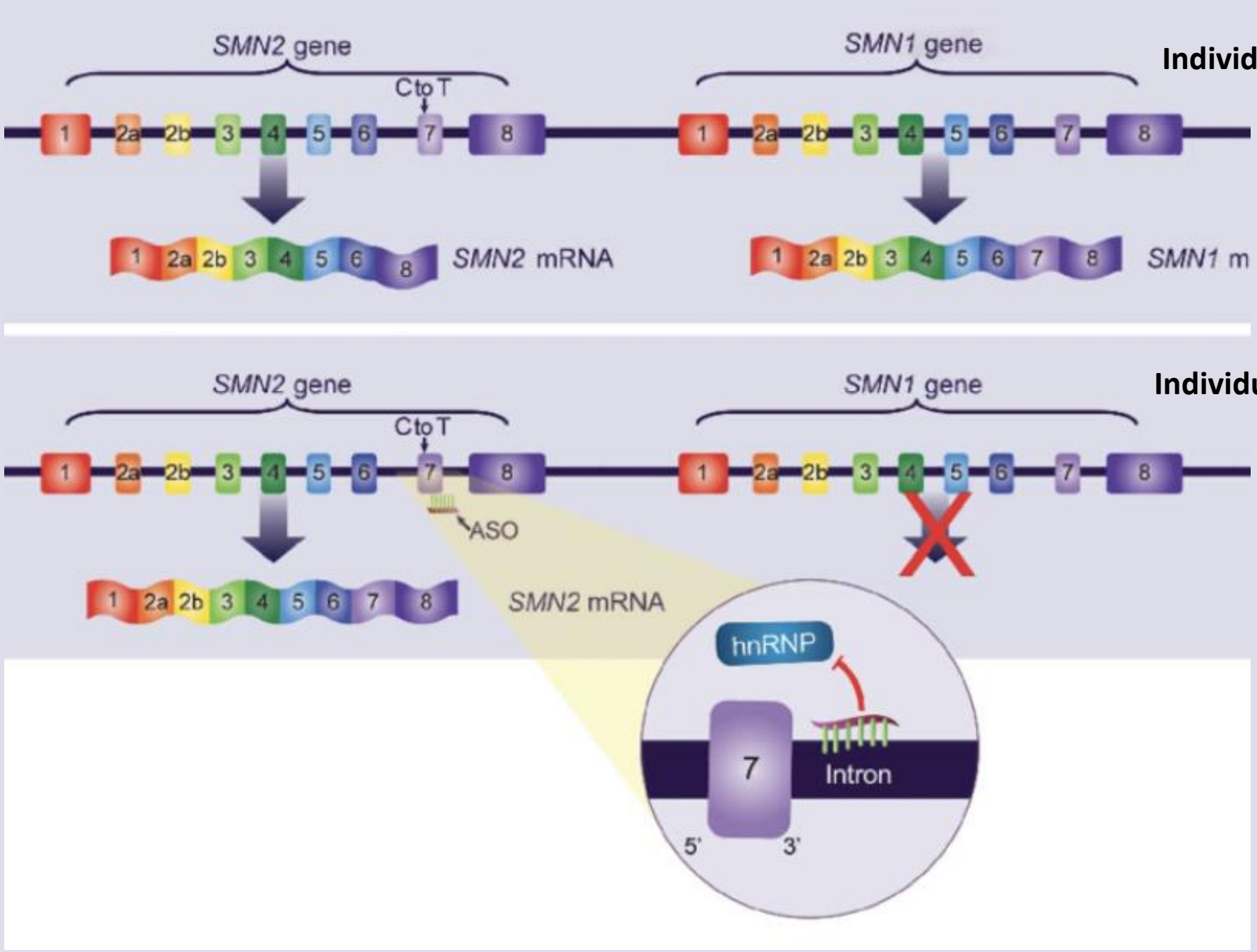
Possiamo regolare lo splicing di SMN2 in modo da fargli fare una proteina Smn funzionale?



Adrian Krainer



SMN2


SMN2 gene
C to T
1 2a 2b 3 4 5 6 7 8
SMN2 mRNA
SMN1 gene
1 2a 2b 3 4 5 6 7 8
SMN1 m
Individuo sano
SMN2 gene
C to T
1 2a 2b 3 4 5 6 7 8
ASO
1 2a 2b 3 4 5 6 7 8
SMN2 mRNA
SMN1 gene
1 2a 2b 3 4 5 6 7 8
Individuo malato di SMA
hnRNP
7
Intron
5'
3'

N ENGL J MED 378;7 NEJM.ORG FEBRUARY 15, 2018

ORIGINAL ARTICLE

Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy

E. Mercuri, B.T. Darras, C.A. Chiriboga, J.W. Day, C. Campbell, A.M. Connolly, S.T. Iannaccone, J. Kirschner, N.L. Kuntz, K. Saito, P.B. Shieh, M. Tulinius, E.S. Mazzone, J. Montes, K.M. Bishop, Q. Yang, R. Foster, S. Gheuens, C.F. Bennett, W. Farwell, E. Schneider, D.C. De Vivo, and R.S. Finkel, for the CHERISH Study Group*

ABSTRACT

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy

R.S. Finkel, E. Mercuri, B.T. Darras, A.M. Connolly, N.L. Kuntz, J. Kirschner, C.A. Chiriboga, K. Saito, L. Servais, E. Tizzano, H. Topaloglu, M. Tulinius, J. Montes, A.M. Glanzman, K. Bishop, Z.J. Zhong, S. Gheuens, C.F. Bennett, E. Schneider, W. Farwell, and D.C. De Vivo, for the ENDEAR Study Group*

ABSTRACT

N ENGL J MED 377;18 NEJM.ORG NOVEMBER 2, 2017

Received: 28 August 2020 | Revised: 19 January 2021 | Accepted: 24 January 2021

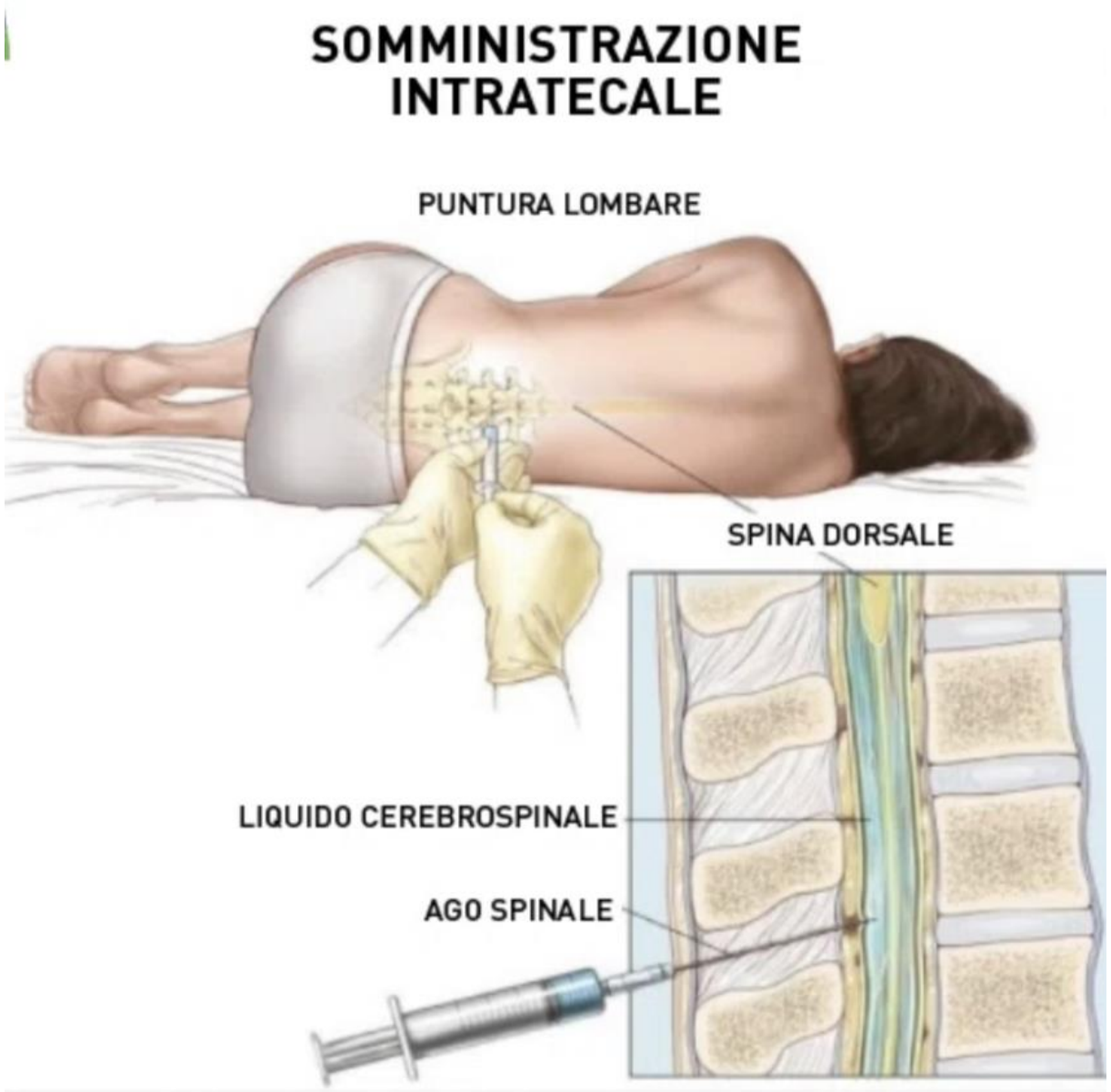
DOI: 10.1002/mus.27187

CLINICAL RESEARCH ARTICLE

MUSCLE&NERVE WILEY

Safety and efficacy of nusinersen in spinal muscular atrophy: The EMBRACE study

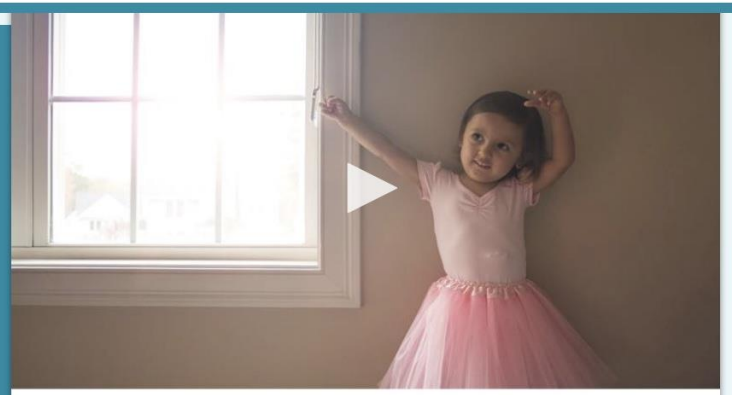
Gyula Acsadi MD, PhD[1] | Thomas O. Crawford MD[2] | Wolfgang Müller-Felber MD[3] | Perry B. Shieh MD[4] | Randal Richardson MD[5] | Niranjana Natarajan MD[6] | Diana Castro MD[7] | Daniela Ramirez-Schrempp MD[8] | Giulia Gambino MSc[9] | Peng Sun PhD[8] | Wildon Farwell MD[8]



SPINRAZA

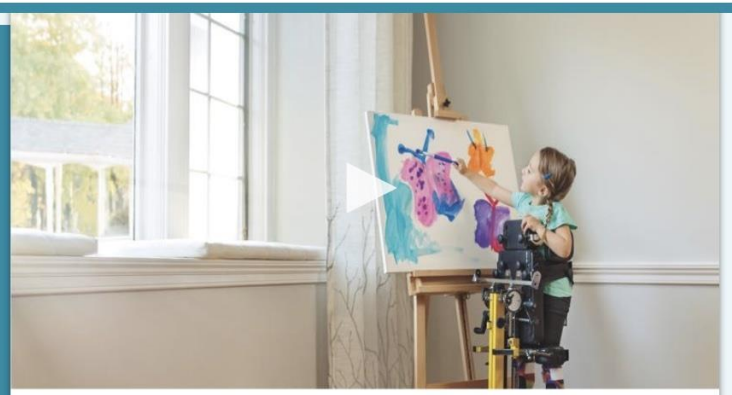
14000 persone trattate negli ultimi 8 anni

2016



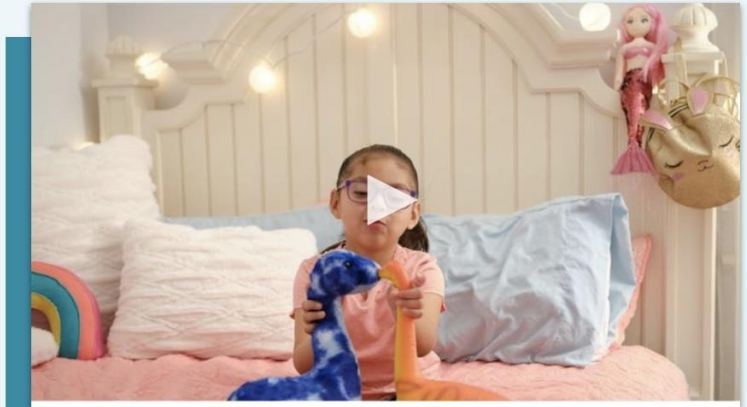
SOFIA // AGE 2 // EARLY-ONSET SMA

“She has got so much joy and so much love and so much drive.”



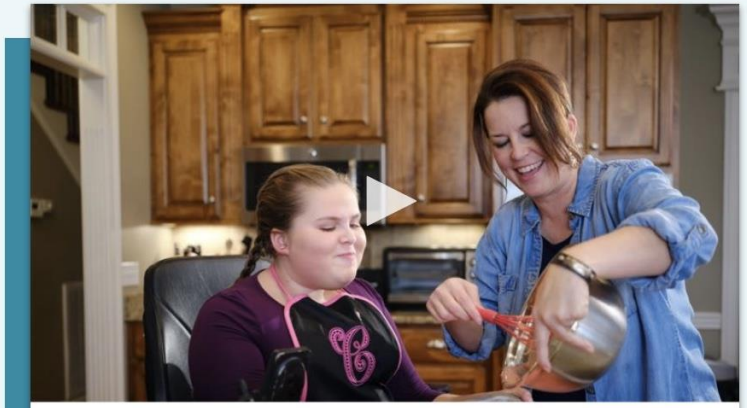
RUBY // AGE 4 // LATER-ONSET SMA

“We feel like we have this tool to fight back with.”



ASHLEY // AGE 7 // LATER-ONSET SMA

“The little gains mean so much.”



CARLEE // AGE 11 // LATER-ONSET SMA

“This is hope for her future.”

Milasen: n-of-one clinical studies

the development of **milasen** was achieved in record time, but it took a high risk/high reward gamble, relying on the safety of IT administration of a chemistry already approved for nusinersen.

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

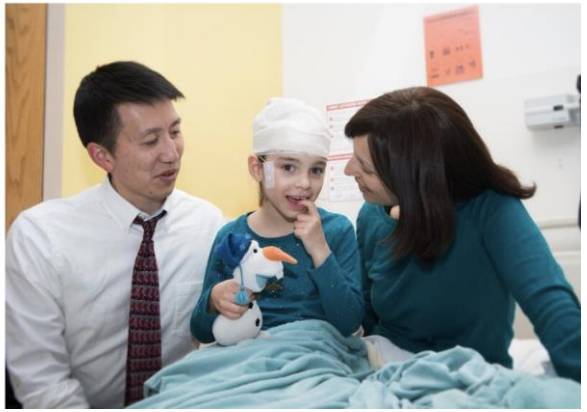
Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease

J. Kim, C. Hu, C. Moufawad El Achkar, L.E. Black, J. Douville, A. Larson, M.K. Pendergast, S.F. Goldkind, E.A. Lee, A. Kuniholm, A. Soucy, J. Vaze, N.R. Belur, K. Fredriksen, I. Stojkovska, A. Tsytsykova, M. Armant, R.L. DiDonato, J. Choi, L. Cornelissen, L.M. Pereira, E.F. Augustine, C.A. Genetti, K. Dies, B. Barton, L. Williams, B.D. Goodlett, B.L. Riley, A. Pasternak, E.R. Berry, K.A. Pflock, S. Chu, C. Reed, K. Tyndall, P.B. Agrawal, A.H. Beggs, P.E. Grant, D.K. Urion, R.O. Snyder, S.E. Waisbren, A. Poduri, P.J. Park, A. Patterson, A. Biffi, J.R. Mazzulli, O. Bodamer, C.B. Berde, and T.W. Yu

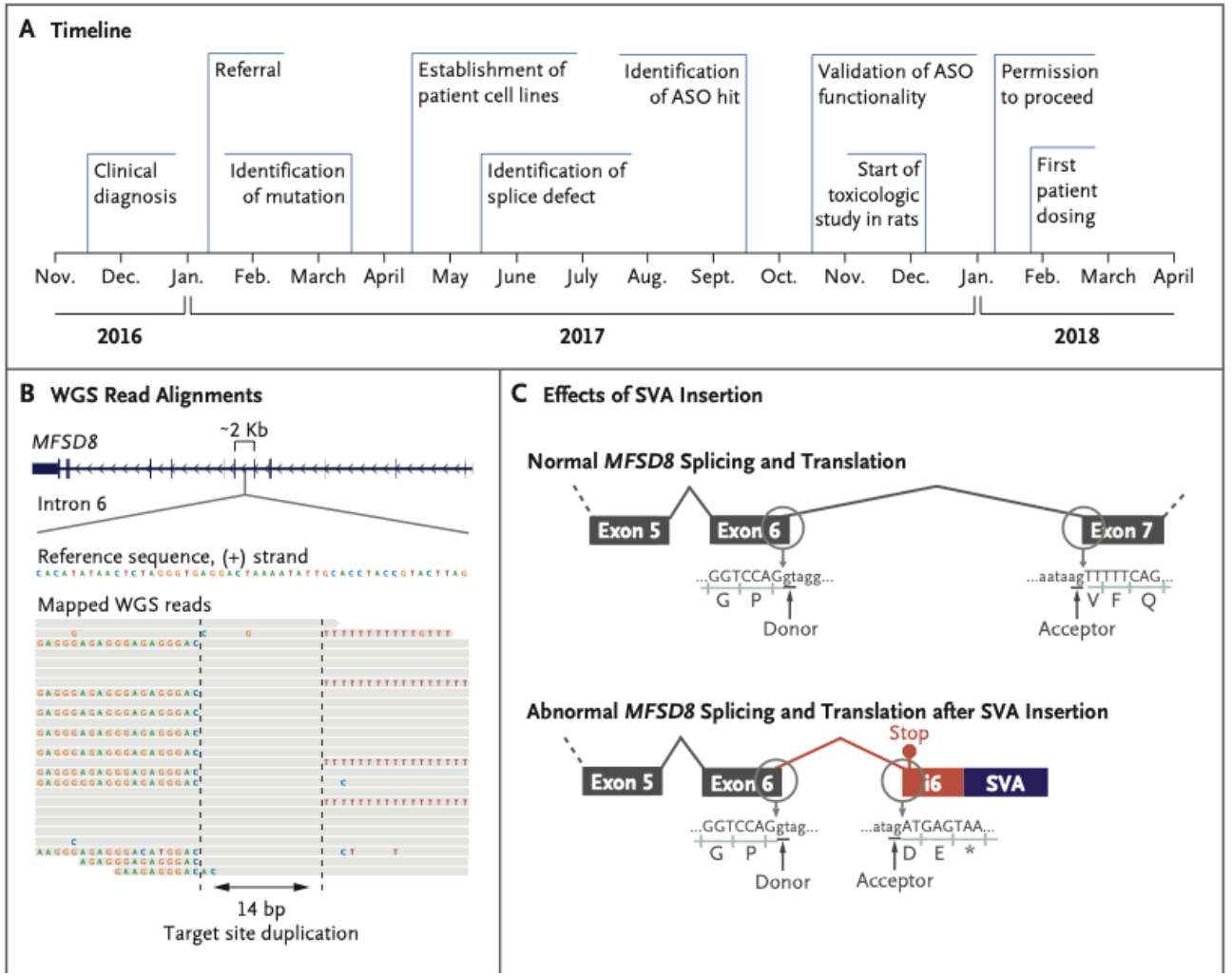
N Engl J Med 2019;381:1644-52.
DOI: 10.1056/NEJMoa1813279

www.Milasmiracle.org

Tim Yu, Mila Makovec. Julia Vitariello



Milasen: n-of-one clinical studies



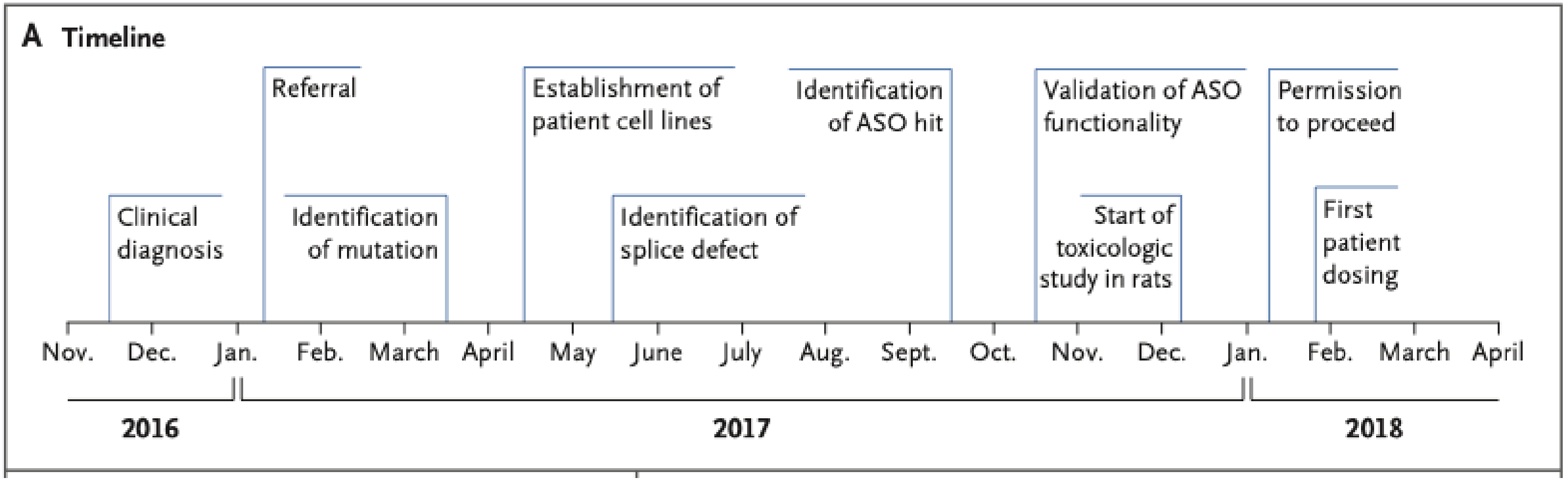
www.Milasmiracle.org

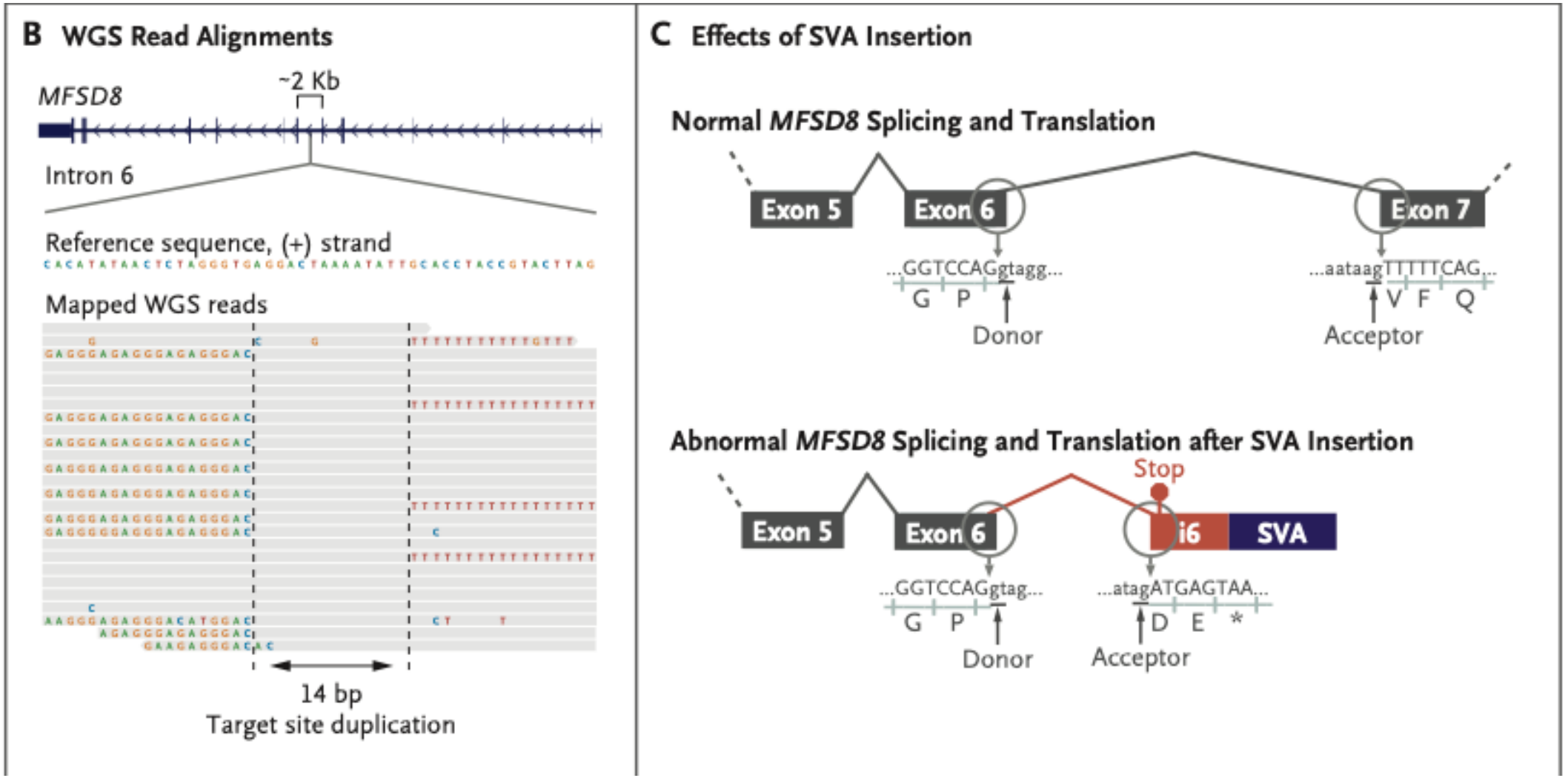
Mila Makovec passed away on February 11, 2021.

Patient-customized ASO

Batten disease

Ceroidolipofuscinosi neuronale giovanile (JNCL)


A Timeline
Clinical diagnosis
Referral
Identification of mutation
Establishment of patient cell lines
Identification of splice defect
Identification of ASO hit
Validation of ASO functionality
Start of toxicologic study in rats
Permission to proceed
First patient dosing
Nov.
Dec.
Jan.
Feb.
March
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
Feb.
March
April
2016
2017
2018


B WGS Read Alignments
MFSD8
~2 Kb
Intron 6
Reference sequence, (+) strand
Mapped WGS reads
14 bp
Target site duplication
C Effects of SVA Insertion
Normal MFSD8 Splicing and Translation
Exon 5
Exon 6
Exon 7
...GGTCCAGgtagg...
G P
Donor
...aataagTTTTTCAG...
V F Q
Acceptor
Abnormal MFSD8 Splicing and Translation after SVA Insertion
Stop
Exon 5
Exon 6
i6
SVA
...GGTCCAGgtag...
G P
Donor
...atagATGAGTAA...
D E *
Acceptor

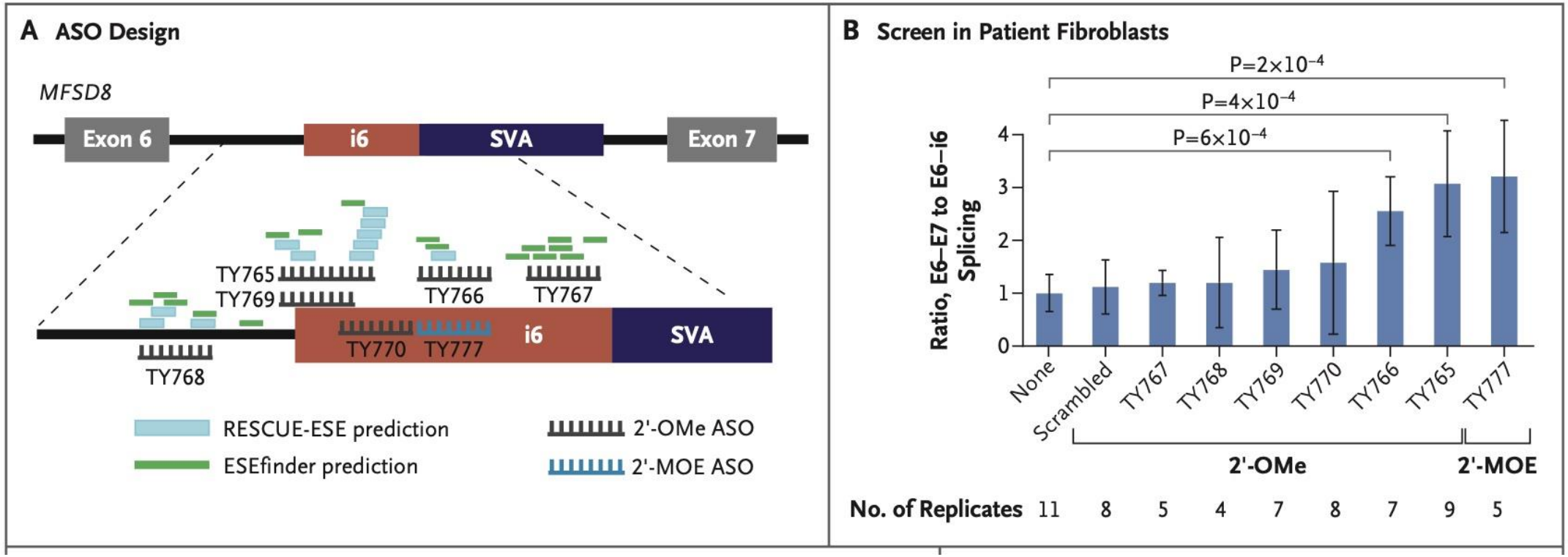
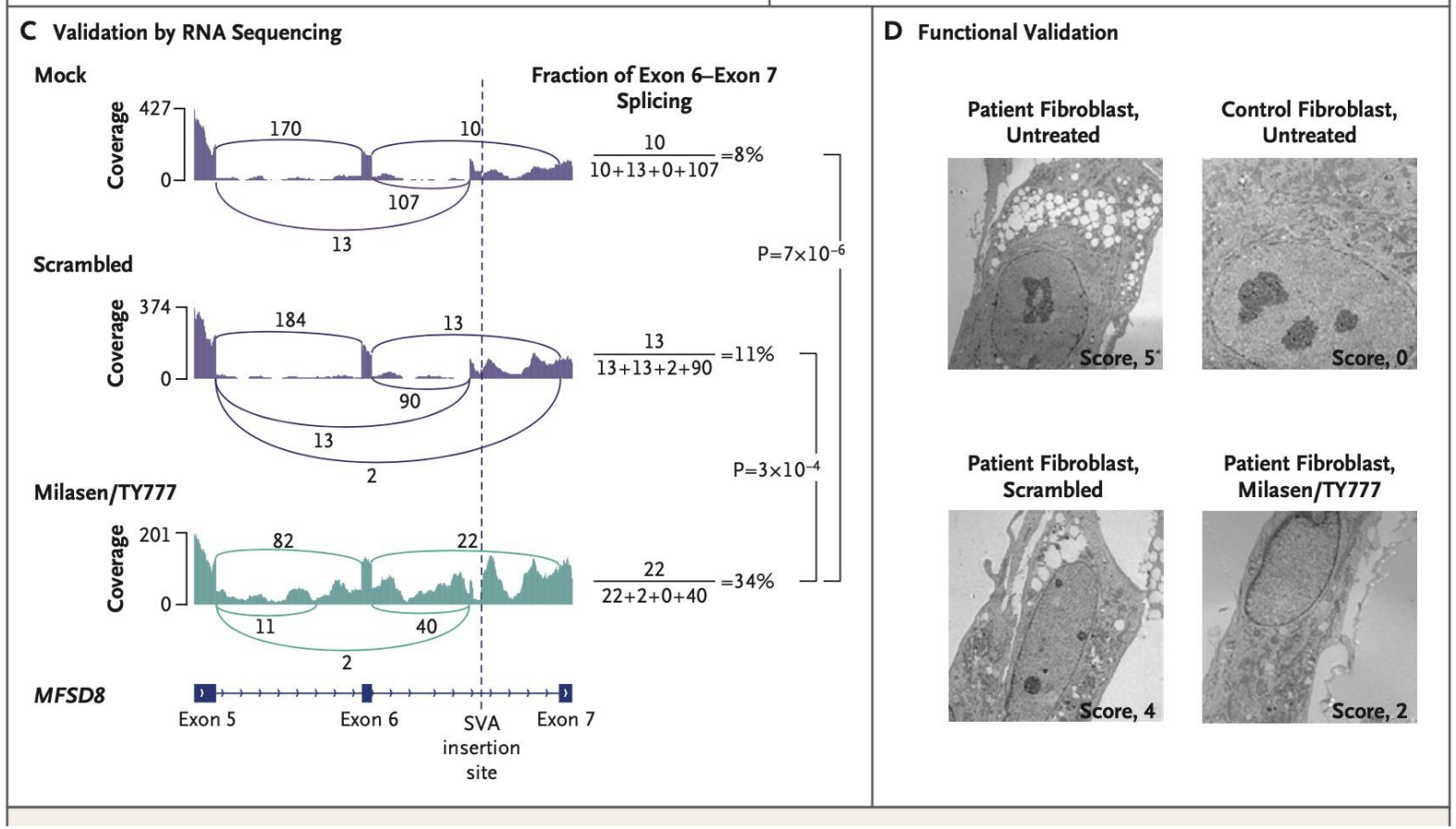


Figure 2. Antisense Oligonucleotide Drug Development.

Panel A shows the location and chemistry of the ASOs that were designed to block the i6.SA splice acceptor site or exonic splicing enhancer (ESE) elements. (Additional details are provided in Table S1.) The ESE elements were predicted with RESCUE-ESE and ESEfinder.[14,15] 2′-MOE denotes 2′-*O*-methoxyethyl, and 2′-OMe 2′-*O*-methyl. Panel B shows the ratio of the normal exon 6–exon 7 (E6–E7) splicing to the abnormal exon 6–intron 6 (E6–i6) splicing (normalized to a no-transfection control), measured in patient fibroblasts that were transfected (for 24 hours at 100 nmol per liter) as indicated. To measure splice isoform-specific levels, multiplex reverse-transcriptase polymerase chain reactions were conducted with isoform-specific primer sets, and then the intensity of the isoform-specific bands was quantified by gel electrophoresis (Fig. S6). "Scrambled" indicates a nontargeting oligonucleotide (TY772). I bars indicate 95% confidence intervals of the means. P values were calculated by two-sided t-test. Panel C shows RNA sequencing (RNA-seq) analysis valida-



quantified by gel electrophoresis (Fig. S6). "Scrambled" indicates a nontargeting oligonucleotide (TY772). I bars indicate 95% confidence intervals of the means. P values were calculated by two-sided t-test. Panel C shows RNA sequencing (RNA-seq) analysis validation of the splice-correcting effect of milasen (TY777). For the calculation of the fraction of normal splicing (exon 6–exon 7), three other splicing events that are mutually exclusive with the normal splicing were considered. Splicing events supported by only one read are not shown. P values were calculated by Fisher's exact test. Panel D shows intracellular vacuoles, visualized by electron microscopy, in control fibroblasts (*MFSD8* wild-type human foreskin fibroblast; BJ cell line) and in patient fibroblasts that are either untreated or transfected with the indicated oligonucleotide. Scoring was performed on a scale of 0 to 5, with 0 representing the lowest and 5 representing the highest level of vacuole accumulation.

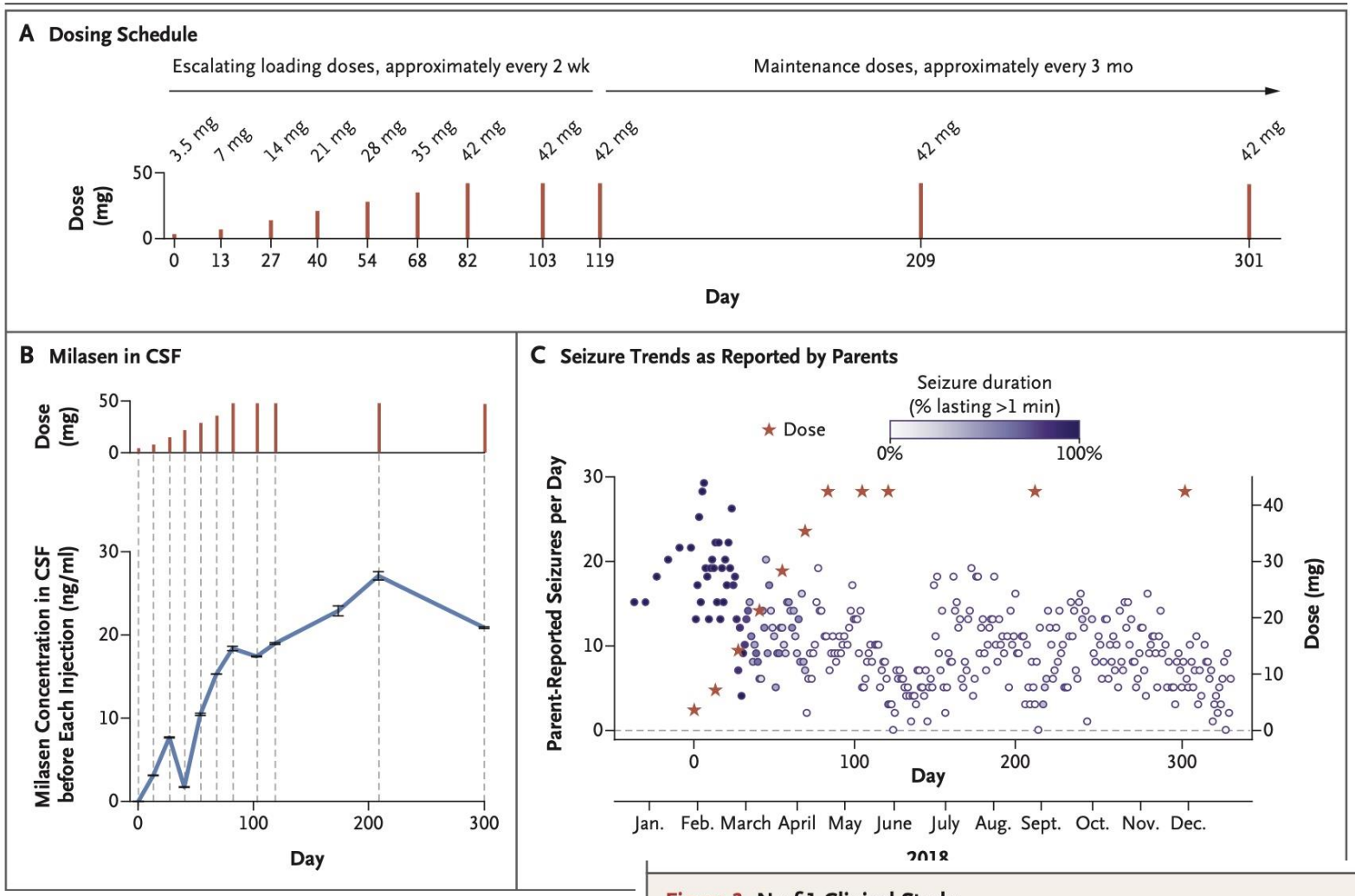
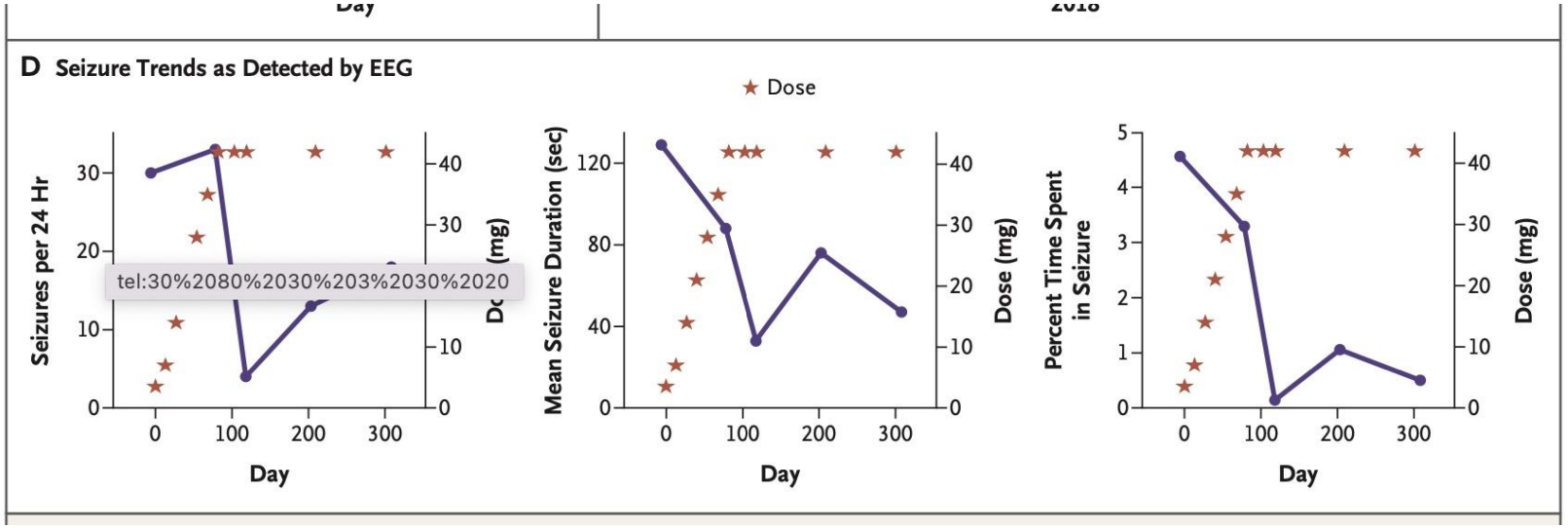


Figure 3. N-of-1 Clinical Study.

Panel A shows the dosing schedule. (Additional details are provided in Fig. S14A.) Panel B shows the concentration of milasen in cerebrospinal fluid (CSF) before each administration (trough). An additional measurement of the concentration in CSF was obtained at day 174 (without concurrent dose administration). I bars indicate the minimum and maximum values of duplicate measurements. Trough levels rose steadily in a dose-proportional fashion until day 40, at which point they dropped to 1.7 ng per milliliter and then resumed their rise with repeated dosing up to a plateau of 18 to 27 ng per milliliter. The dip at day 40 may have been due to a CSF leak, given its coincident timing with a post–lumbar puncture headache after the previous dose. A similar plateauing of CSF trough levels was observed in a previous study of intrathecally delivered nusinersen (9 to 11 ng per milliliter after four repeated doses of 12 mg).[8] Panel C shows the trends in seizure frequency and duration as reported in a seizure diary recorded by the parents. Seizures were all of the same type: sudden startle followed by uncontrollable, untriggered laughter that was different from the patient's natural laugh, at times accompanied by an increase in the nonspecific repetitive hand movements she had at baseline. Panel D shows the trends in seizure activity as



panied by an increase in the nonspecific repetitive hand movements she had at baseline. Panel D shows the trends in seizure activity as detected by electroencephalography (EEG). In a comparison of the means of the initial two recordings and the subsequent three recordings, the daily seizure count, seizure duration, and percent cumulative time spent in seizure decreased by 63% (from 31.5 to 11.7 per day), 52% (from 108 seconds to 52 seconds), and 85% (from 3.9% to 0.6%), respectively.

www.Milasmiracle.org

Mila Makovec passed away on February 11, 2021.

Ultrarare diseases: N-of-1 studies

https://www.oligotherapeutics.org/will-n-of-1-drugs-play-a-role-in-the-future-of-medicine/

https://www.rnatherapy.nl

Overview n-lorem FOUNDATION

https://www.nlorem.org

N=1

https://www.n1collaborative.org

International Collaborative Network for N-of-1 Trials and Single-Case Designs

https://www.nof1sced.org