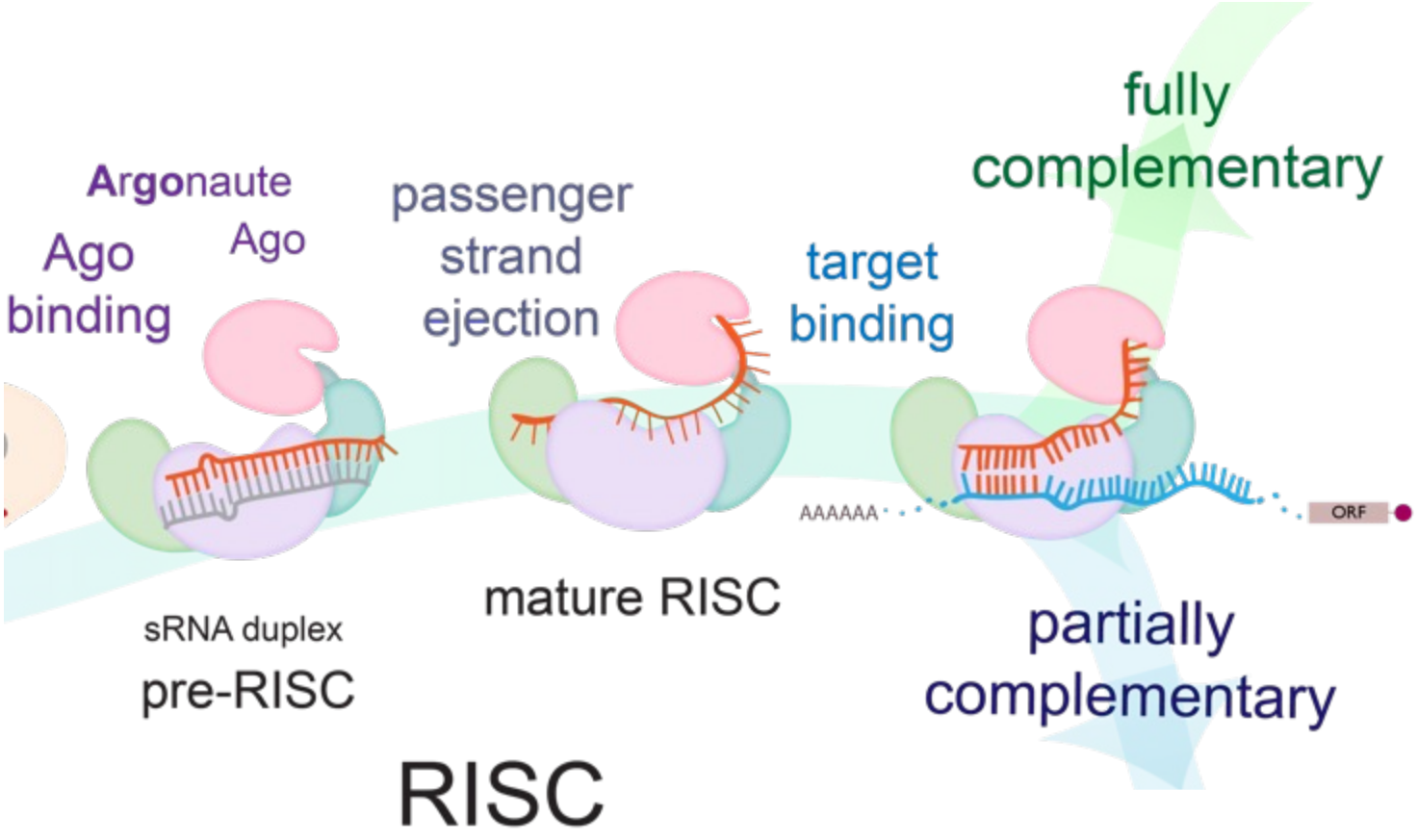
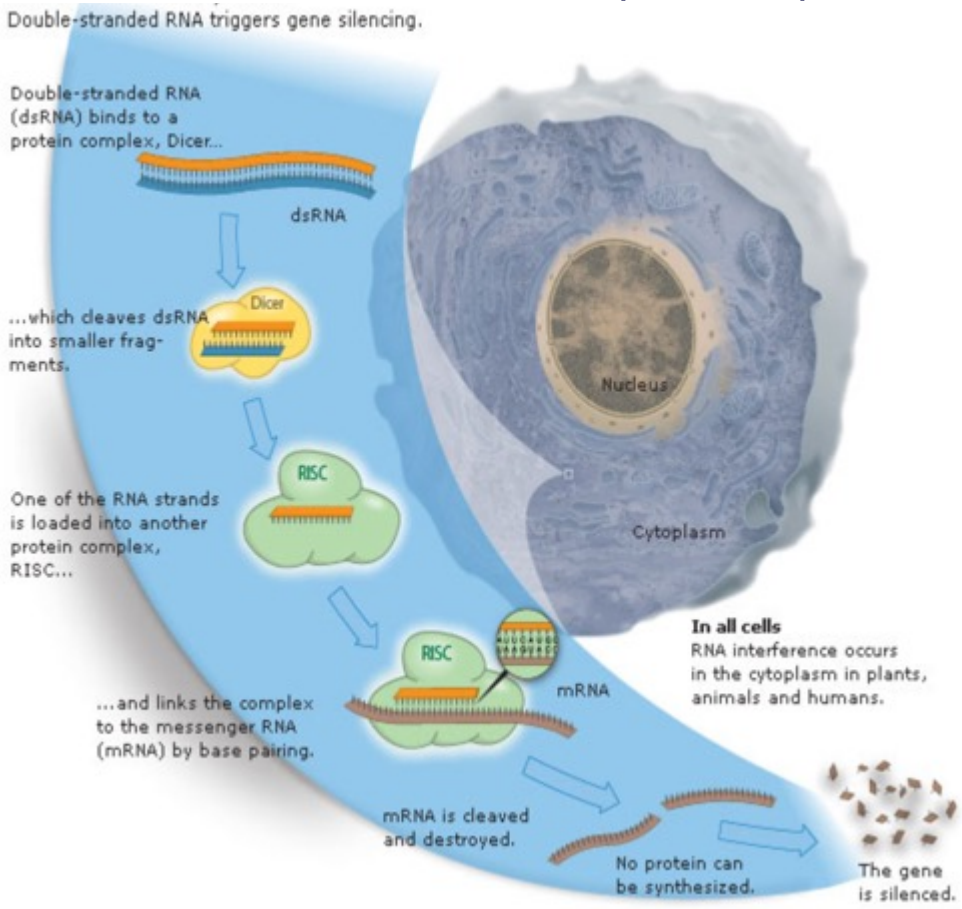

Argonaute
Ago
Ago
binding
passenger
strand
ejection
target
binding
fully
complementary
AAAAAA
ORF
sRNA duplex
pre-RISC
mature RISC
partially
complementary
RISC

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

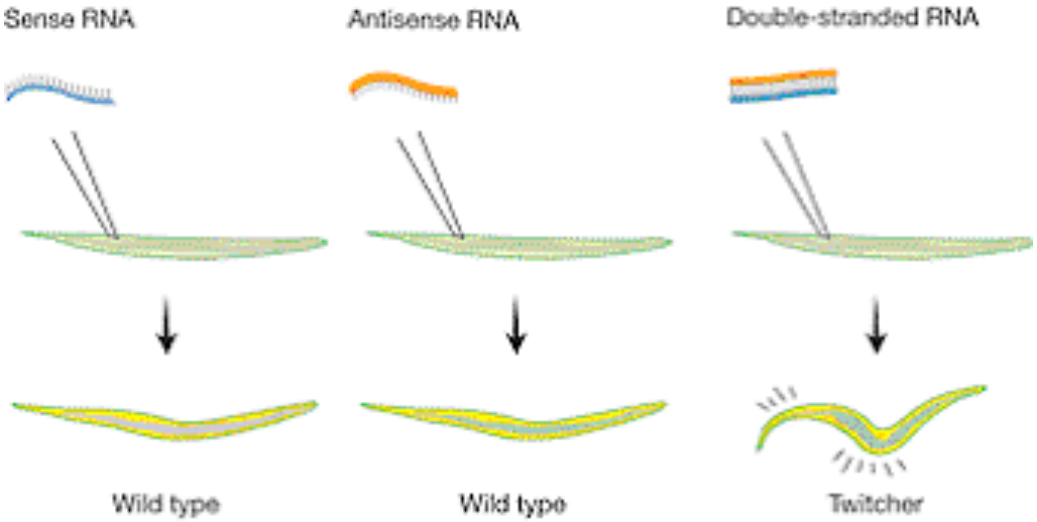
Key breakthrough

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‡

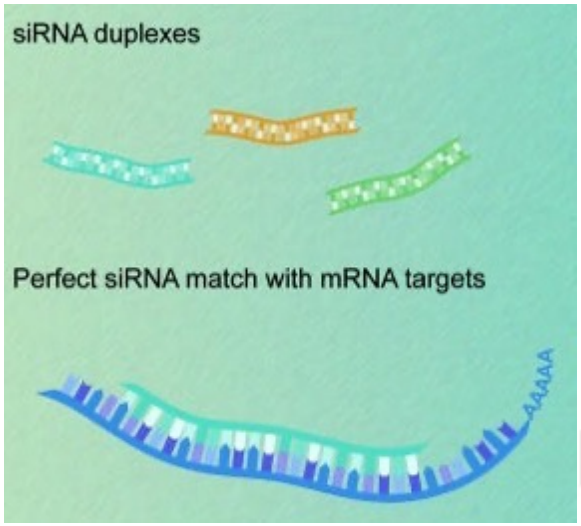


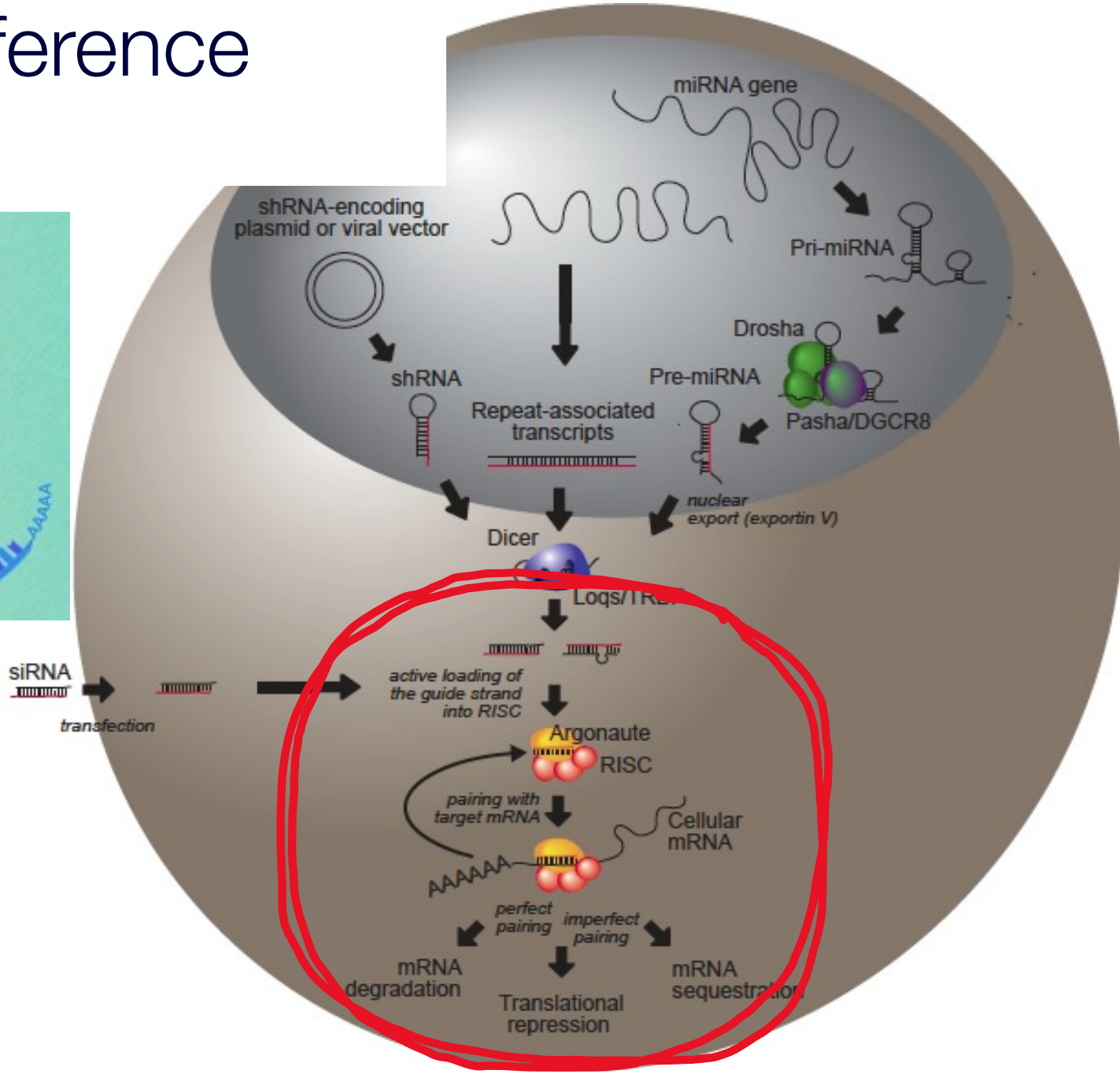
*The **unc-22** gene encodes a myofilament protein. Decrease in unc-22 activity is known to produce severe twitching movements.*

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)





miRNAs typically inhibit the translation of many different mRNAs with similar sequences. In contrast, siRNAs typically inhibit only a single, specific target.

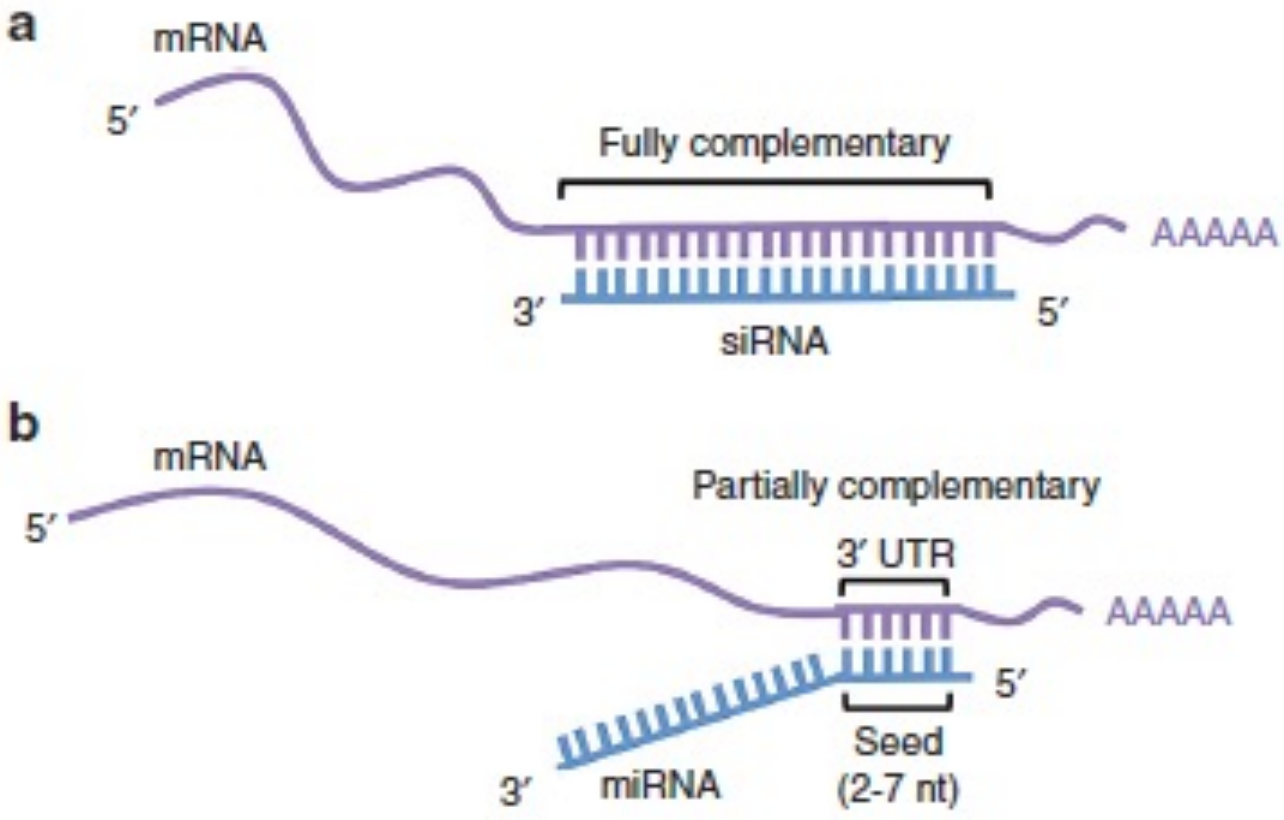


Table 1 Comparison of general properties between siRNA and miRNA

	siRNA	miRNA
Prior to Dicer processing	Double-stranded RNA that contains 30 to over 100 nucleotides	Precursor miRNA (pre-miRNA) that contains 70–100 nucleotides with interspersed mismatches and hairpin structure
Structure	21–23 nucleotide RNA duplex with 2 nucleotides 3'overhang	19–25 nucleotide RNA duplex with 2 nucleotides 3'overhang
Complementary	Fully complementary to mRNA	Partially complementary to mRNA, typically targeting the 3' untranslated region of mRNA
mRNA target	One	Multiple (could be over 100 at the same time)
Mechanism of gene regulation	Endonucleolytic cleavage of mRNA	Translational repression
		Degradation of mRNA
		Endonucleolytic cleavage of mRNA (rare, only when there is a high level of complementary between miRNA and mRNA)
Clinical applications	Therapeutic agent	Drug target
		Therapeutic agent
		Diagnostic and biomarker tool

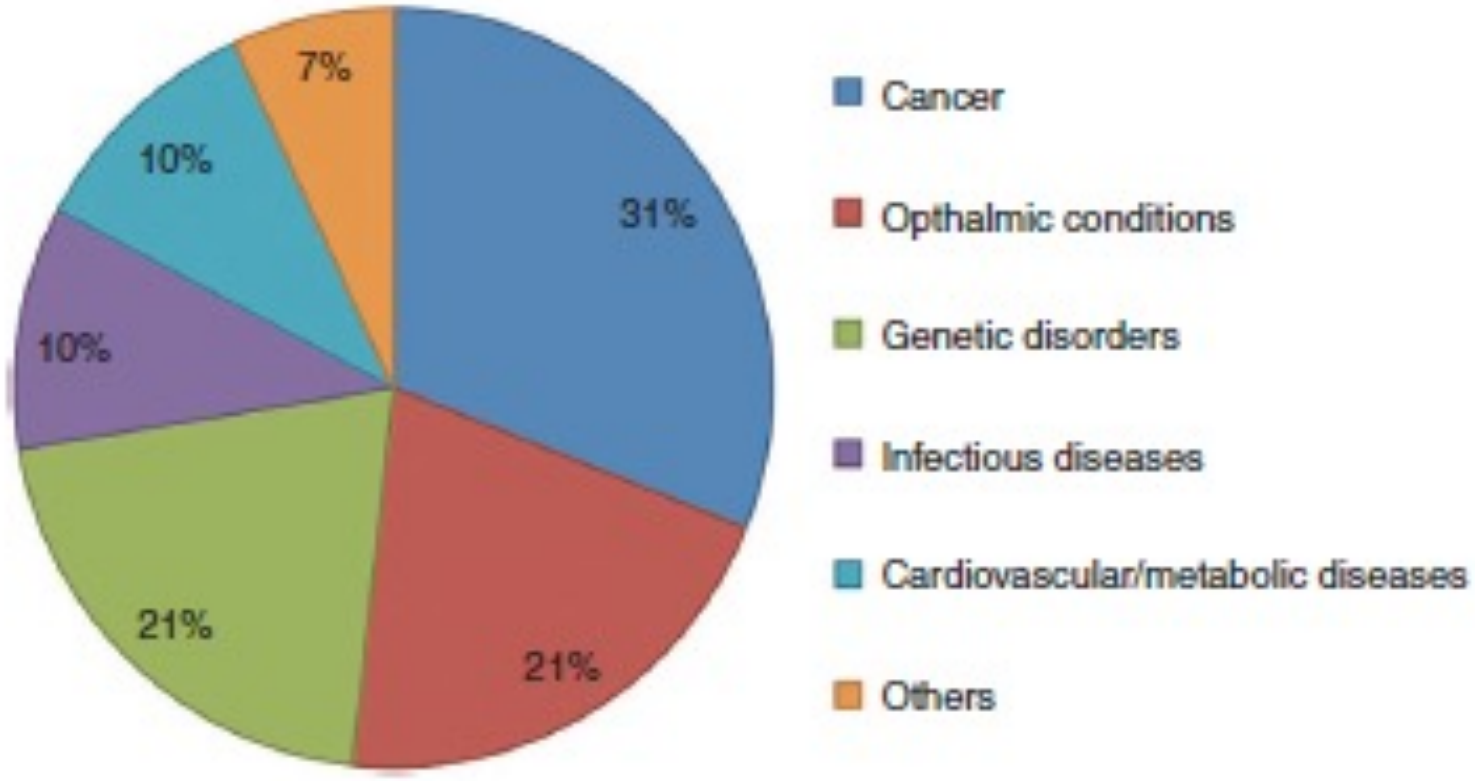
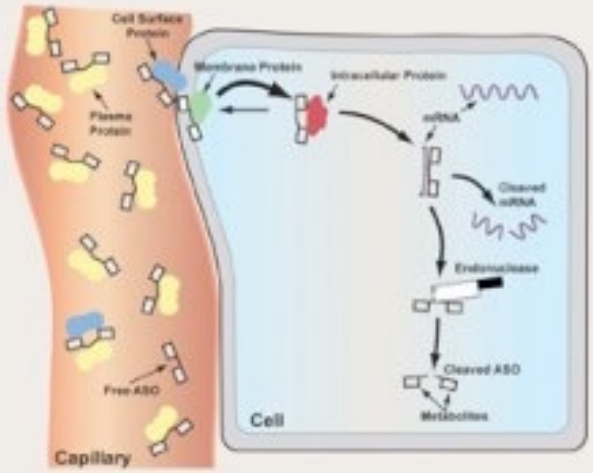


Figure 4 Therapeutic indications of siRNA and miRNA therapeutics.

Second Edition

Antisense Drug Technology

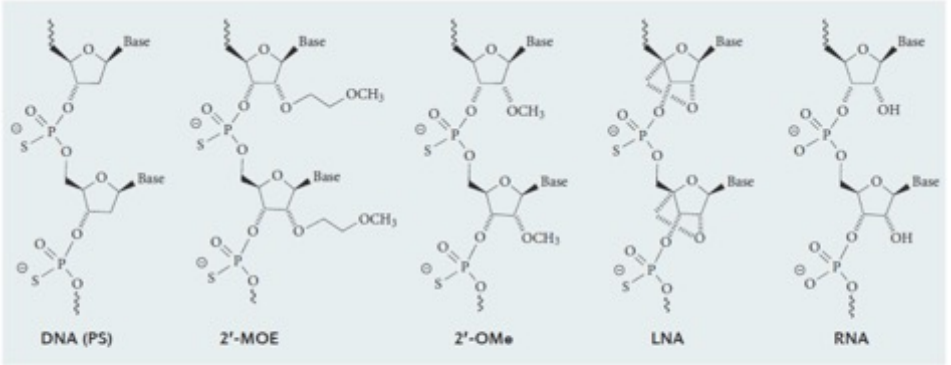
Principles, Strategies, and Applications

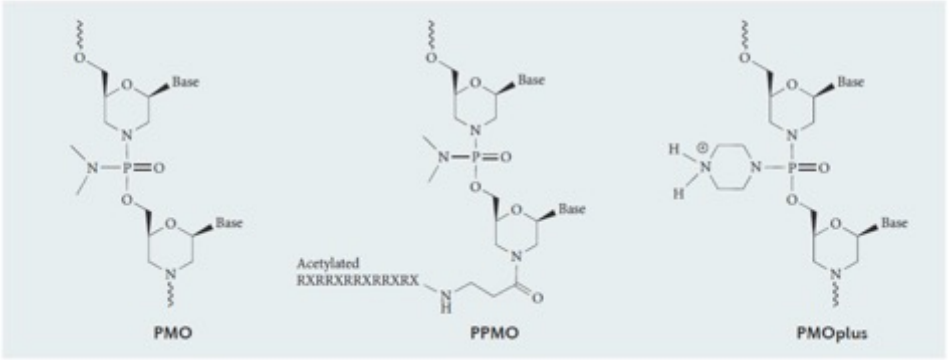


Edited by

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.

pioneering **microRNA Replacement Therapy**

miRna THERAPEUTICS

MRX34 is a first-in-class cancer therapy and the first microRNA mimic to enter clinical trials.

Mirna has secured an exclusive license from Marina Biotech, Inc. to the patent estate covering the SMARTICLES® liposomal delivery technology for several of our lead microRNA product candidates, including miR-34, let-7 and two other undisclosed targets. The SMARTICLES formulation offers key efficacy and safety benefits, including the ability to deliver high numbers of microRNA mimic molecules to cancers cells in the liver, spleen and other highly vascularized tissues, as well as bone marrow and malignant lymphocytes.

Program	Key Oncogenic Targets	Indication	In Vivo Formulation: Discovery	Preclinical	Phase 1	Phase 2
MRX34 miR-34 mimic	BCL2, E2F3, HDAC1, MET, MEK1, CDK4/6, PDGFR-α, WNT1/3, NOTCH-1	Primary liver cancer & solid cancers with liver metastases				
		Hematological malignancies				
miR-Rxlet-7 let-7 mimic	RAS, MYC, HMGA2, TGFBR1, MYCN, Cyclin D2, IL6, ITGB3					
miR-Rx06	UNDISCLOSED					
miR-Rx07	UNDISCLOSED					
miR-Rx16 miR-16 mimic	BCL2, VEGF-A, Cyclin-D1, HMGA1, FGFR1, CDK6, BMI1					

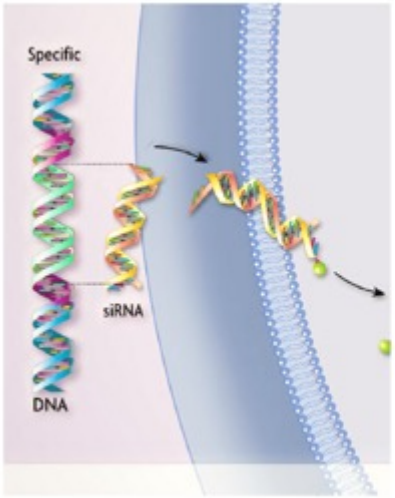
The NEW ENGLAND JOURNAL of MEDICINE

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

Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis

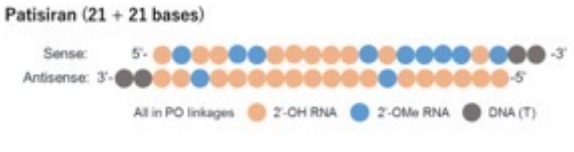
D. Adams, A. Gonzalez-Duarte, W.D. O'Riordan, C.-C. Yang, M. Ueda, A.V. Kristen, I. Tournev, H.H. Schmidt, T. Coelho, J.L. Berk, K.-P. Lin, G. Vita, S. Attarian, V. Planté-Bordeneuve, M.M. Mezei, J.M. Campistol, J. Buades, T.H. Brannagan III, B.J. Kim, J. Oh, Y. Parman, Y. Sekijima, P.N. Hawkins, S.D. 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** was the first RNAi therapeutic approved in the United States and Europe in August 2018 (siRNA LNP against liver transthyretin)



FDA APPROVES FIRST RNA-BASED THERAPEUTIC

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

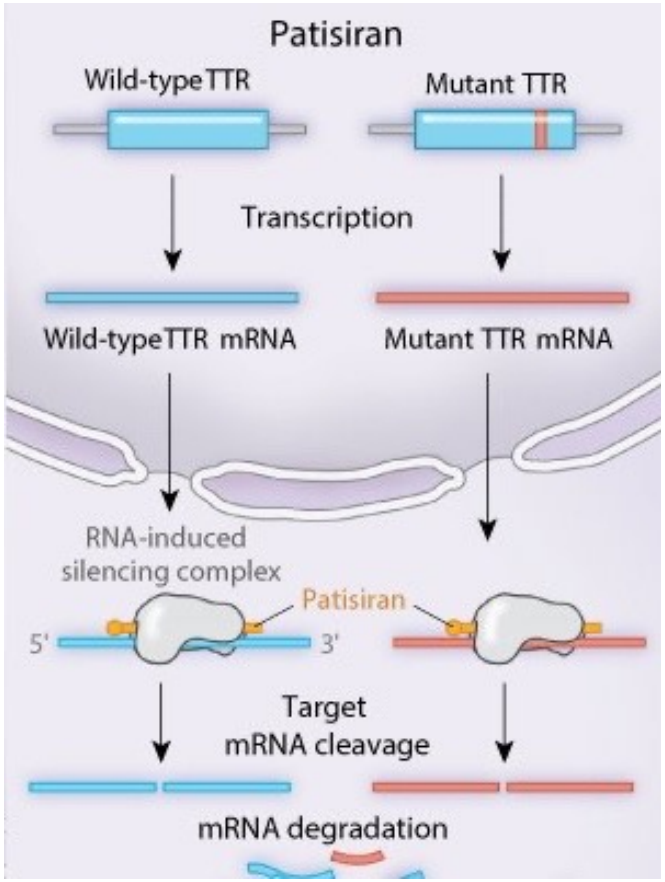


How does ONPATTRO™ (patisiran) work?

ONPATTRO infusion

ONPATTRO decreases the amount of TTR protein made in the liver

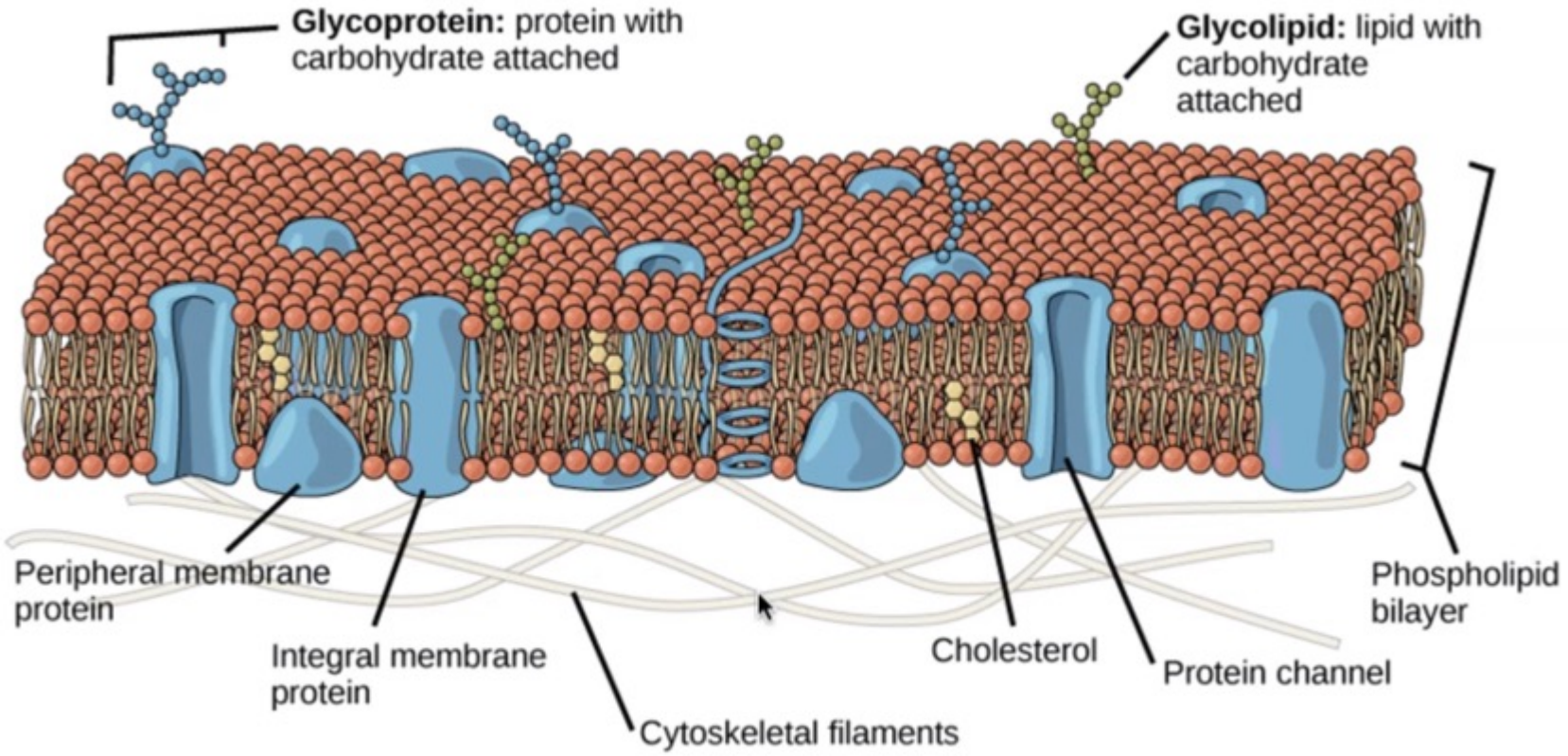
This helps to decrease the amount of amyloid deposits and may result in fewer symptoms

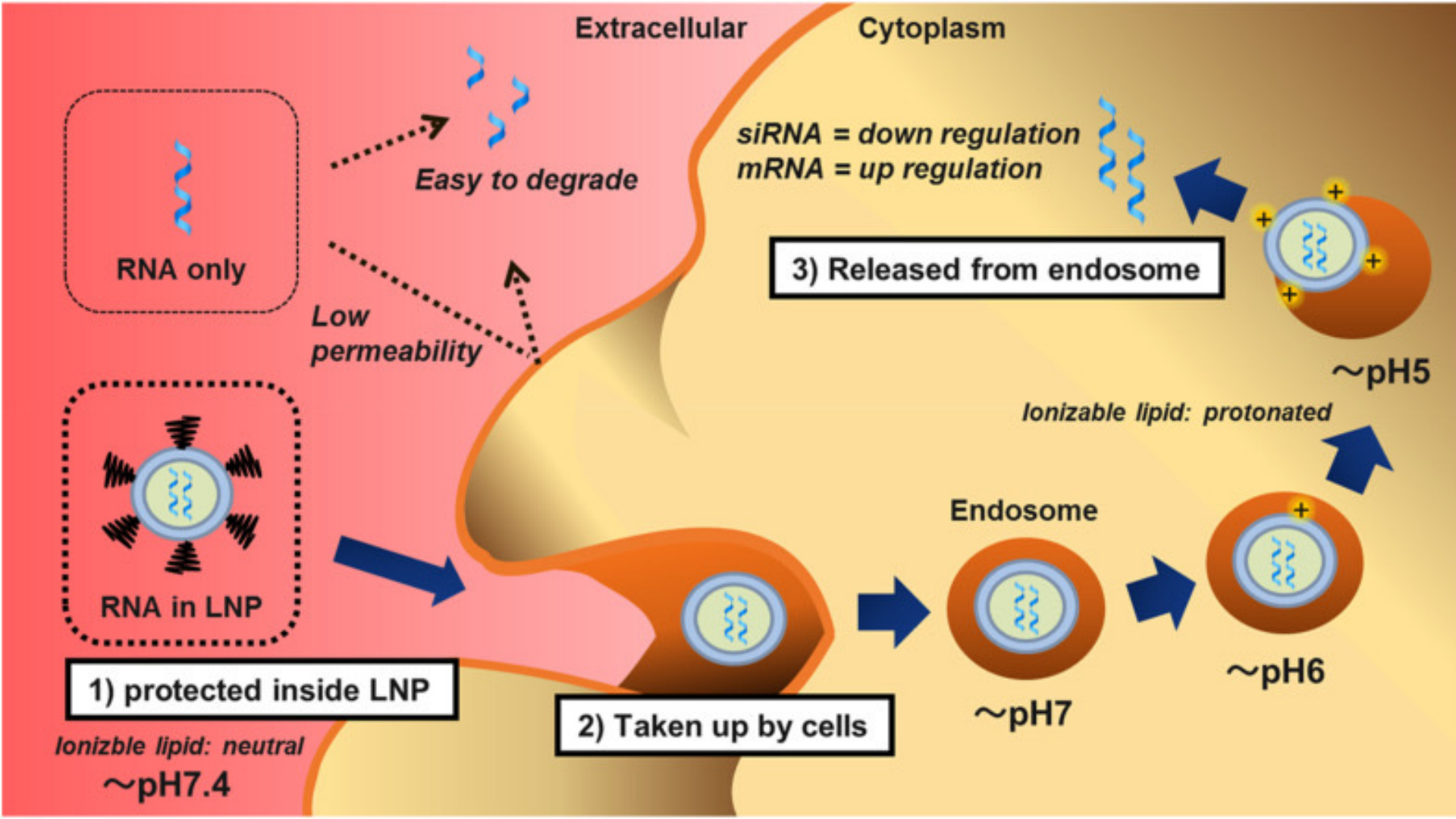


FDA/EMA approved ASOs and siRNAs (Jan 2022)

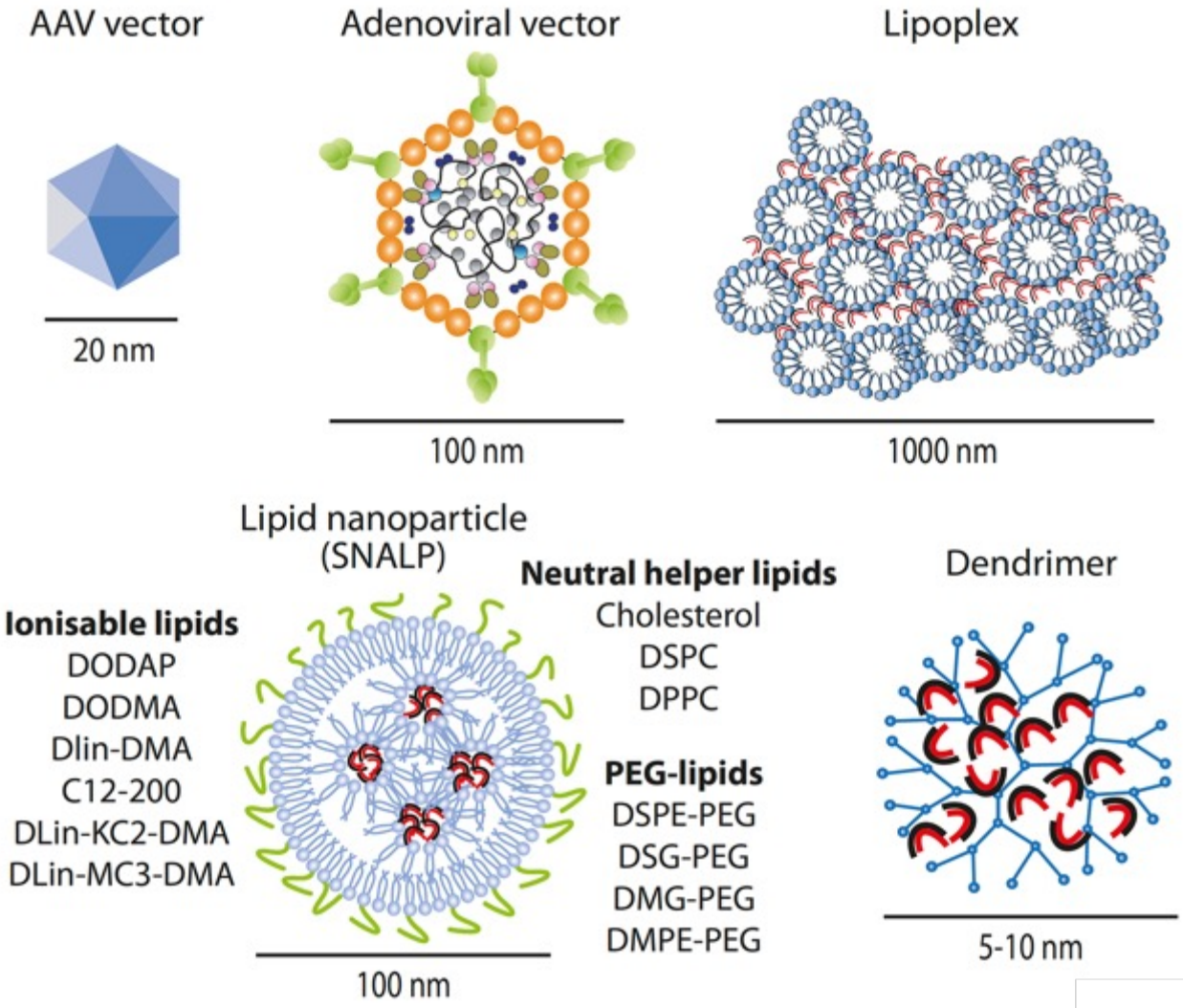
Product (Commercial name; Developer/Manufacturer)	Length	Modifications	Vehicle	Route of administration	Indication	Target organ	Target gene and mechanism	Year of approval
Antisense oligonucleotides (ASOs)								
Fomivirsen (Vitravene; Isis Pharmaceuticals, Novartis)	21-mer	PS	None	Intravitreal	CMV retinitis	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 (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)
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 (ALAS1) 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 (HAO1) mRNA	2020 (EMA), 2020 (FDA)

The plasma membrane is an unsurmountable barrier for nucleic acids

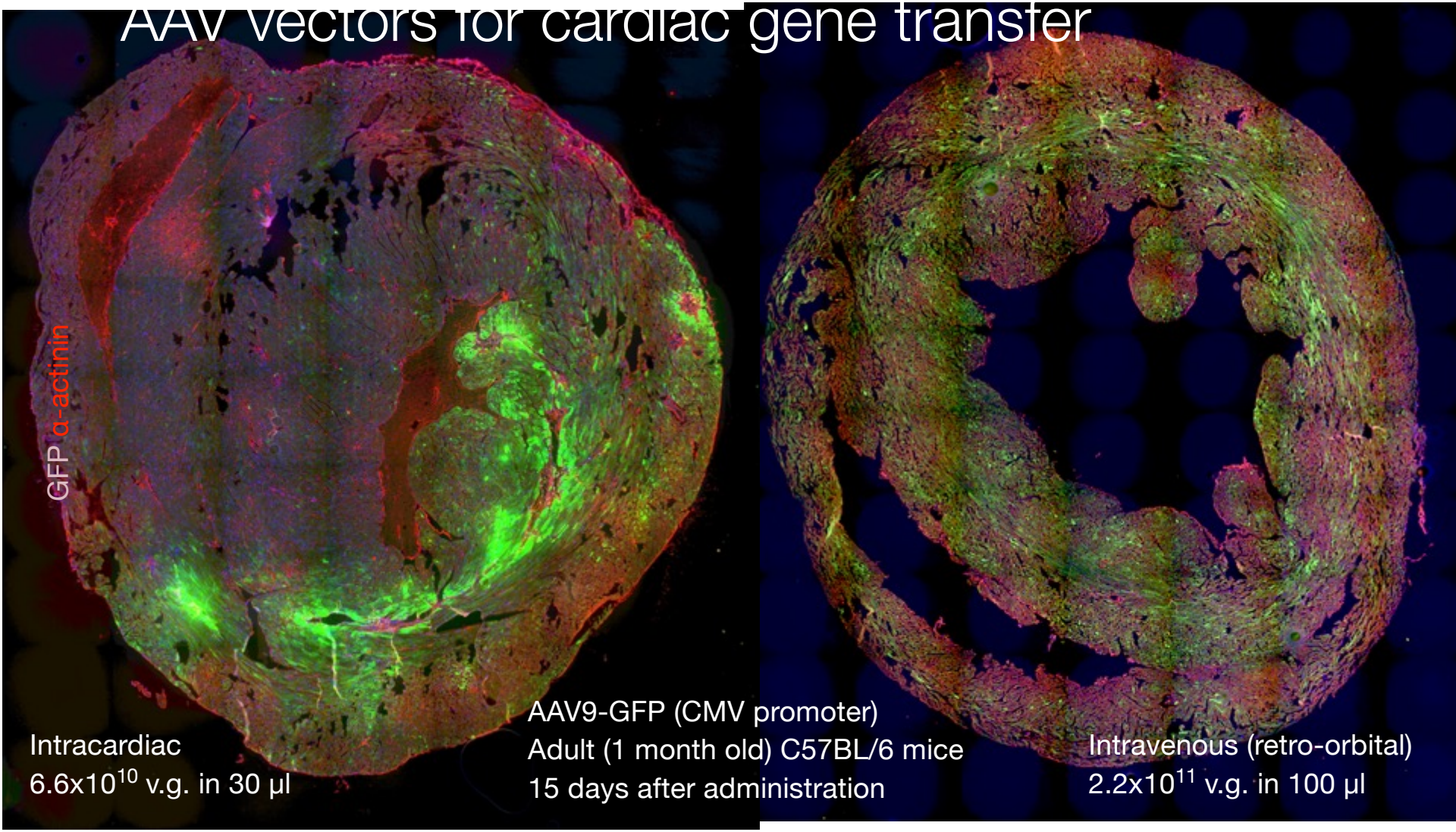



Extracellular
Cytoplasm
RNA only
Easy to degrade
Low permeability
siRNA = down regulation
mRNA = up regulation
3) Released from endosome
~pH5
Ionizable lipid: protonated
RNA in LNP
Endosome
~pH6
~pH7
1) protected inside LNP
2) Taken up by cells
Ionizble lipid: neutral
~pH7.4

Size of nucleic acid delivery vehicles



AAV vectors for cardiac gene transfer



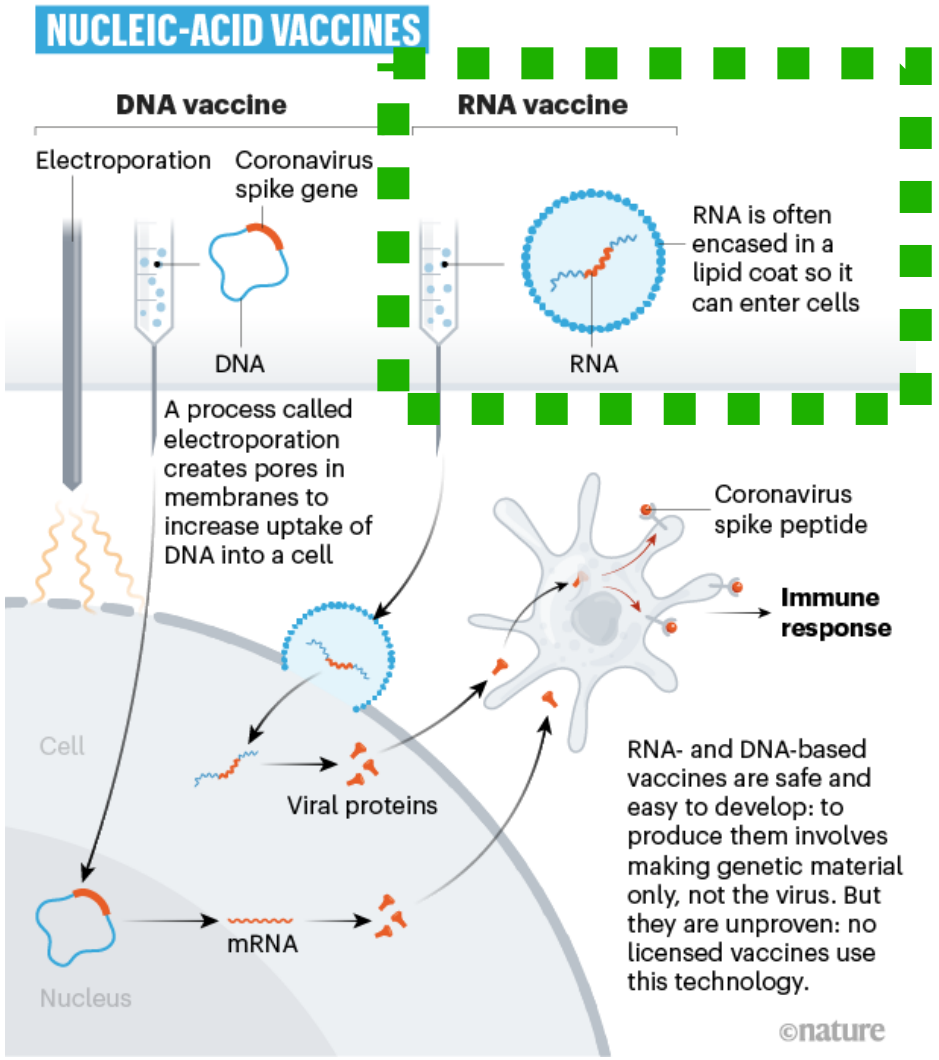
THE RACE FOR CORONAVIRUS VACCINES

By Ewen Callaway; design by Nik Spencer.

Moderna

Pfizer/BioNTech

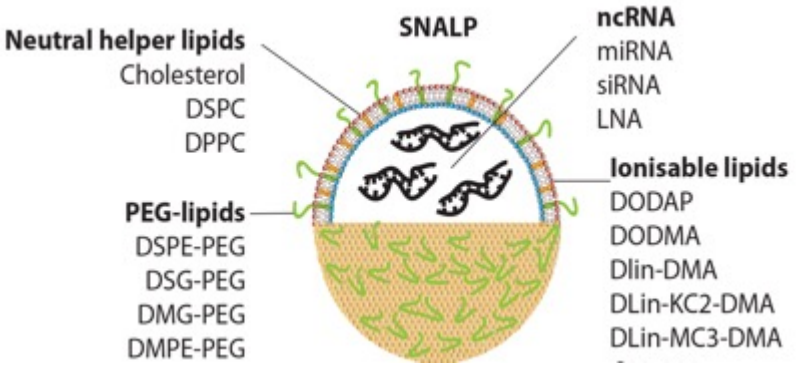
CureVac



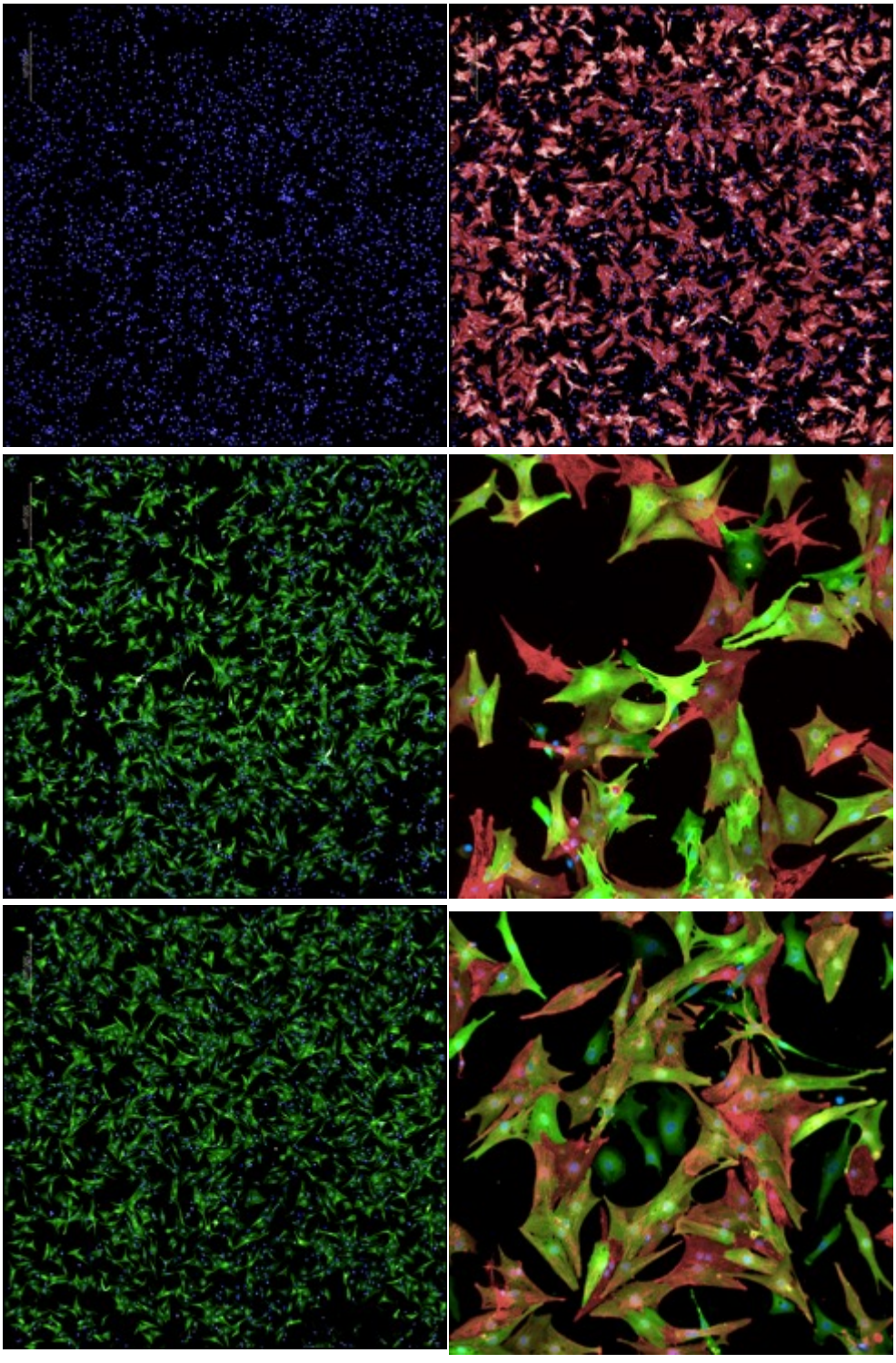
Nature **580**, 576-577 (2020)

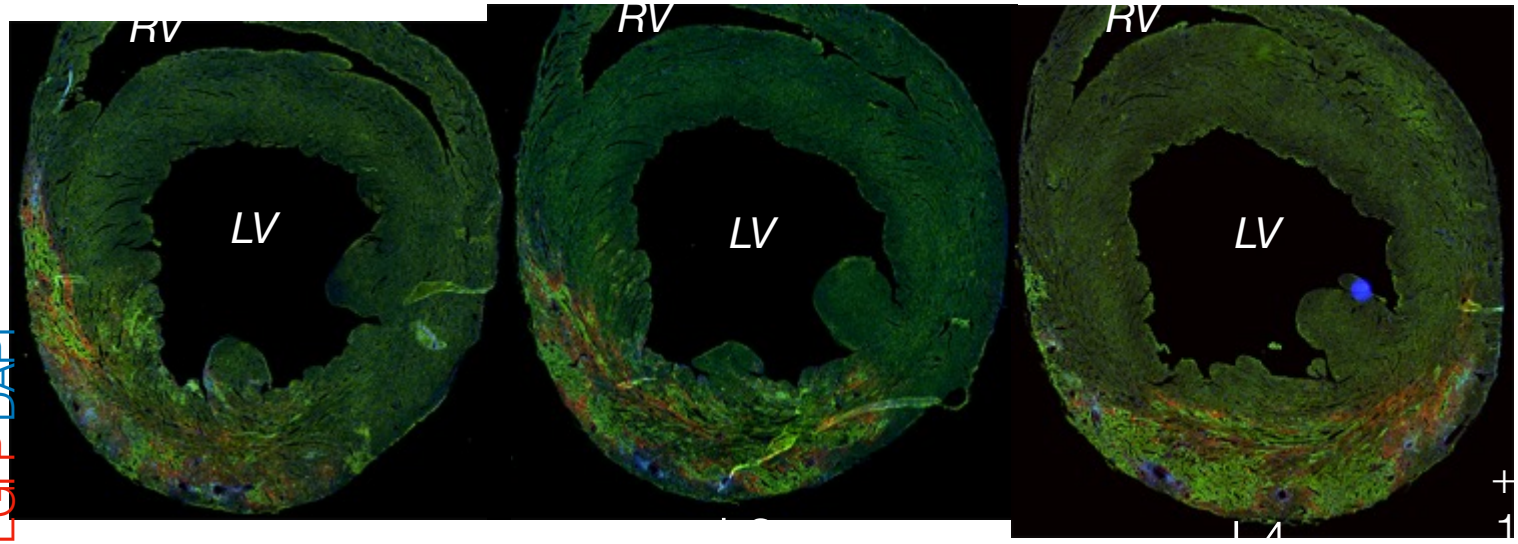
Stable Nucleic Acid-Lipid nanoParticles (SNALPs)

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]

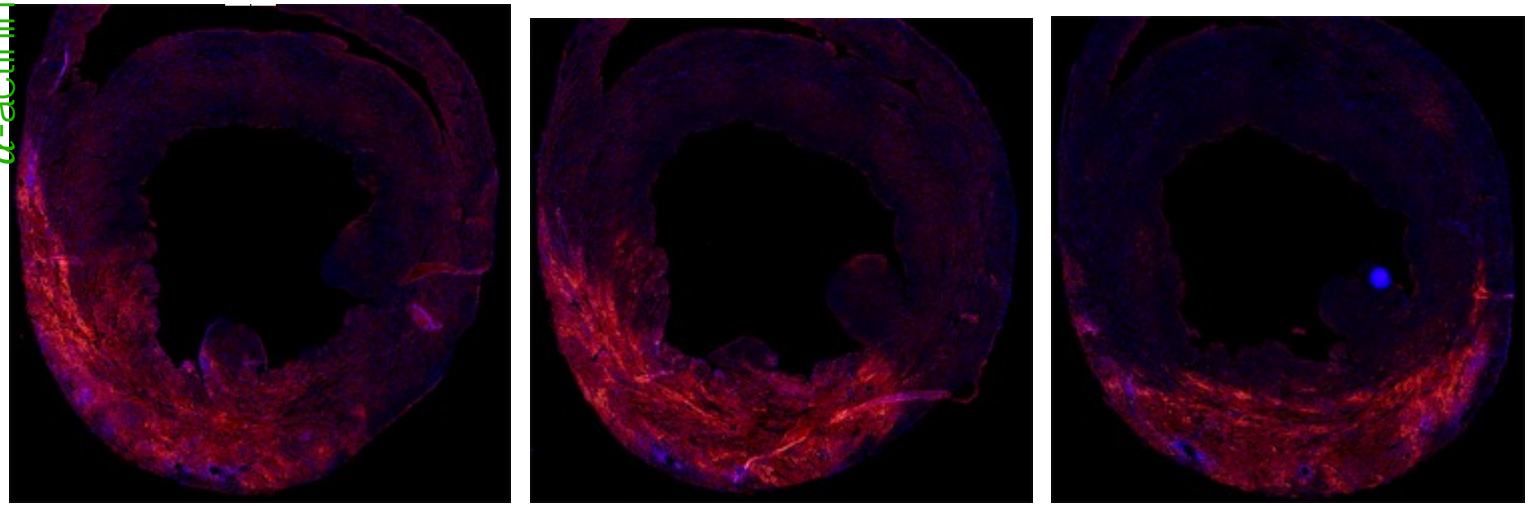


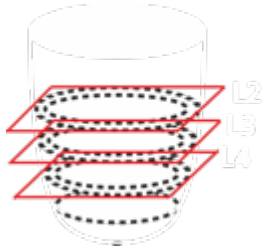
α-Actinin GFP DAPI

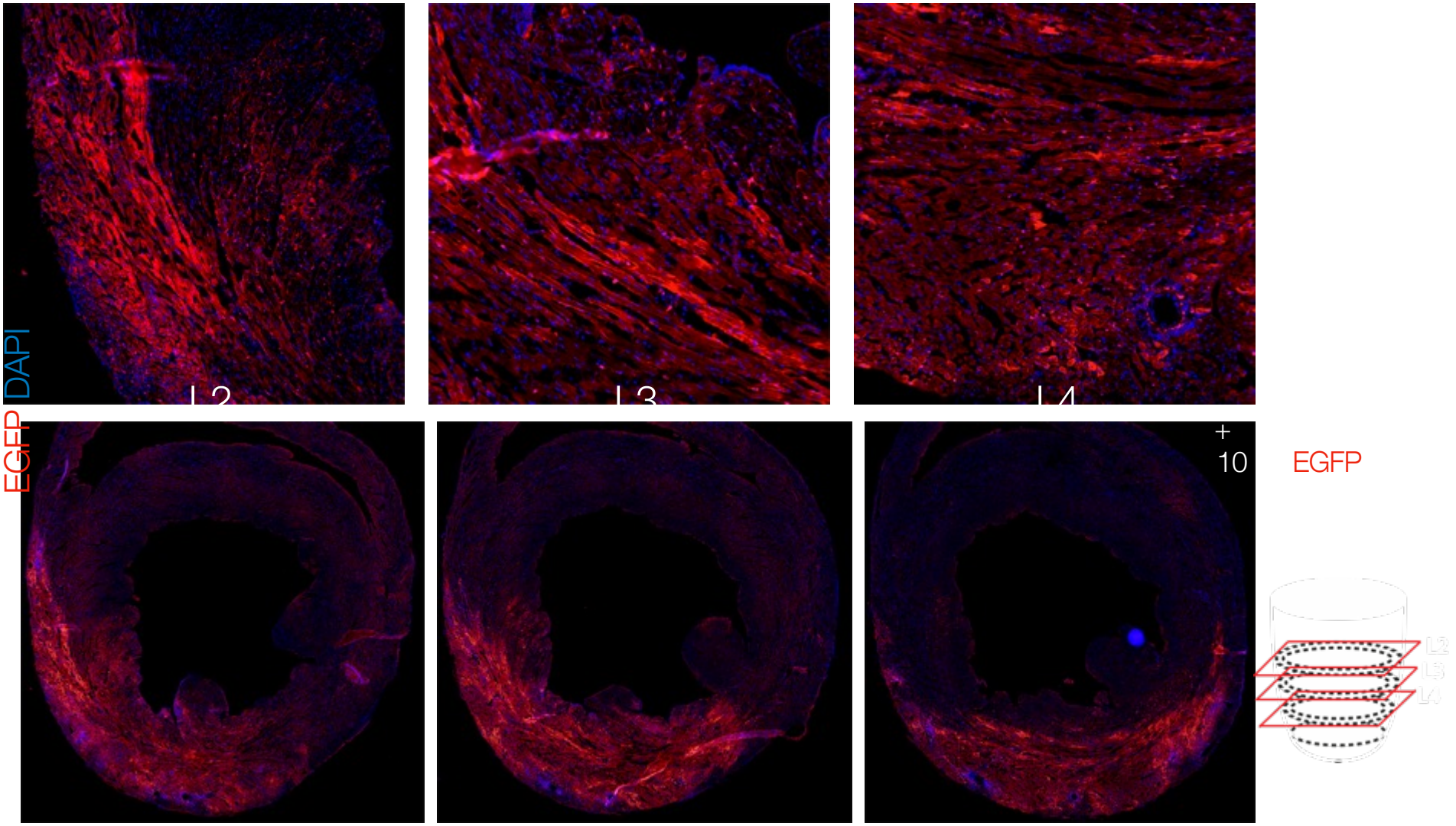




EGFP

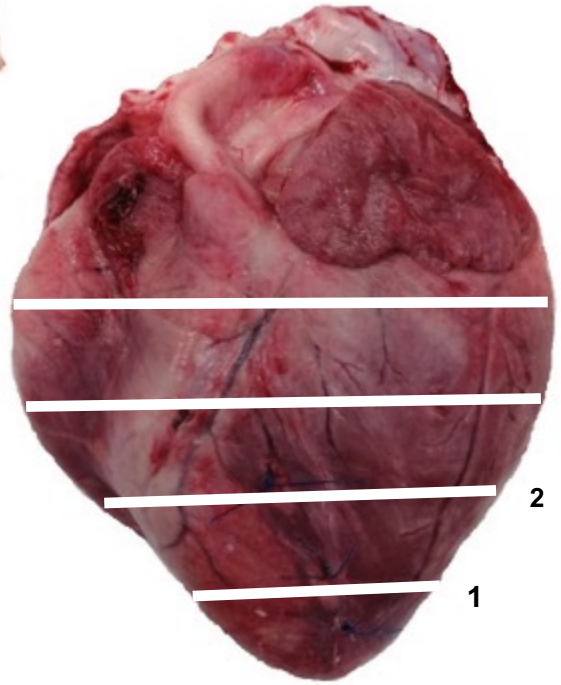


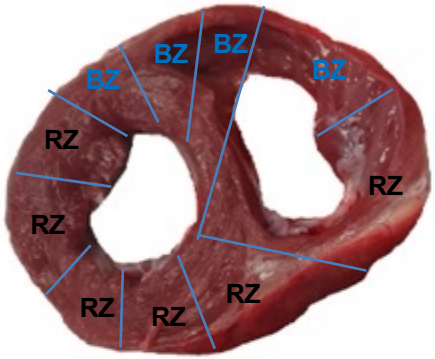


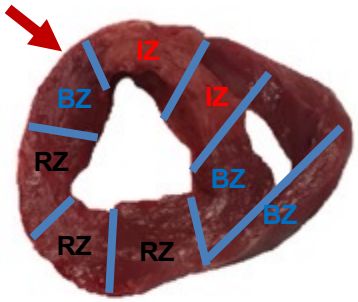

DAPI
L2
L3
L4
EGFP
+
10
EGFP
L2
L3
L4

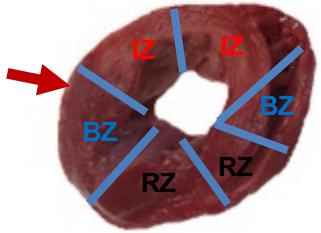
Intramyocardial delivery of JSNALP9-GFP

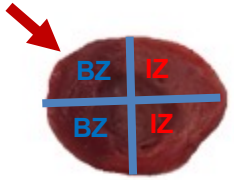
With:
Khatia Gabisonia
Fabio Recchia
Pisa, Italy







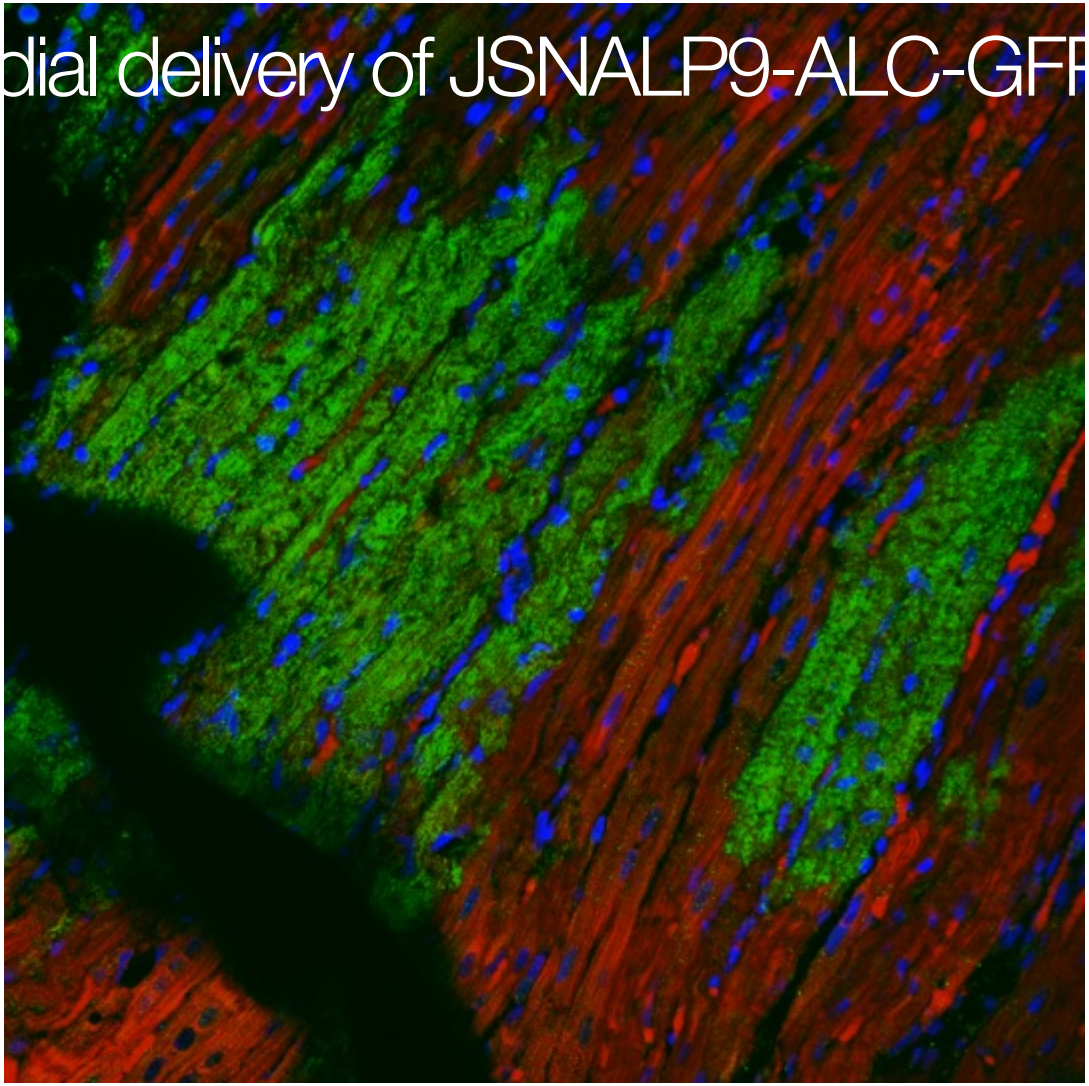




Sacrifice 24h
after injection

Nano 31
Nano 33

30 kg
Heart 240 g



Anti-GFP-antibody
647 nm filter (far red)
shown in green

Anti-a-actinin antibody
488 nm filter
Shown in red

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