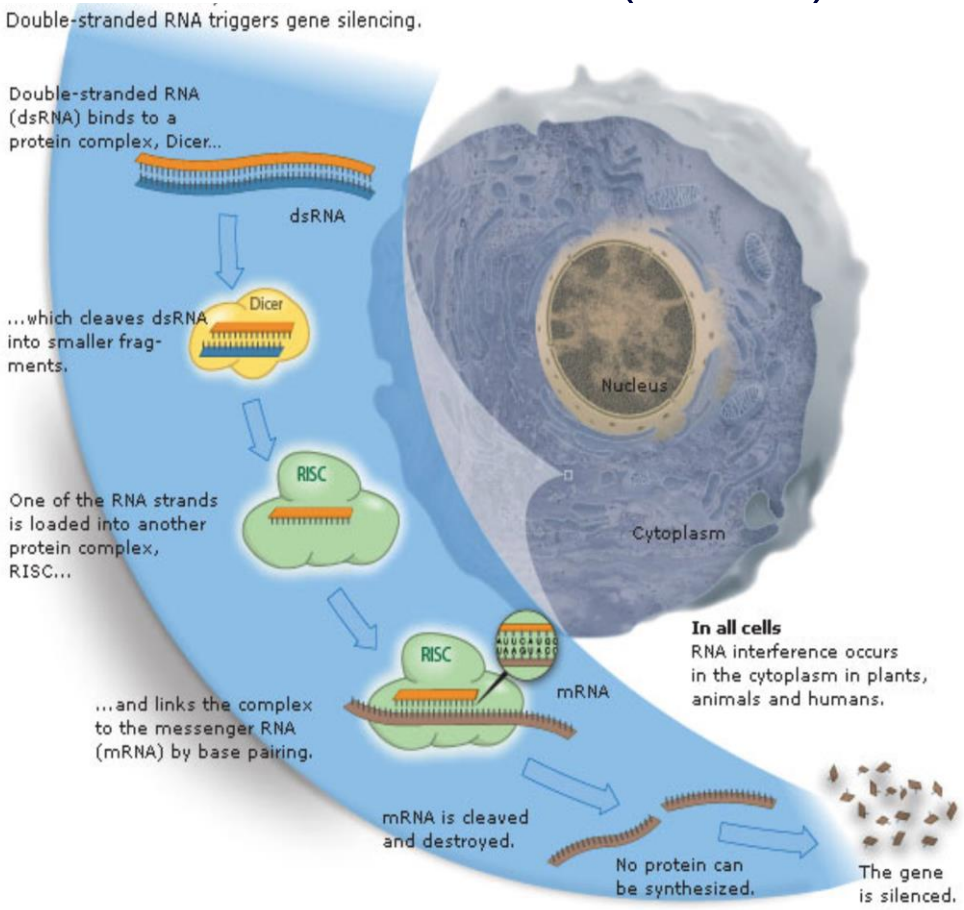
RNA interference (RNAi)



The Nobel Prize in Physiology or Medicine 2006

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

Photo: L. Cicero/Stanford

Andrew Z. Fire

1/2 of the prize

USA

Stanford University School of Medicine
Stanford, CA, USA

Photo: R. Carlin/UMMAS

Craig C. Mello

1/2 of the prize

USA

University of Massachusetts Medical School
Worcester, MA, USA

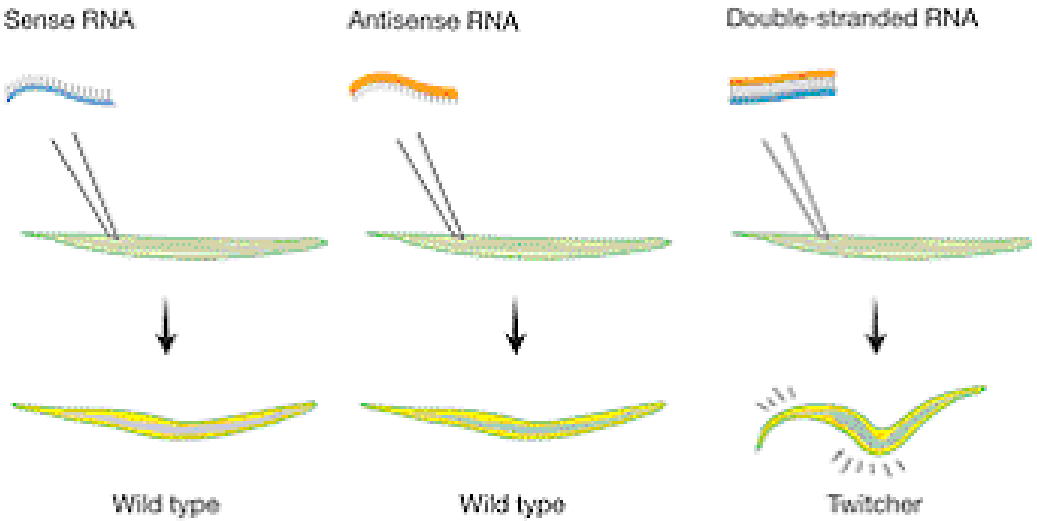
Key breakthrough

dsRNA is the actual trigger of specific mRNA degradation, with the sequence of dsRNA determining which mRNA is degraded

NATURE | VOL 391 | 19 FEBRUARY 1998

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

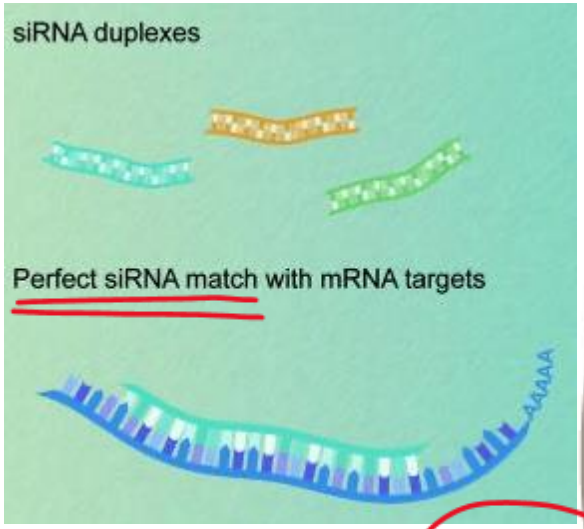


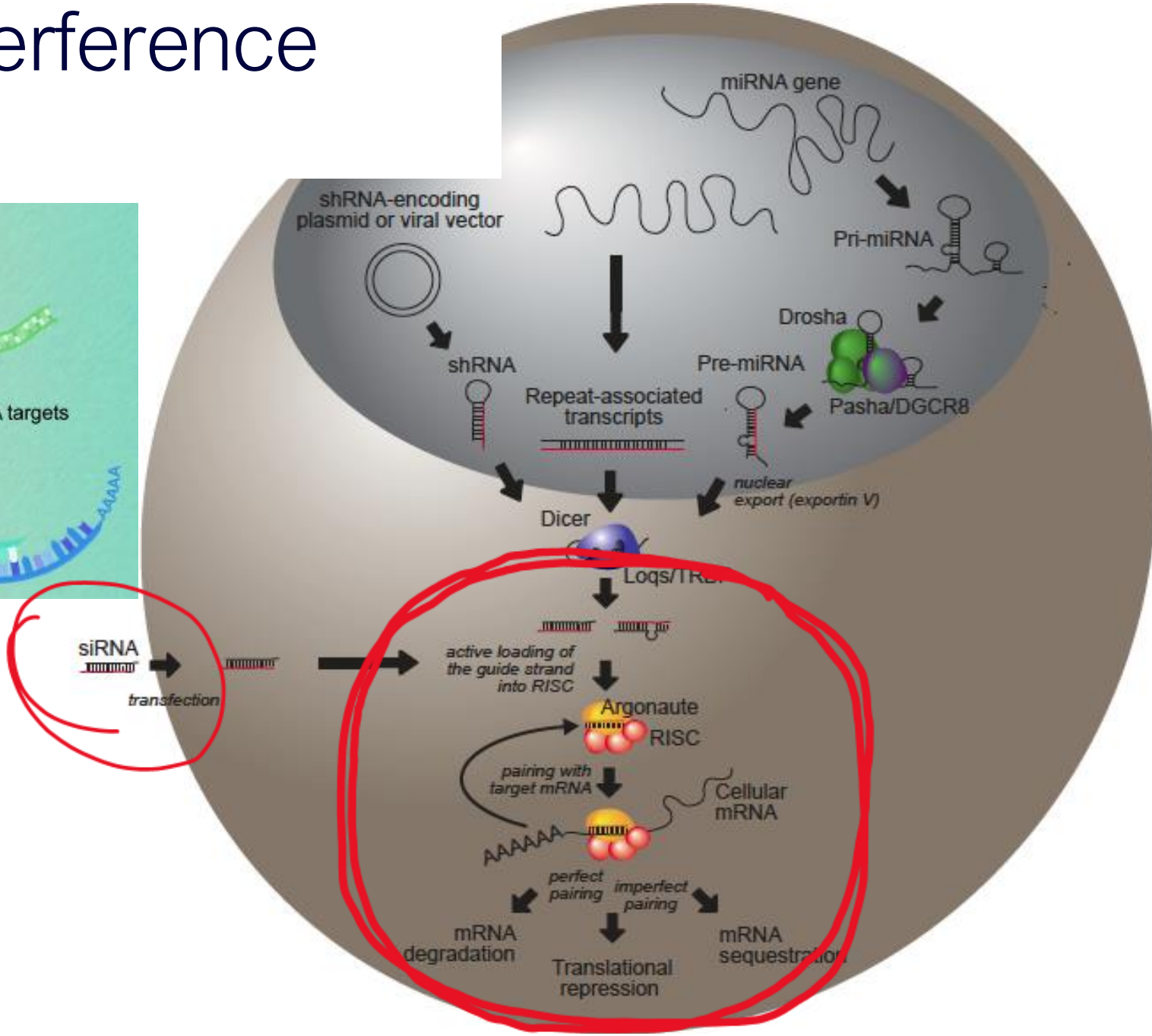
*The **unc-22** gene encodes a myofilament protein. Decrease in unc-22 activity is known to produce severe twitching movements.*

Injected double-stranded RNA, but not single-stranded RNA, induced the twitching phenotype in the progeny.

1) silencing was triggered by injected **dsRNA**, but weakly or not at all by sense or antisense single-stranded RNAs.
2) silencing was **specific** for an mRNA homologous to the dsRNA; other mRNAs were unaffected
3) the dsRNA had to correspond to the mature mRNA sequence; neither intron nor promoter sequences triggered a response. This indicated a **post-transcriptional**, **cytoplasmic** mechanism
4) the targeted mRNA was **degraded**
5) the dsRNA effect could spread between tissues and even to the progeny, suggesting a **transmission** of the effect between cells

RNA interference (RNAi)





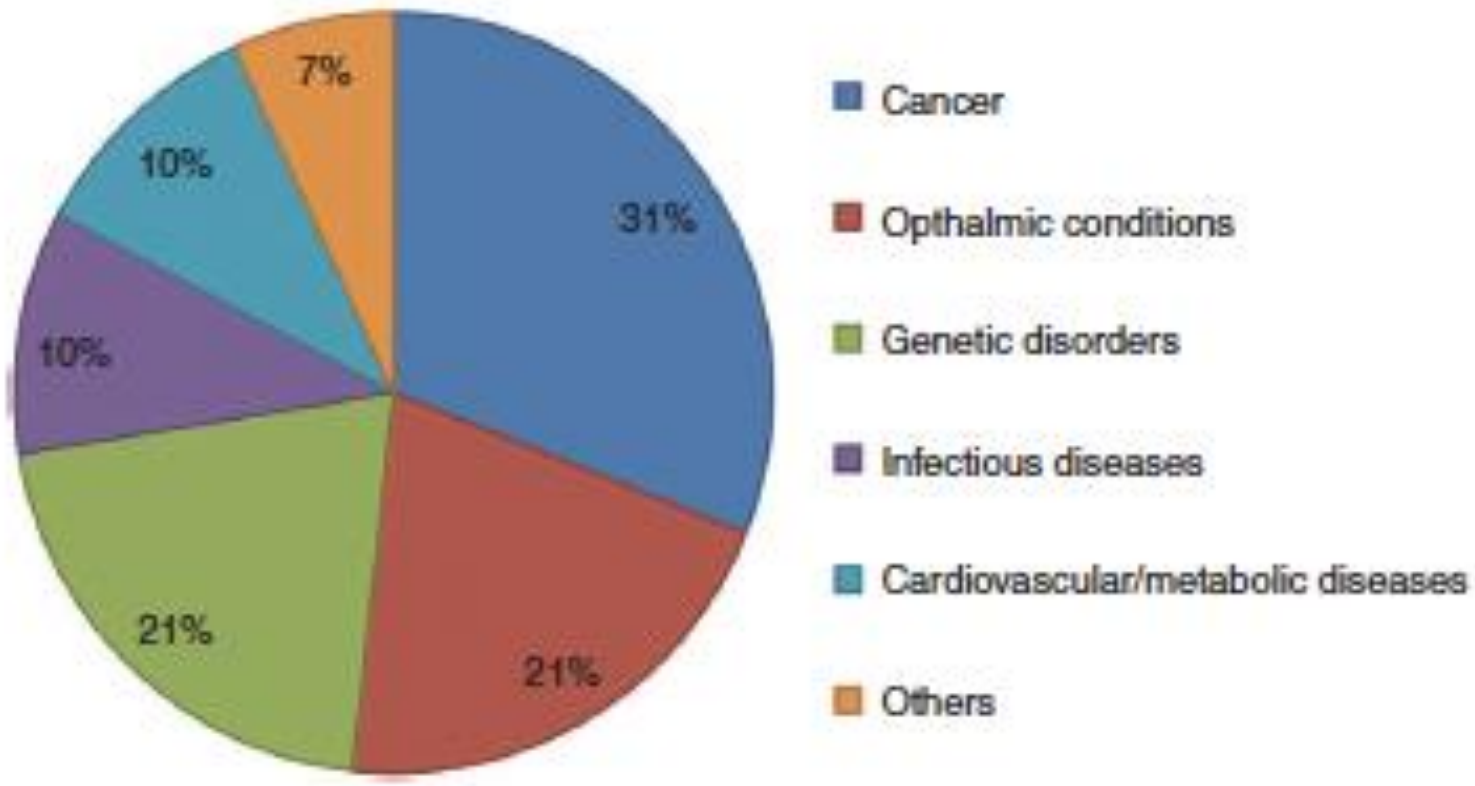
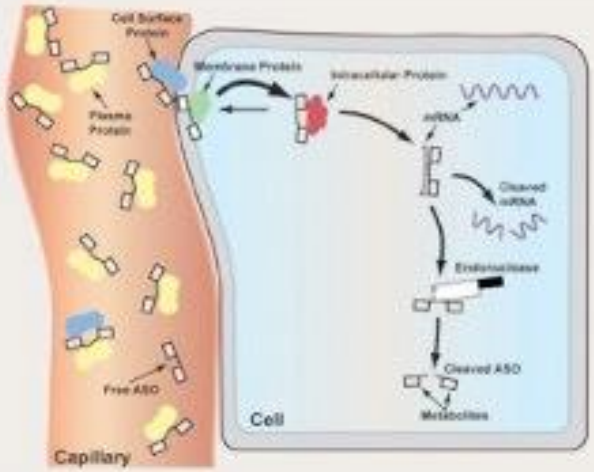


Figure 4 Therapeutic indications of siRNA and miRNA therapeutics.

Second Edition

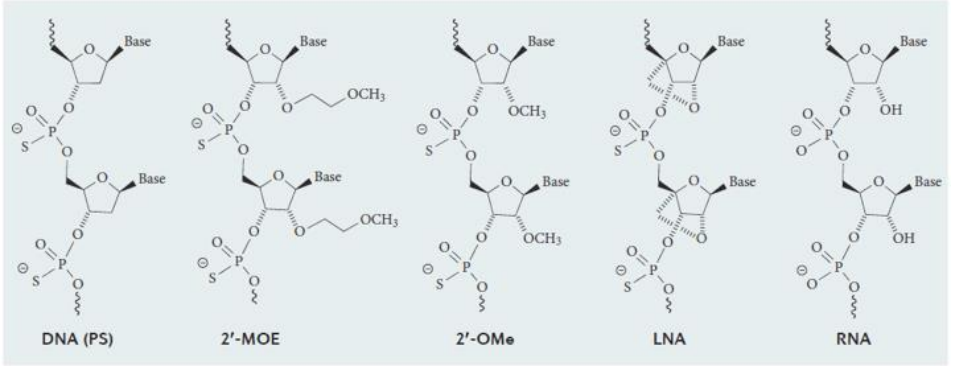
Antisense Drug Technology

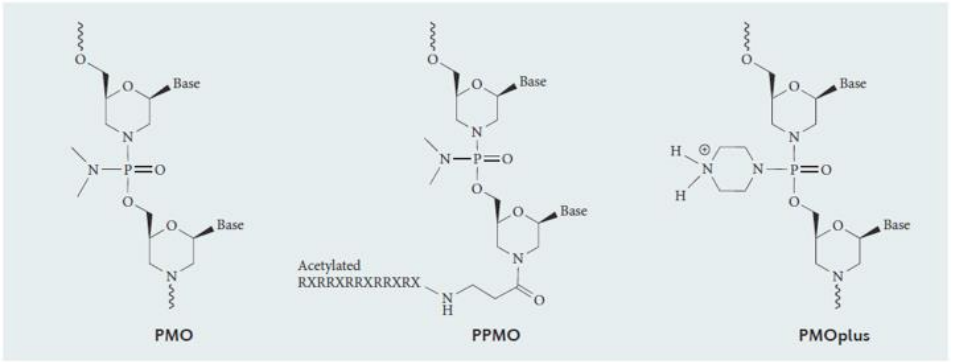
Principles, Strategies, and Applications



Edited by
Stanley T. Crooke

CRC Press



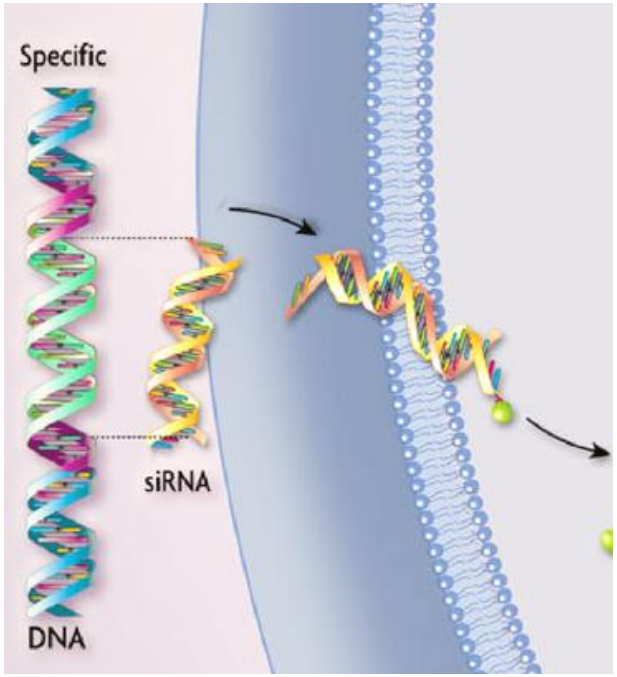


RNA therapeutics: beyond RNA interference and antisense oligonucleotides

Ryszard Kole[1], Adrian R. Krainer[2] and Sidney Altman[3]

Abstract | Here, we discuss three RNA-based therapeutic technologies exploiting various oligonucleotides that bind to RNA by base pairing in a sequence-specific manner yet have different mechanisms of action and effects. RNA interference and antisense oligonucleotides downregulate gene expression by inducing enzyme-dependent degradation of targeted mRNA. Steric-blocking oligonucleotides block the access of cellular machinery to pre-mRNA and mRNA without degrading the RNA. Through this mechanism, steric-blocking oligonucleotides can redirect alternative splicing, repair defective RNA, restore protein production or downregulate gene expression. Moreover, they can be extensively chemically modified to acquire more drug-like properties. The ability of RNA-blocking oligonucleotides to restore gene function makes them best suited for the treatment of genetic disorders. Positive results from clinical trials for the treatment of Duchenne muscular dystrophy show that this technology is close to achieving its clinical potential.

FDA APPROVES FIRST RNA-BASED THERAPEUTIC



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 5, 2018 VOL. 379 NO. 1

Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis

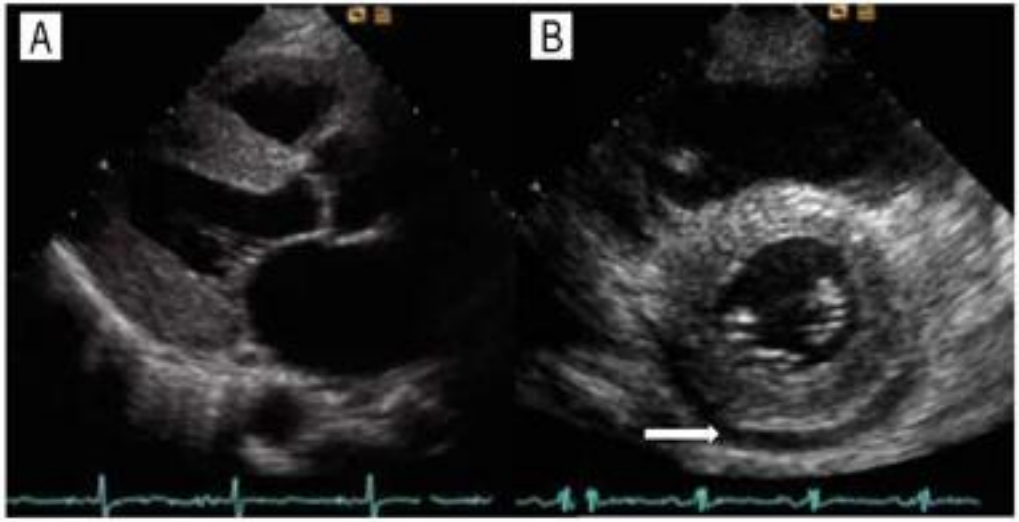
D. Adams, A. Gonzalez-Duarte, W.D. O'Riordan, C.-C. Yang, M. Ueda, A.V. Kristen, I. Tournev, H.H. Schmidt, T. Coelho, J.L. Berk, K.-P. Lin, G. Vita, S. Attarian, V. Planté-Bordeneuve, M.M. Mezei, J.M. Campistol, J. Buades, T.H. Brannagan III, B.J. Kim, J. Oh, Y. Parman, Y. Sekijima, P.N. Hawkins, S.D. Solomon, M. Polydefkis, P.J. Dyck, P.J. Gandhi, S. Goyal, J. Chen, A.L. Strahs, S.V. Nochur, M.T. Sweetser, P.P. Garg, A.K. Vaishnaw, J.A. Gollob, and O.B. Suhr

Patisiran is the first clinical treatment for polyneuropathy of hereditary transthyretin-mediated amyloidosisin adult patients.

- L'amiloidosi ereditaria da transtiretina (ATTRv) è una malattia genetica rara a trasmissione autosomica dominante.

- Le varianti amiloidogeniche del gene TTR riducono la stabilità della proteina circolante innescando una sequenza di eventi molecolari che ne determinano la progressiva deposizione, a livello extracellulare, in forma di fibre di **amiloide**.

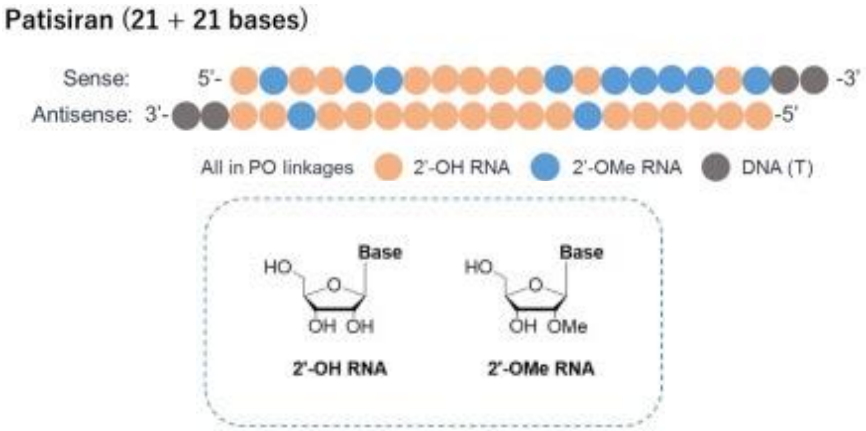
- I depositi di amiloide da transtiretina sono sistemici e causano un danno d'organo ingravescente e inesorabilmente fatale se la malattia non viene riconosciuta e trattata tempestivamente.



(A) Proiezione parasternale asse lungo: si può notare l'aumento degli spessori parietali del ventricolo sinistro in assenza di dilatazione della camera (geometria concentrica). (B) Proiezione parasternale asse corto: aspetto "granular sparkling" del miocardio, ispessimento dei lembi valvolari mitralici, lieve versamento pericardico (freccia). (C) Doppler pulsato transmitralico: pattern di tipo restrittivo indicativo di disfunzione

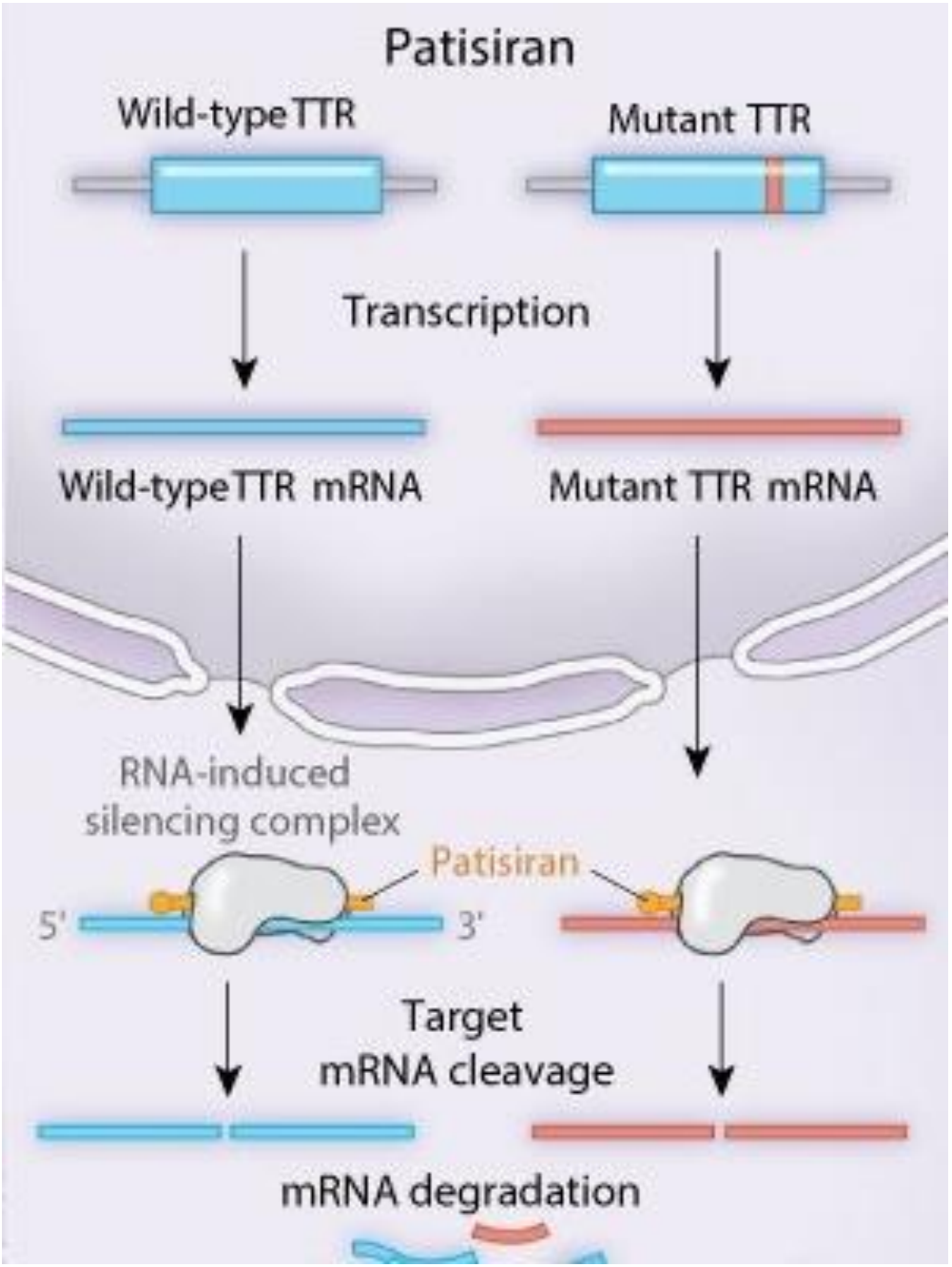
L'amiloidosi da transtiretina causa neuropatia periferica sensitivomotoria e neuropatia autonomica, malattia renale cronica e cardiomiopatia.

L'amiloidosi da transtiretina wild type (ATTRwt) è sempre più riconosciuta come causa di cardiomiopatia infiltrativa negli anziani.



Patisiran è una molecola di acido ribonucleico interferente a doppio filamento (RNAi) che si lega al messaggero RNA (mRNA) di TTR per bloccarne la produzione nel fegato. Usato per l'amiloidosi sia causata da TTR mutata che da TTR normale.

Questo processo **elimina i depositi di amiloide negli organi ripristinandone la funzione**.



Taylor & Francis
Taylor & Francis Group

Amyloid
The Journal of Protein Folding Disorders

ISSN: 1350-6129 (Print) 1744-2818 (Online) Journal homepage: www.tandfonline.com/journals/iamy20

Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial

David Adams, Ivailo L. Tournev, Mark S. Taylor, Teresa Coelho, Violaine Planté-Bordeneuve, John L. Berk, Alejandra González-Duarte, Julian D. Gillmore, Soon-Chai Low, Yoshiki Sekijima, Laura Obici, Chongshu Chen, Prajakta Badri, Seth M. Arum, John Vest, Michael Polydefkis & The HELIOS-A Collaborators

To cite this article: David Adams, Ivailo L. Tournev, Mark S. Taylor, Teresa Coelho, Violaine Planté-Bordeneuve, John L. Berk, Alejandra González-Duarte, Julian D. Gillmore, Soon-Chai Low, Yoshiki Sekijima, Laura Obici, Chongshu Chen, Prajakta Badri, Seth M. Arum, John Vest, Michael Polydefkis & The HELIOS-A Collaborators (2023) Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial, Amyloid, 30:1, 18-26, DOI: 10.1080/13506129.2022.2091985

Vutrisiran, a successor to patisiran, came on the market in 2022.

It uses the same RNAi mechanism but it is coupled to N-acetylgalactosamine, which increases its uptake in liver cells and allows the administration of lower dose.

While patisiran required intravenous injection every 3 weeks, treatment with vutrisiran involves only one subcutaneous injection every 3 months.

FDA/EMA approved ncRNAs

Table 1 Antisense oligonucleotides and short interfering RNAs approved by the European Medicines Agency and/or the Food and Drug Administration and their main characteristics

Product (commercial name; developer/manufacturer)	Length	Modifications	Vehicle	Route of administration	Indication	Target organ	Target gene and mechanism	Year of approval
Antisense oligonucleotides (ASOs)								
Fomivirsen (Vitravene; Isis Pharmaceuticals, Novartis)	21-mer	PS	None	Intravitreal	CMV retinitis	Eye	CMV IE-2 mRNA	1998 (FDA), 1999 (EMA); 2002 withdrawn
Mipomersen (Kynamro; Ionis Pharmaceuticals, Kastle Therapeutics)	20-mer	PS, 2′-MOE, GapmeR	None	Subcutaneous	Familiar hypercholesterolaemia (FH)	Liver	Apolipoprotein B (ApoB) mRNA	2013 (FDA); 2019 withdrawn
Nusinersen (Spinraza; Ionis Pharmaceuticals, Biogen)	18-mer	PS, 2′-MOE	None	Intrathecal	Spinal muscular atrophy		Survival of motoneuron 2 (SMN2) pre-mRNA splicing (exon 7 inclusion)	2017 (EMA), 2016 (FDA)
Eteplirsen (Exondys 51, Sarepta Therapeutics)	30-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Skeletal muscle	Dystrophin pre-mRNA splicing (exon 51 skipping)	2016 (FDA)
Inotersen (Tesgedi; Ionis Pharmaceuticals, Akcea Therapeutics)	20-mer	PS, 2′-MOE, GapmeR	None	Subcutaneous	Hereditary transthyretin amyloidosis	Liver	Transthyretin (TTR) mRNA	2018 (EMA), 2018 (FDA)
Golodirsen (Vyondys 53; Sarepta Therapeutics)	25-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Muscle	Dystrophin pre-mRNA splicing (exon 53 skipping)	2019 (FDA)
Viltolarsen (Viltepso, NS Pharma)	21-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Muscle	Dystrophin pre-mRNA splicing (exon 53 skipping)	2020 (FDA) 2020 (EMA)
Volanesorsen (Waylivra; Ionis Pharmaceuticals, Akcea Therapeutics)	20-mer	PS, 2′-MOE, GapmeR	None	Subcutaneous	Familiar chylomicronaemia syndrome (FCS)	Liver	Apolipoprotein C3 (ApoC3I) mRNA	2019 (EMA)
Casimersen (Amondys 45; Sarepta Therapeutics)	22-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Muscle	Dystrophin pre-mRNA splicing (exon 45 skipping)	2021 (FDA)
Small interfering RNAs (siRNAs)								
Patisiran (Onpattro; Anylam Pharmaceuticals)	21-nt ds	2′-*O*-Me	SNALP LNP	Intravenous	Hereditary transthyretin amyloidosis	Liver	Transthyretin mRNA	2018 (EMA), 2019 (FDA)
Givosiran (Givlaari; Anylam Pharmaceuticals)	21-nt ds	PS, 2′-*O*-Me, 2′-F, GalNAc-conjugated	None	Subcutaneous	Acute hepatic porphyria (AHP)	Liver	Delta aminolevulinic acid synthase 1 mRNA	2020 (EMA), 2019 (FDA)
Inclisiran (Leqvio; Novartis Pharmaceuticals)	22-nt ds	PS, 2′-*O*-Me, 2′-F, GalNAc-conjugated	None	Subcutaneous	Primary hypercholesterolaemia or mixed dyslipidaemia	Liver	Proprotein convertase subtilisin/kexi)*n* type 9 (PCSK9) mRNA	2020 (EMA) 2021 (FDA)
Lumasiran (Oxlumo; Anylam Pharmaceuticals)	21-nt ds	PS, 2′-*O*-Me, 2′-F, GalNAc-conjugated	None	Subcutaneous	Primary hyperoxaluria type 1 (PH1)	Liver	Hydroxyacid oxidase-1 mRNA	2020 (EMA), 2020 (FDA)

ASO, antisense oligonucleotide; ds, double stranded; GalNAc, *N*-acetylgalactosamine; PMO, phosphoroamidate morpholino oligomer; PS, phosphorothioate modification; siRNA, short interfering RNA.

9 ASOs; 4 siRNAs (2022)

↓

10 ASOs & 6 siRNAs (July 2022)

M. Giacca & A.M. Shah, 2022, Eur. Heart J.

Antisense therapeutics

1978

Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 285–288, January 1978
Biochemistry

Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide

(*in vitro* protein synthesis/nucleic acid hybridization/DNA nucleotidyltransferase)

MARY L. STEPHENSON AND PAUL C. ZAMECNIK

The John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Massachusetts 02114

Contributed by Paul C. Zamecnik, November 10, 1977

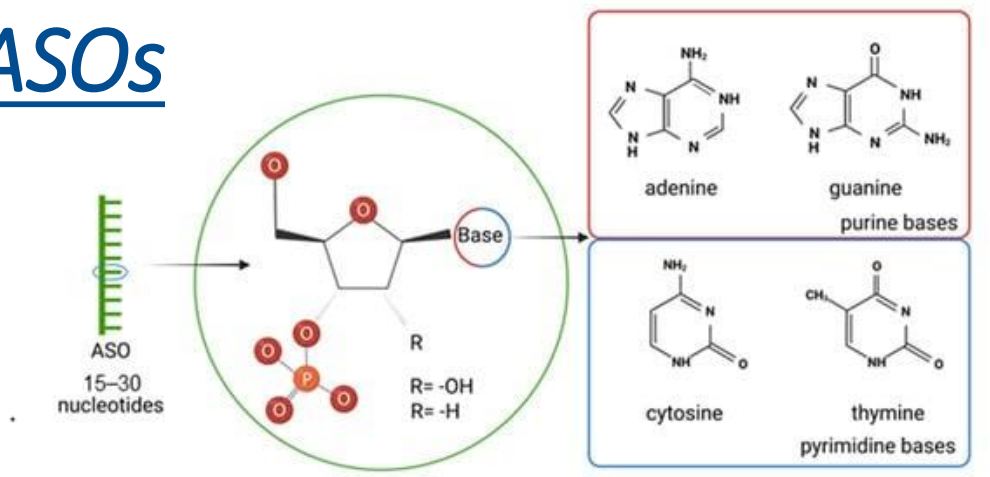
2002

AMERICAN JOURNAL OF OPHTHALMOLOGY

A Randomized Controlled Clinical Trial of Intravitreous Fomivirsen for Treatment of Newly Diagnosed Peripheral Cytomegalovirus Retinitis in Patients With AIDS

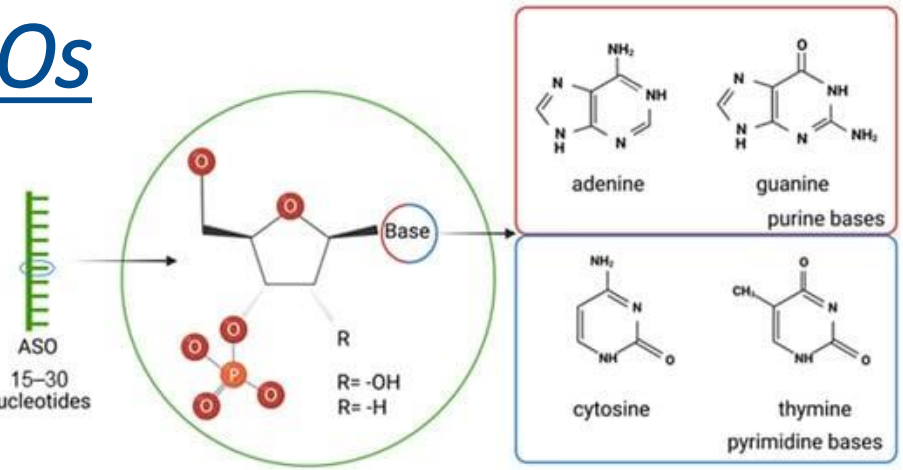
THE VITRAVENE STUDY GROUP

ASOs

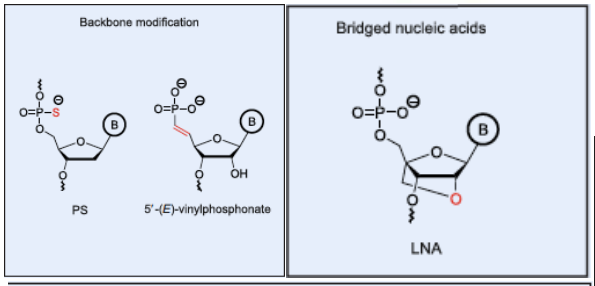


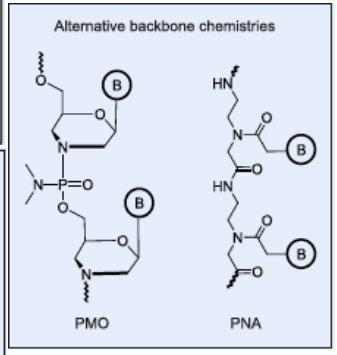
Rapidly degraded

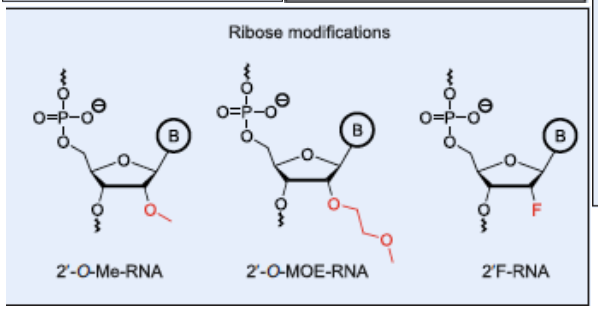
ASOs



Rapidly degraded



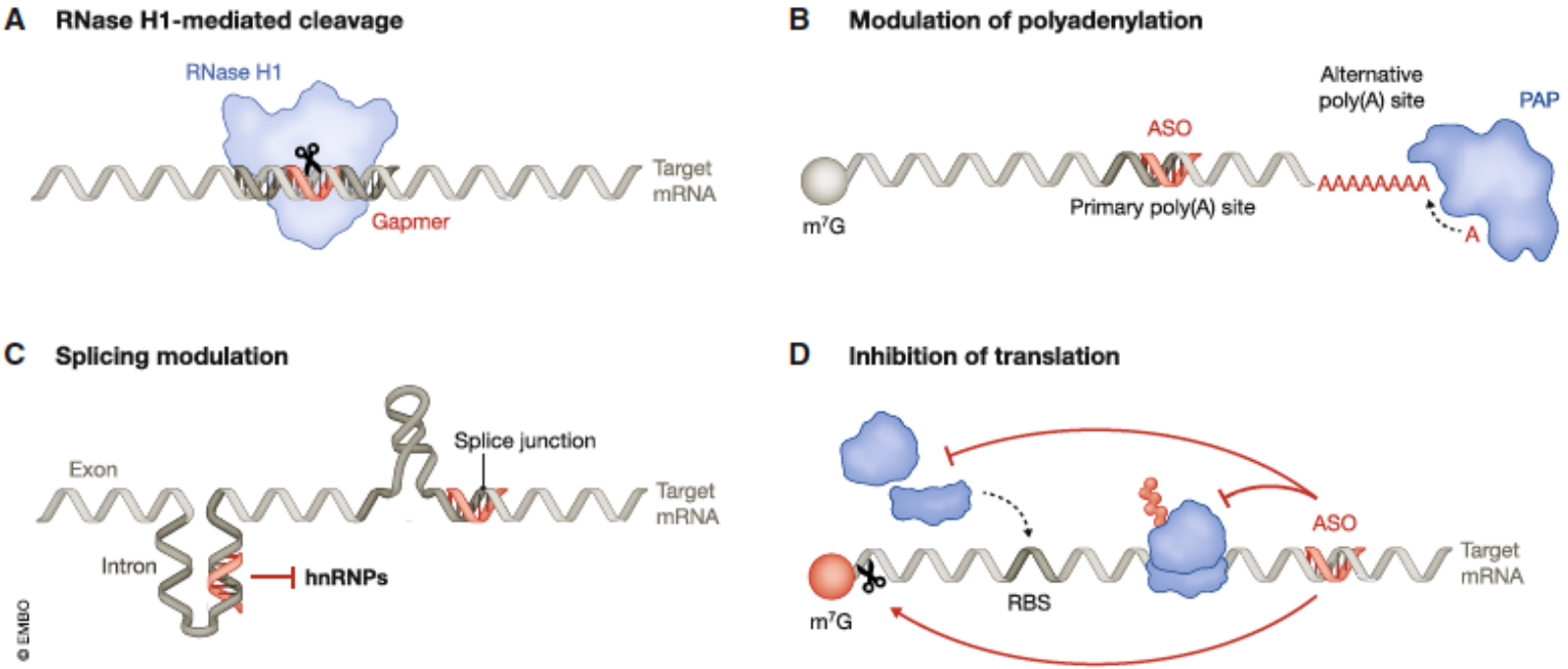




The goals of oligonucleotide medicinal chemistry are:

- to increase resistance to nucleases,
- to enhance affinity to the target,
- to improve pharmacokinetics
- to reduce pro-inflammatory responses.

Molecular Mechanisms of ASOs



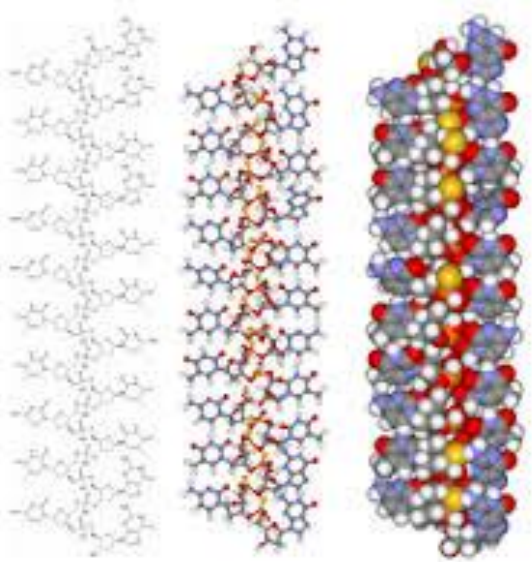
(A) RNase H1-mediated RNA cleavage induced by gapmers, which have a central core of deoxyribonucleotides (pink) flanked by 20 modified nucleotides at both the 5' and 3 ends (B) modulation of polyadenylation, (C) modulation of splicing (D) inhibition of translation by blocking ribosome scanning, interfering with translation initiation factors or causing the cleavage of the 5' cap structure (m7G).

Table 1. FDA-approved RNA therapeutics.

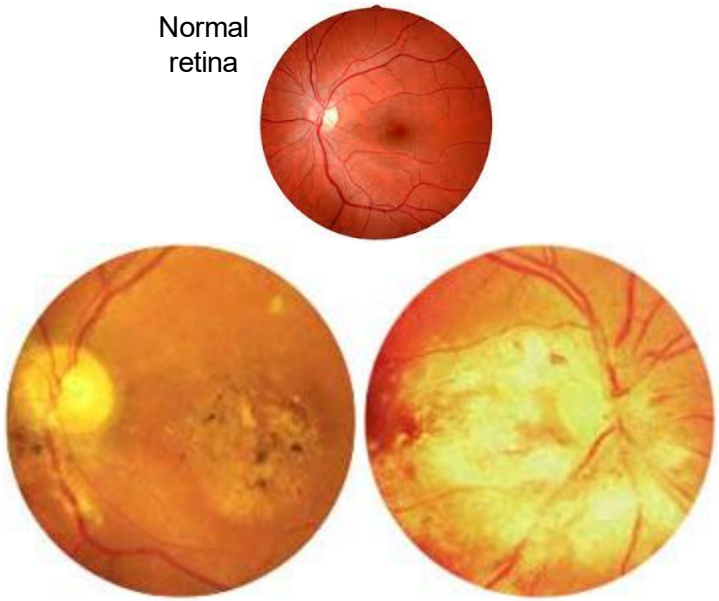
Product	Target	Mechanism of action	Indication	Route of delivery	Company	Approval year
ASOs						
Formivirsen	CMV mRNA	Downregulation	CMV retinitis	IVT	Ionis Pharmaceuticals, Novartis	1998 (withdrawn 2002)
Mipomersen	Apolipoprotein B-100 mRNA	Downregulation	Familial Hypercholesterolemia	SC	Ionis Pharmaceuticals	2013
Nusinersen	SMN2 pre-mRNA	Splicing modulation	Spinal muscular atrophy	ITH	Ionis Pharmaceuticals, Biogen	2016
Eteplirsen	Exon 51 of dystrophin pre-mRNA	Splicing modulation	DMD	IV	Sarepta Therapeutics	2016
Inotersen	TTR mRNA	Downregulation	Transthyretin-mediated amyloidosis	SC	Ionis Pharmaceuticals	2018
Golodirsen	Exon 53 of *DMD*	Splicing modulation	DMD	IV	Sarepta Therapeutics	2019
Volanesoren	Apolipoprotein CIII mRNA	Downregulation	Familial chylomicronemia syndrome	SC	Ionis Pharmaceuticals, Akcea	2019
Viltolarsen	Exon 53 of dystrophin pre-mRNA	Splicing modulation	DMD	IV	NS Pharma, Inc	2020
Casimersen	Exon 45 of dystrophin pre-mRNA	Splicing modulation	DMD	IV	Sarepta Therapeutics	2021

Fomivirsen

Vitravene (Fomivirsen) sodium is a phosphorothioate oligonucleotide, twenty-one nucleotides in length, indicated for the local treatment of cytomegalovirus (CMV) infections, and in particular of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS), who are intolerant of or have a contraindication to other treatment(s) for CMV retinitis or who were insufficiently responsive to previous treatment(s) for CMV retinitis.

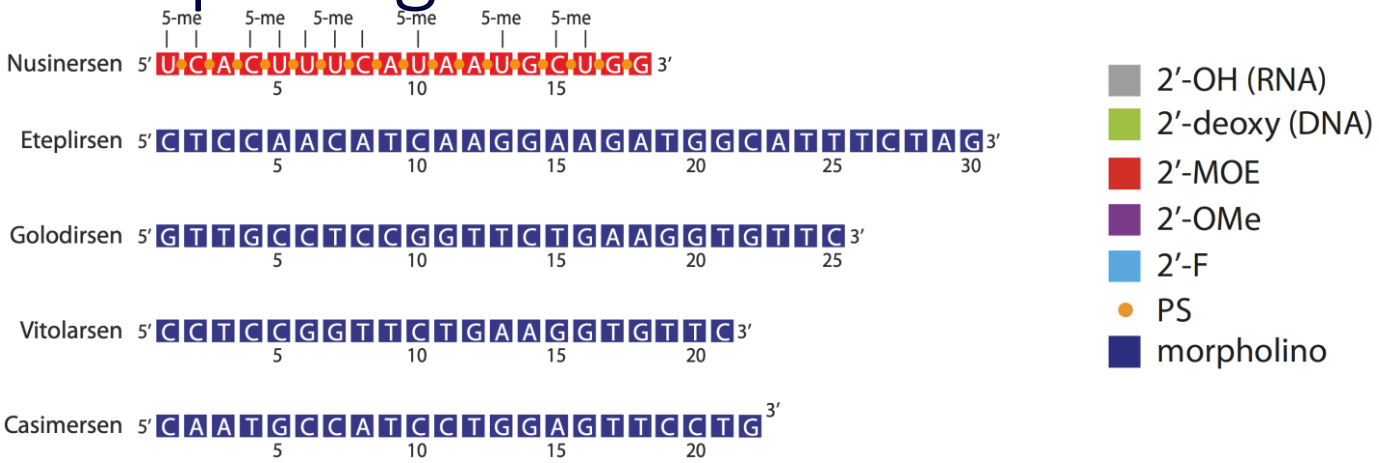


Target sequence: IE2 gene of the CMV genome



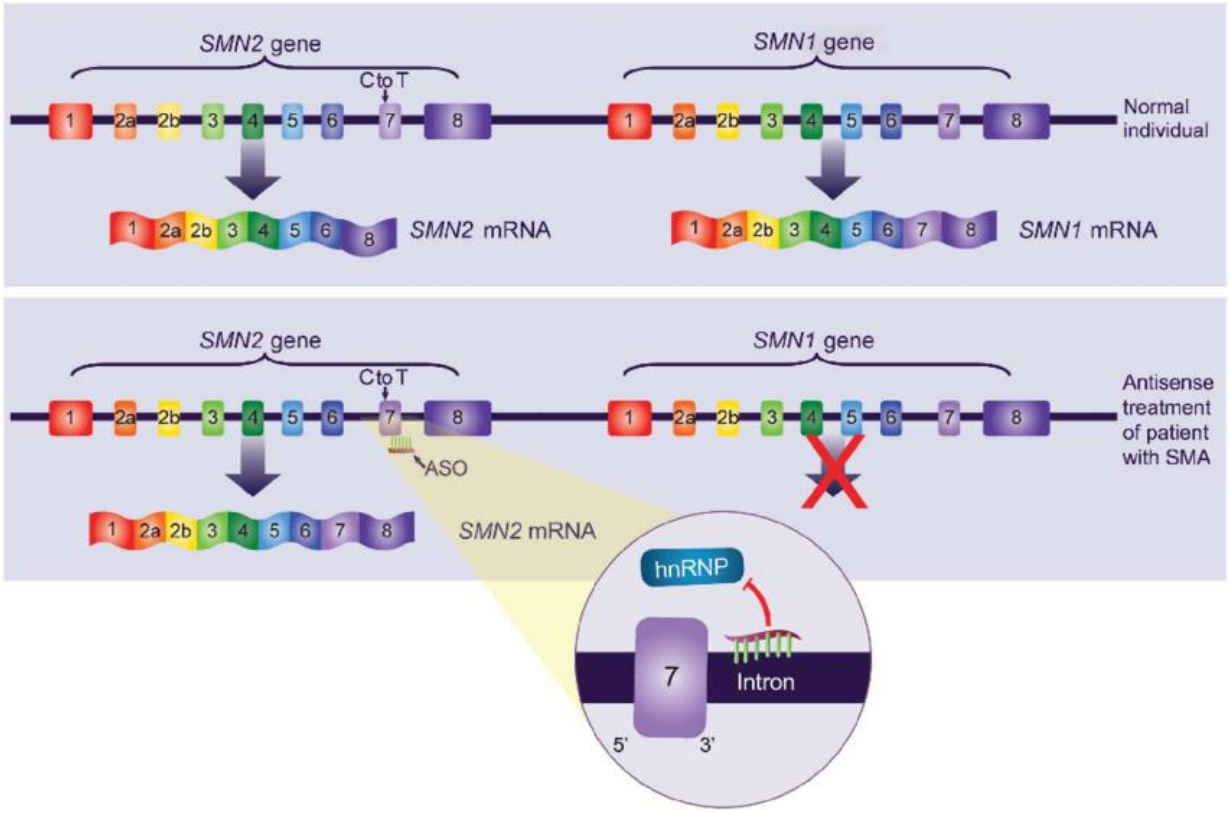
CMV retinitis

Clinically approved ASOs that regulate pre-mRNA splicing

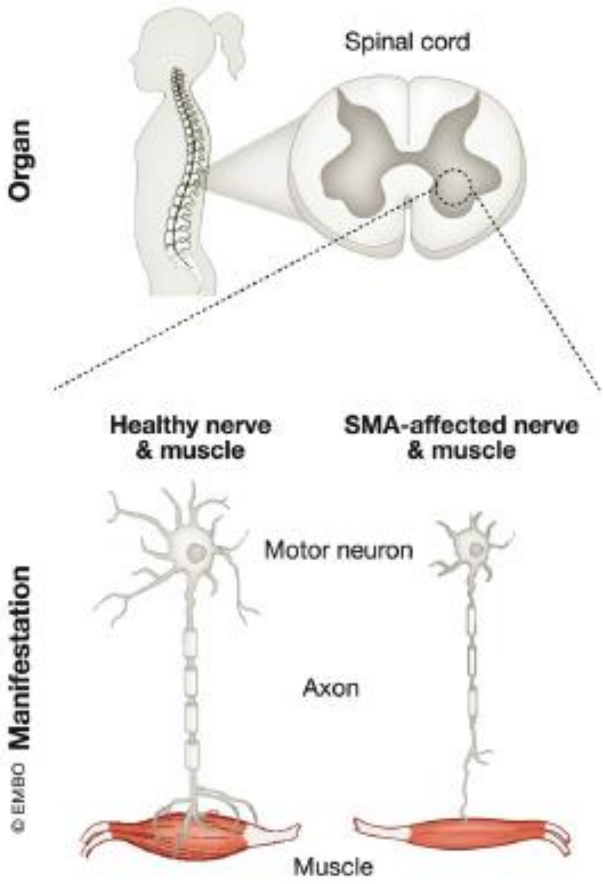


Product (Commercial name; Developer/Manufacturer)	Length	Modifications	Vehicle	Route of administration	Indication	Target organ	Target gene and mechanism	Year of approval
Antisense oligonucleotides (ASOs)								
Nusinersen (Spinraza; Ionis Pharmaceuticals, Biogen)	18-mer	PS, 2'-MOE	None	Intrathecal	Spinal muscular atrophy (SMA)		SMN2 pre-mRNA splicing (exon 7 inclusion)	2017 (EMA), 2016 (FDA)
Eteplirsen (Exondys 51, Sarepta Therapeutics)	30-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Skeletal muscle	Dystrophin pre-mRNA splicing (exon 51 skipping)	2016 (FDA)
Golodirsen (Vyondys 53; Sarepta Therapeutics)	25-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Muscle	Dystrophin pre-mRNA splicing (exon 53 skipping)	2019 (FDA)
Viltolarsen (Viltepso, NS Pharma)	21-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Muscle	Dystrophin pre-mRNA splicing (exon 53 skipping)	2020 (FDA) 2020 (EMA)
Casimersen (Amondys 45; Sarepta Therapeutics)	22-mer	PMO	None	Intravenous	Duchenne muscular dystrophy (DMD)	Muscle	Dystrophin pre-mRNA splicing (exon 45 skipping)	2021 (FDA)

Nusinersen for antisense pre-mRNA splicing modulation for spinal muscular atrophy

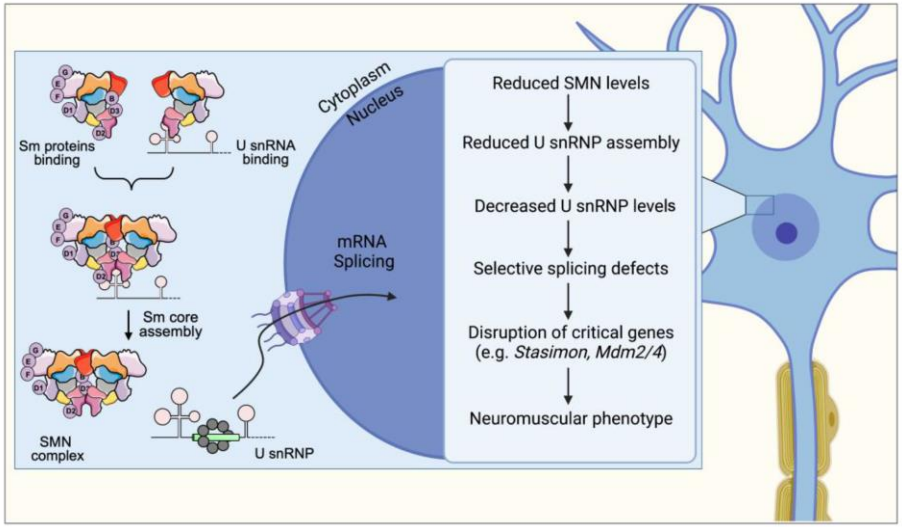


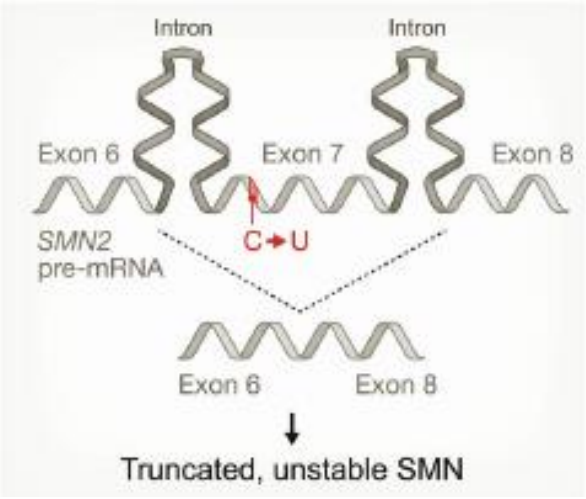
SMA



SMA is an autosomal recessive neuromuscular disease caused by deletions or loss-of-function mutations in the gene survival motor neuron1 (SMN1)

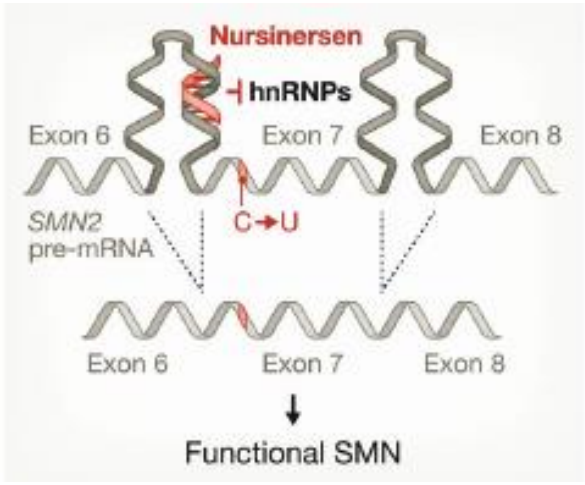
Without functional SMN, the motor neurons in the spinal cord and brain stem degenerate, resulting in muscle weakness and atrophy. Of the infants born with the most severe form of SMA, 60% show symptoms before 6 months of age and the median life expectancy is less than 2 years.





Humans have a second gene, SMN2, that encodes an identical SMN protein. SMN2 contains a synonymous C-to-T substitution within exon 7 that weakens the binding of splice activators to the SMN2 pre-mRNA leading to aberrant splicing, with 90% of mature SMN2 transcripts lacking exon 7 and producing a truncated, unstable polypeptide.

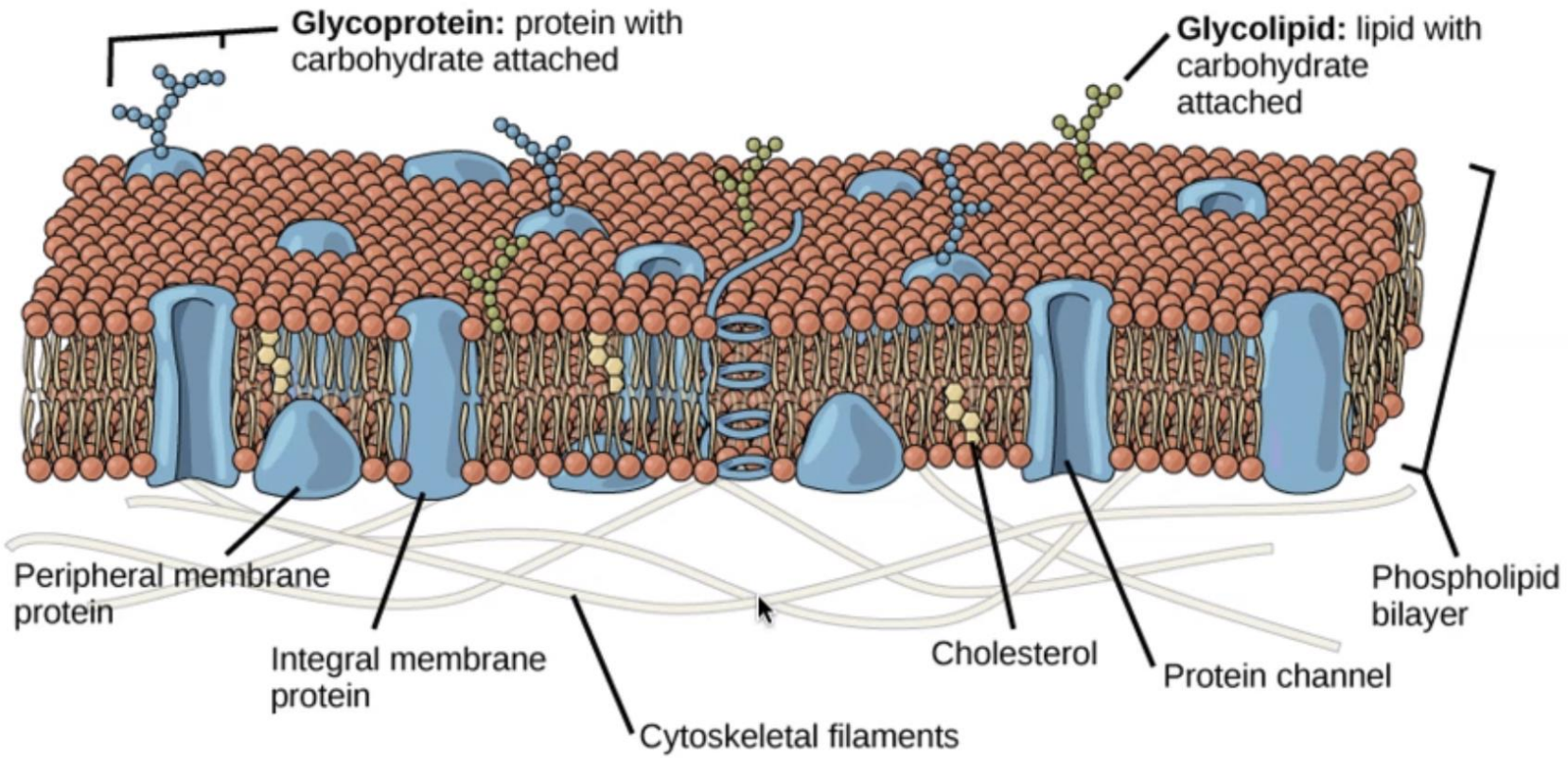
Therapy

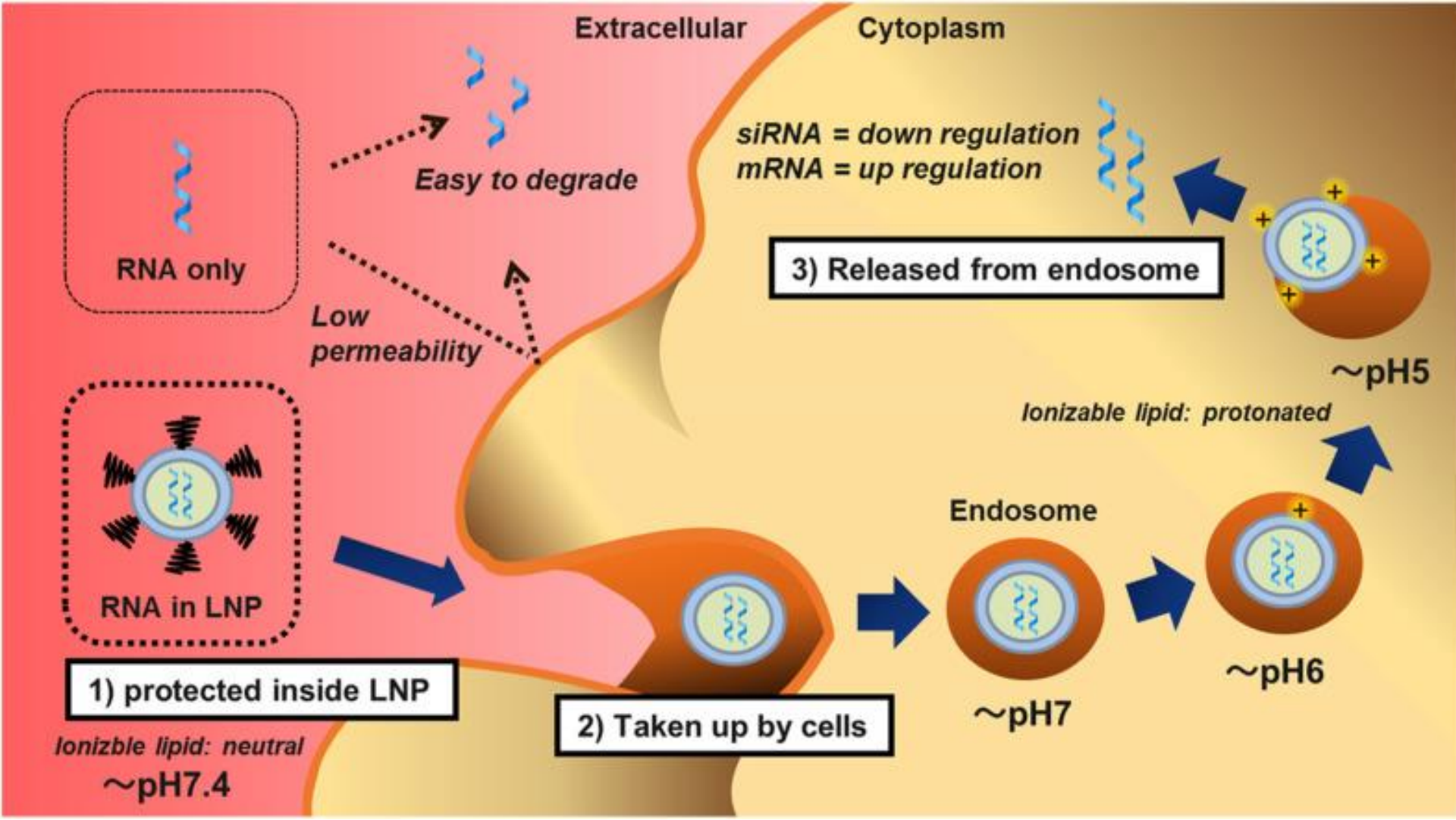


Binding of the ASO nusinersen to the SMN2 pre-mRNA displaces the splice repressor hnRNP, resulting in the production of a mature mRNA that includes exon 7 and translation of the full-length SMN protein.

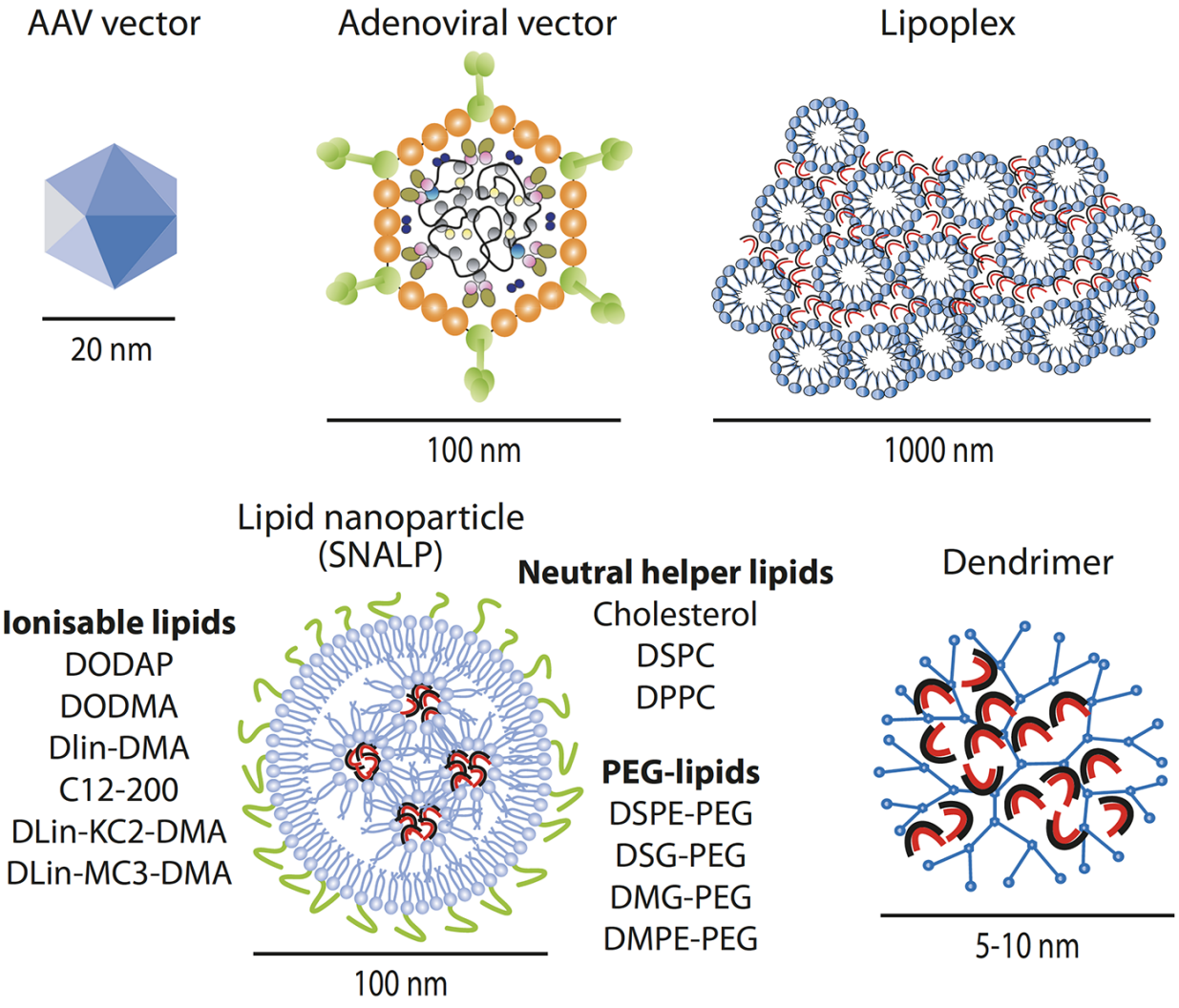
The drug was approved for use in the US in 2016. It has since become available in over 40 countries.

The plasma membrane is an unsurmountable barrier for nucleic acids



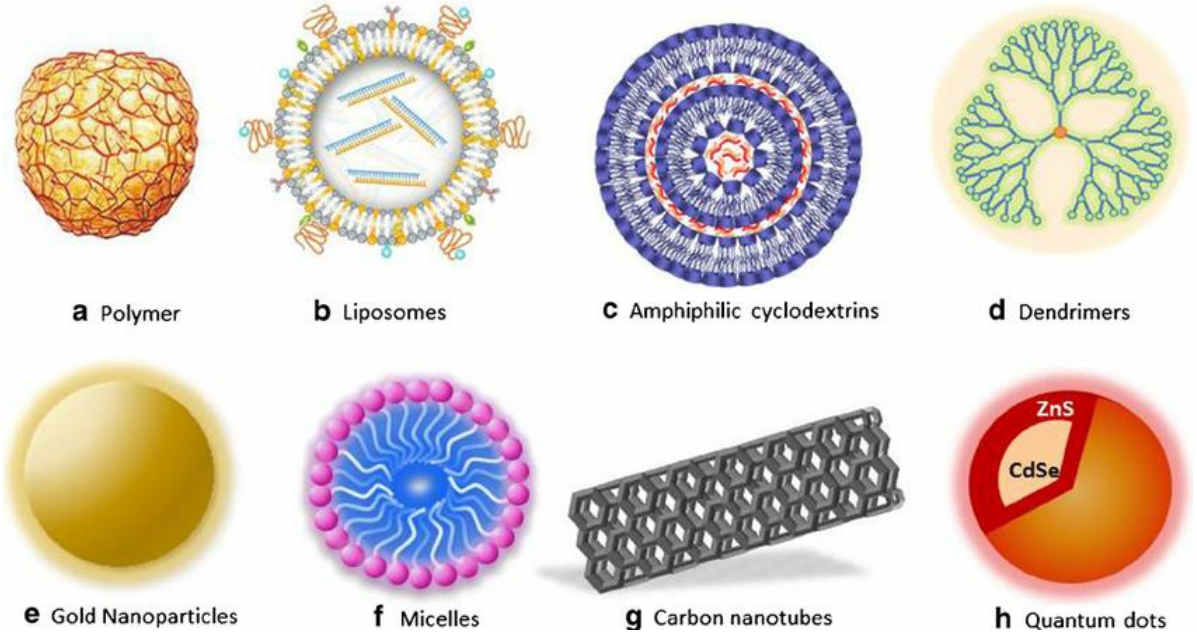

Extracellular
Cytoplasm
RNA only
Easy to degrade
Low permeability
siRNA = down regulation
mRNA = up regulation
3) Released from endosome
~pH5
Ionizable lipid: protonated
RNA in LNP
Endosome
~pH6
~pH7
1) protected inside LNP
2) Taken up by cells
Ionizble lipid: neutral
~pH7.4

Size of nucleic acid delivery vehicles



Development of a nanocarrier that:

- Encapsulates ncRNAs
- Enters into cardiomyocytes efficiently
- Releases the ncRNAs in the cytosol after endo-lysosomal escape
- Is biocompatible
- Is simple, cheap and made of approved chemical components



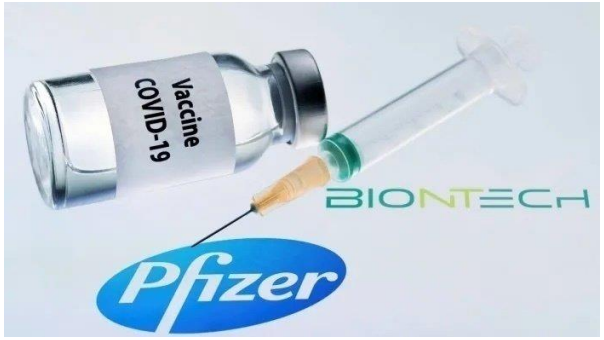
THE RACE FOR CORONAVIRUS VACCINES

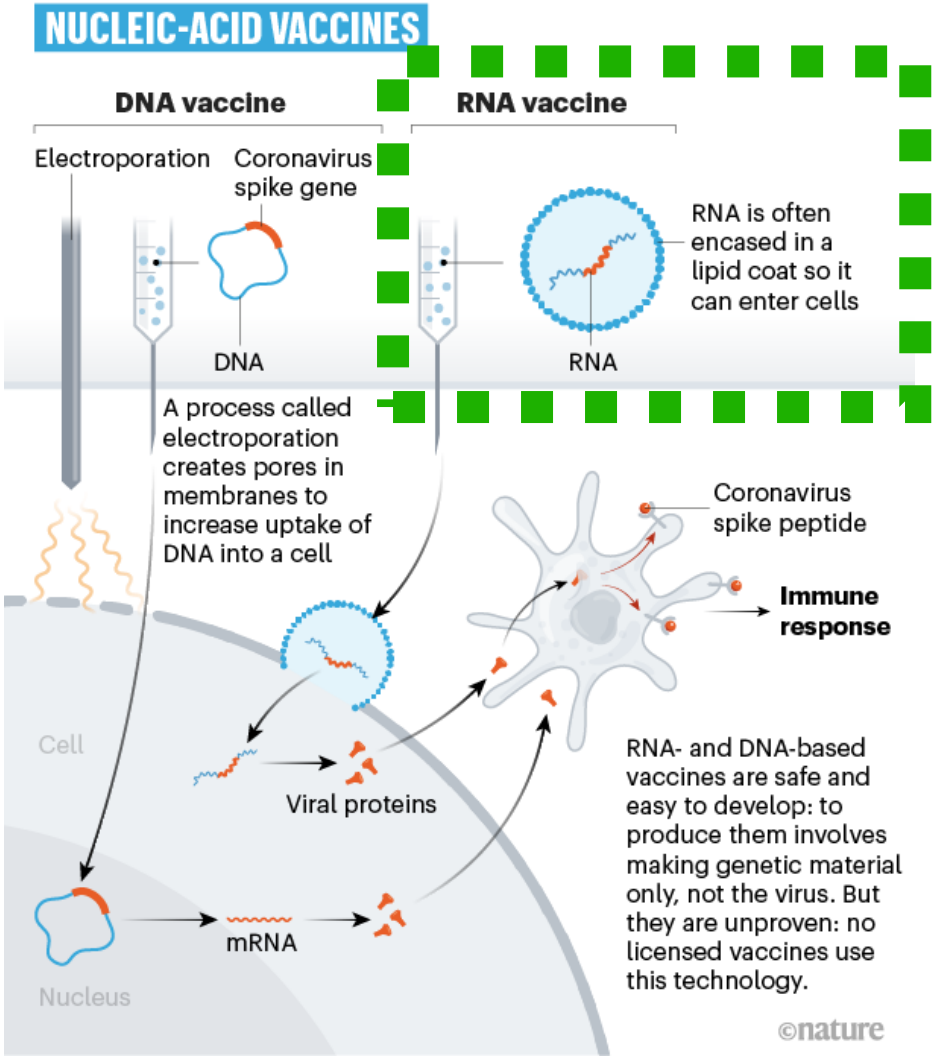
By Ewen Callaway; design by Nik Spencer.

Moderna

Pfizer/BioNTech

CureVac

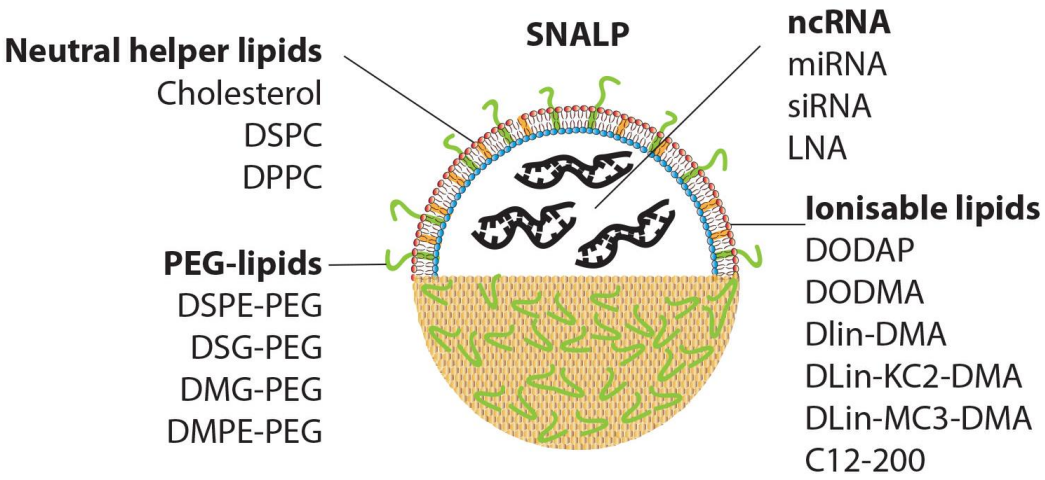




Nature **580**, 576-577 (2020)

Stable Nucleic Acid-Lipid nanoParticles (SNALPs)

Product	**Patisiran**	**BNT162b2** (Pfizer-BioNTech COVID-19 vaccine)	**mRNA-1273** (Moderna COVID-19 vaccine)
LNP technology	SNALP	SNALP	SNALP
Therapeutic RNA	Anti-TTR siRNA	SARS-CoV-2 Spike modified mRNA	SARS-CoV-2 Spike modified mRNA
Ionizable lipids	DLin-MC3-DMA	ALC-0315	SM-102
Neutral lipids	DSPC	DSPC	DSPC
	Cholesterol	Cholesterol	Cholesterol
PEG lipids	PEG_{2000}-C-DMG	PEG_{2000}	PEG_{2000}-C-DMG
Reference	[46]	[35]	[34]



LNP-miRNA therapy for cardiac regeneration

- **Effect transient** - no chronic therapy with long-term side effects
- Can be easily **stored** and **distributed**
- If coronary administration effective, can be **administered by any interventional cardiologist**. Alternatively, through endo-ventricular catheterisation or during bypass surgery or minithoracotomy
- **Drug development** can recapitulate that of siRNAs or mRNA SNALPs