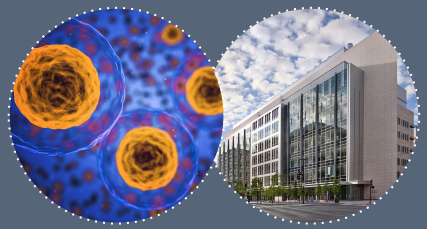
Non-viral Delivery of RNA and Genome Editors



Daniel G. Anderson

Department of Chemical Engineering
Institute for Medical Engineering and Science
Koch Institute for Integrative Cancer Research

KOCH INSTITUTE for Integrative Cancer Research at MIT

HST Harvard–MIT Health Sciences & Technology

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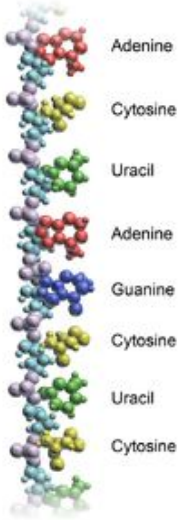
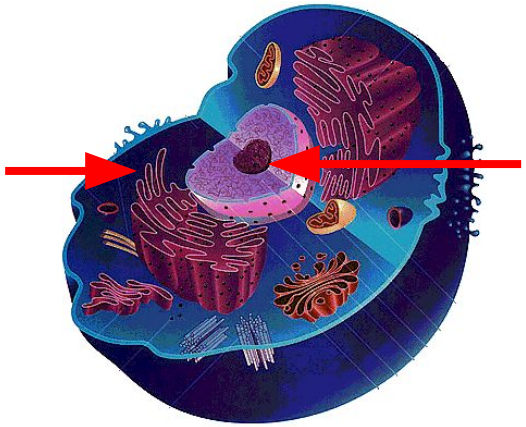
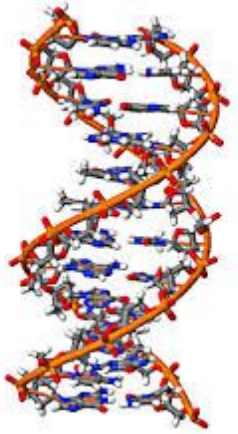
Intracellular Delivery of Nucleic Acids
will Revolutionize Medicine



RNA





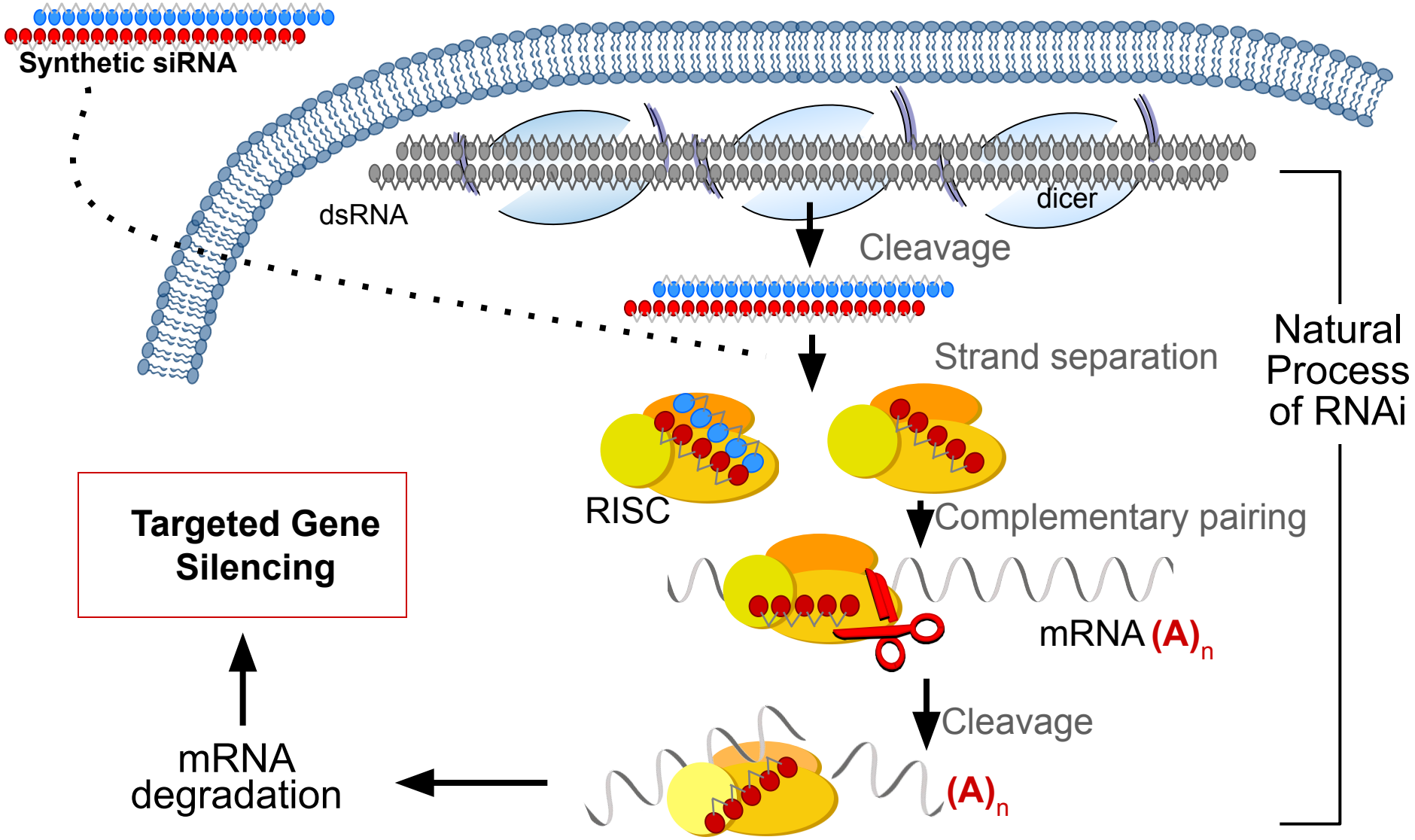
DNA

How can they be delivered inside of cells, in vivo?

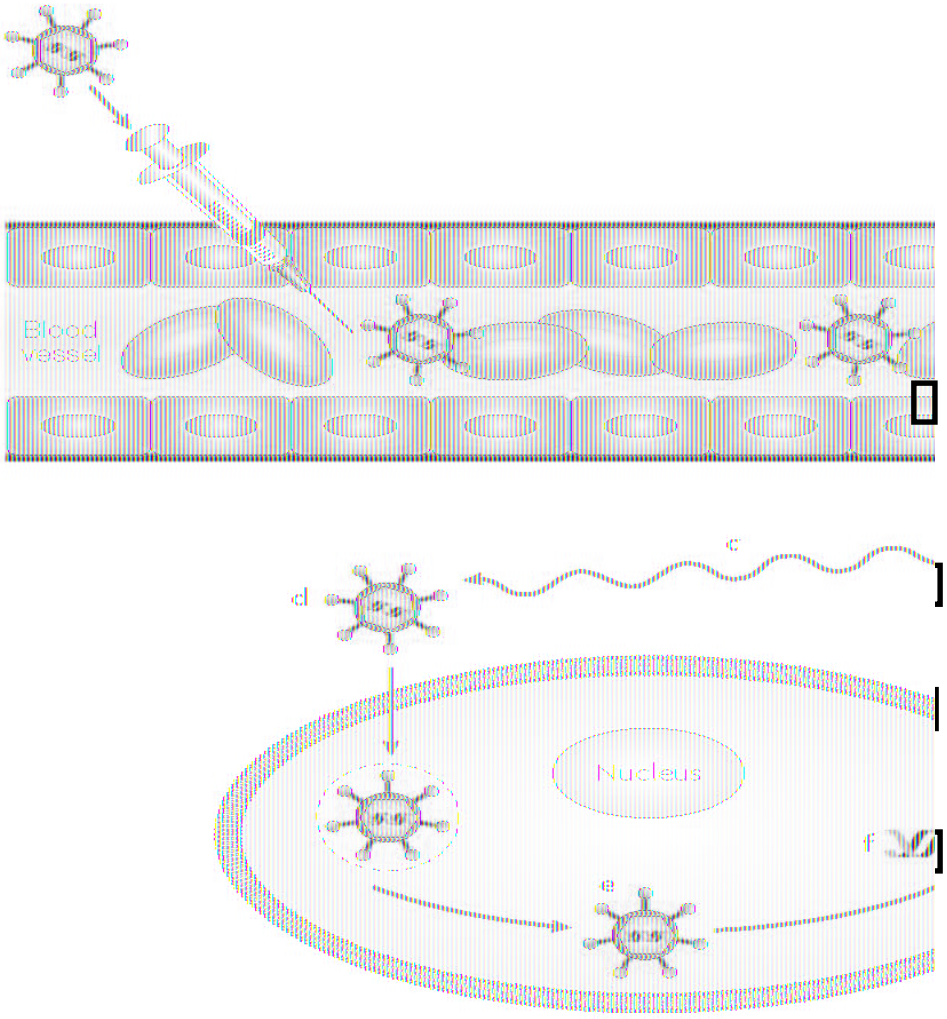
mRNA vaccines for COVID

Formulations inspired in part by efforts to deliver siRNA to the

“Modular Pharmacology” with siRNA: Turning Genes Off via RNA Interference



The barriers to intracellular delivery are substantial!



Nanocomplexation

Transport to cells of interest

Avoidance of non-target cells

Endocytosis

Endosomal escape

Release of RNA from nanoparticle

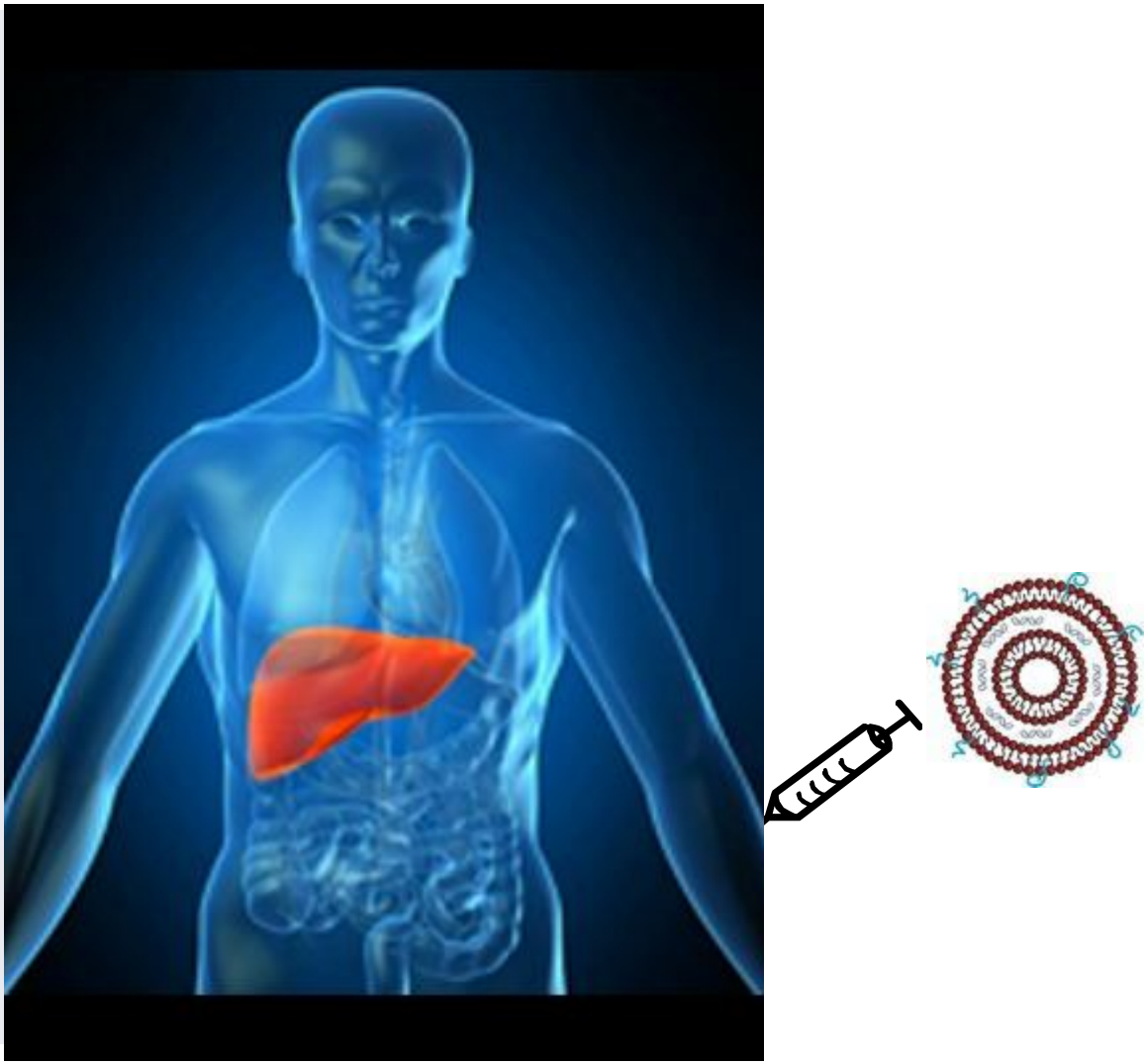
Nuclear transport

What organs are most amenable to targeting?

Systemic Delivery of Nanoparticles

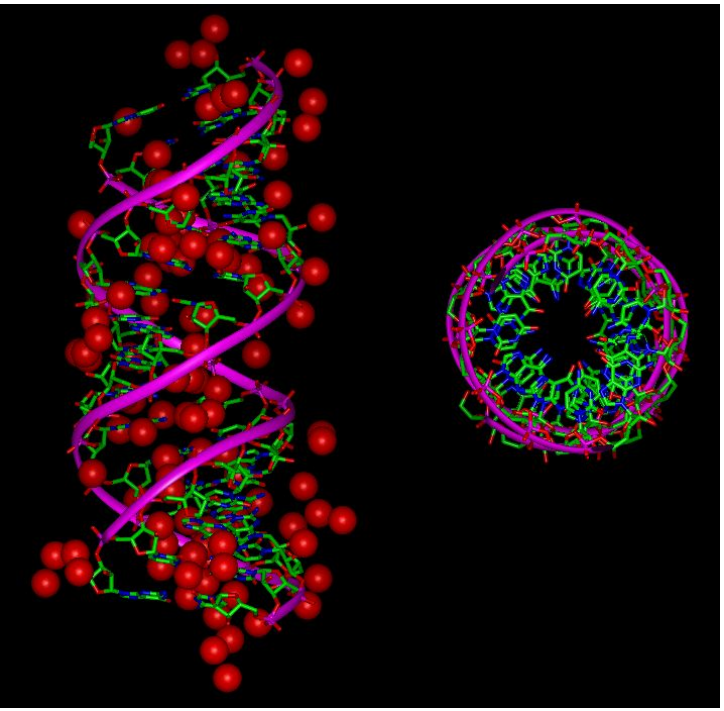
- **Parenteral administration of nanoparticles leads to accumulation in organs with fenestrated endothelium**

- **Liver**
- **Spleen**
- **Bone Marrow**
- **Kidney**
- **Phagocytic cells**



Tissue accumulation is not intracellular delivery

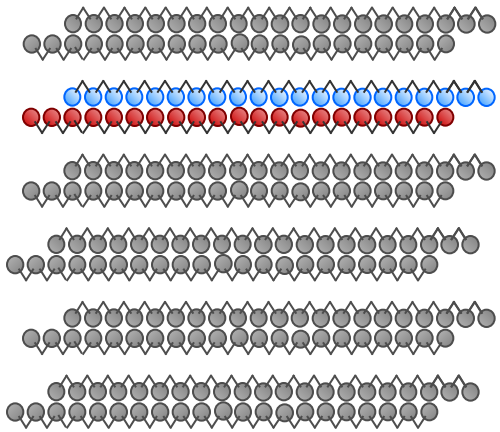
Turning Nucleic Acids into Drugs is not easy!



- **RNA/DNA is a large molecule, and highly charged, and does not cross cellular membranes**
- **RNA/DNA is prone to nuclease degradation**
- **RNA/DNA can induce immune responses (TLR pathways!)**

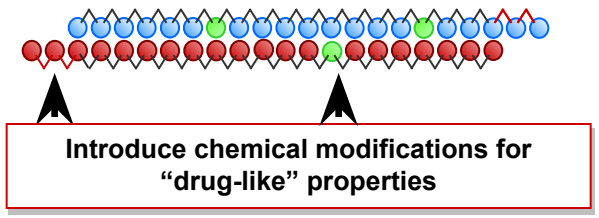
Keys to turning Nucleic Acids into Drugs

Sequence Selection



- Target Specificity and Potency

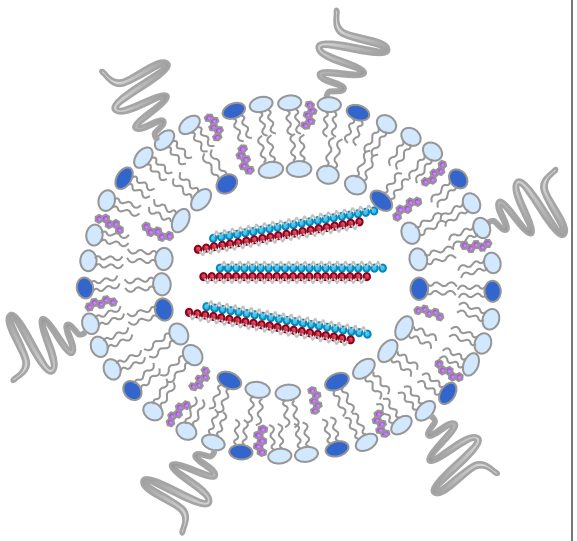
Chemical Modification



Ligand Conjugation

- Stabilization
- Potency
- Selectivity
- Cellular Specific Uptake

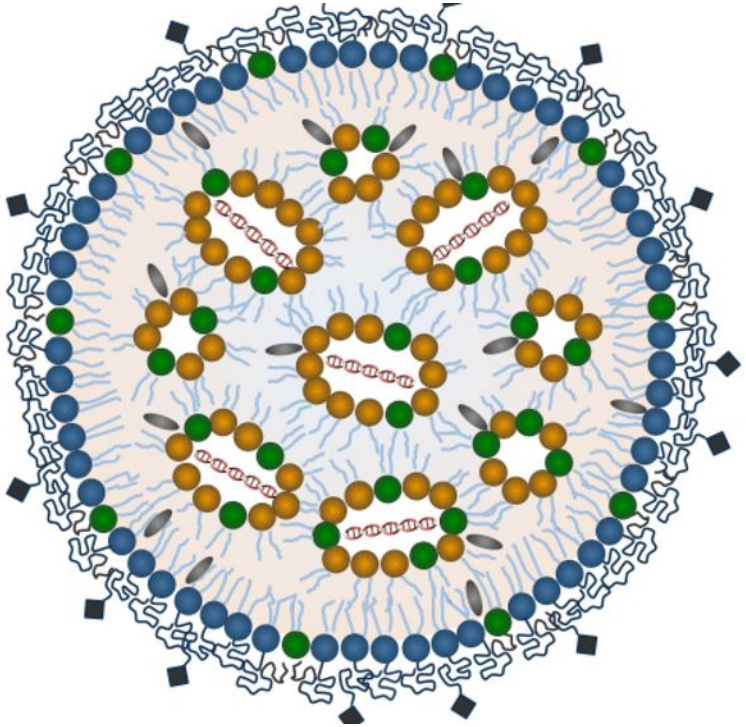
Encapsulation



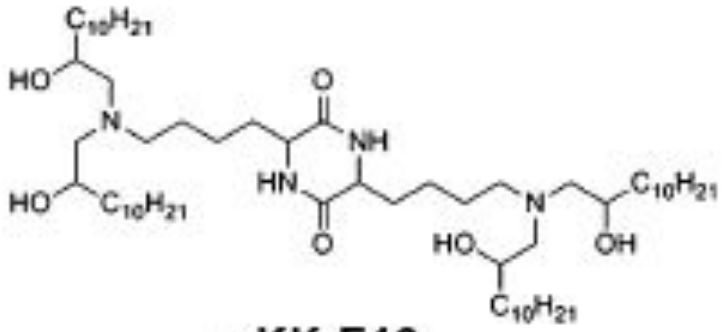
- Particularly Important for Longer RNAs (mRNAs, lncRNAs) where chemical tools are less available

What materials can we use for RNA delivery?

Typical RNA-Lipid Nanoparticles Composition



AMINE/ionizable Lipid



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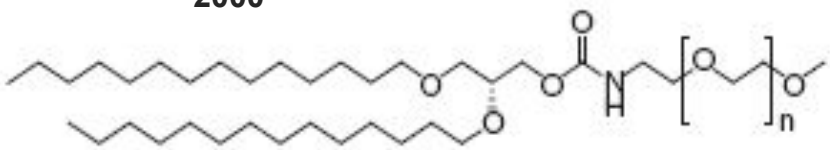
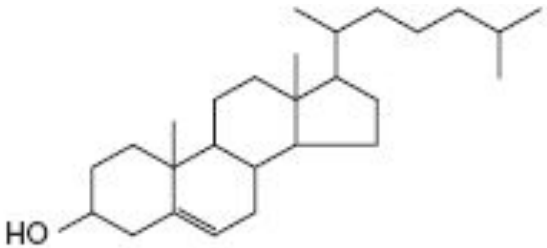
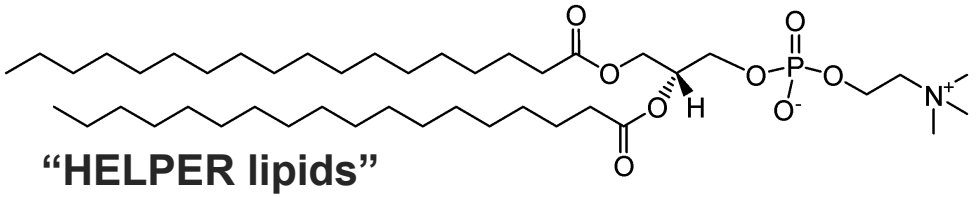
cKK-E12

"PEG –lipid"
$mPEG_{2000}$-C14 glyceride





Cholesterol

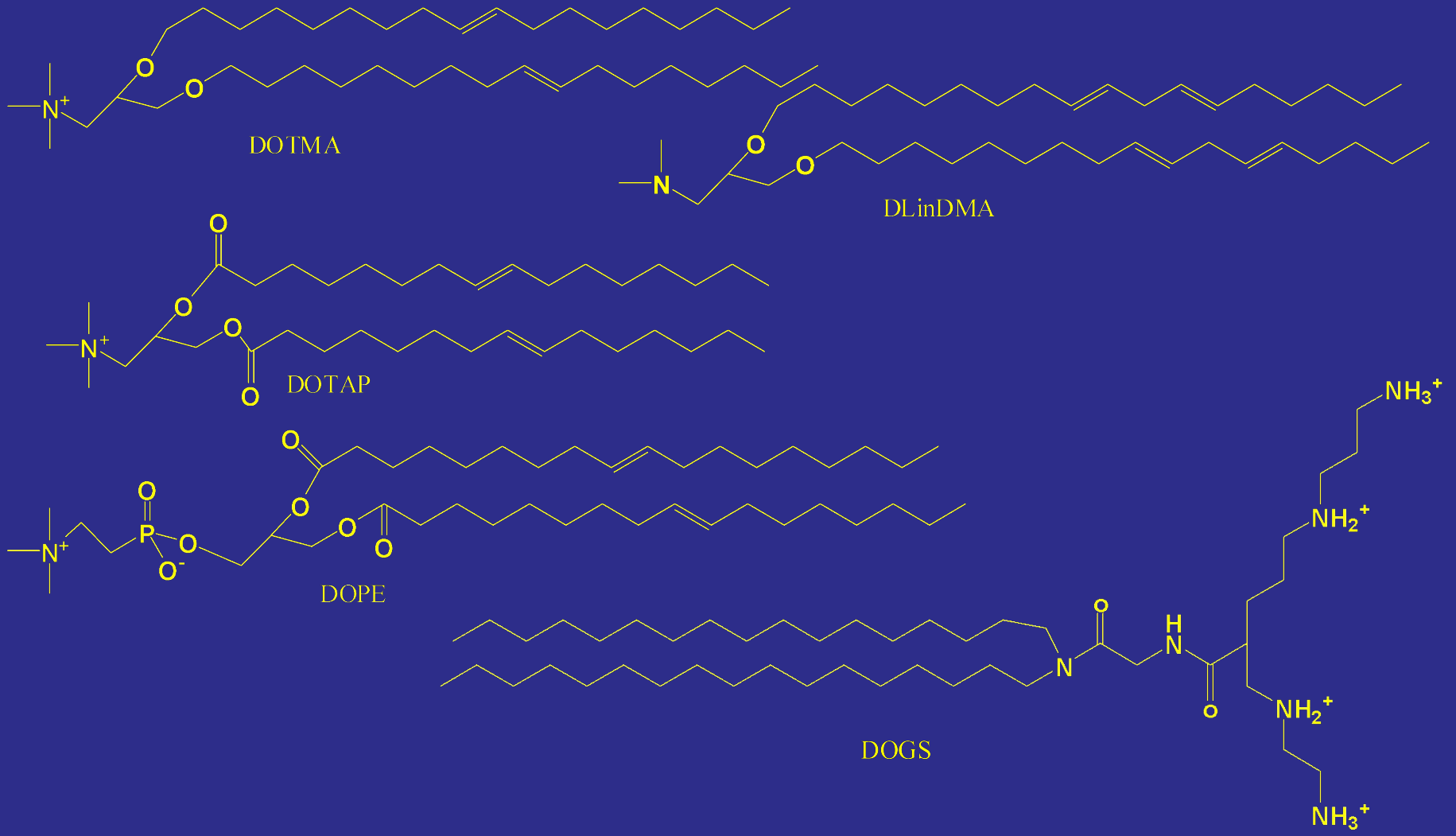


"HELPER lipids"

DSPC

- **Low surface charge**
- **Small uniform size particle**
  <100 nm

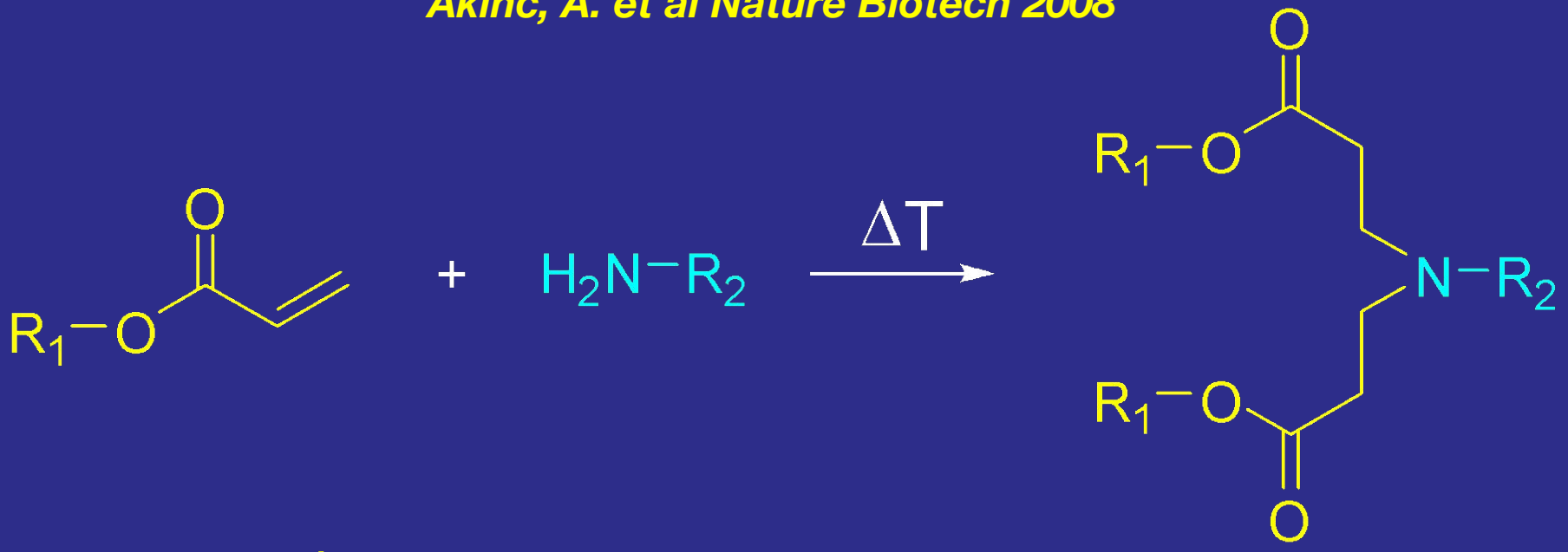
What materials can we use for RNA delivery?



How can we increase diversity?

Combinatorial synthesis of lipid-like materials

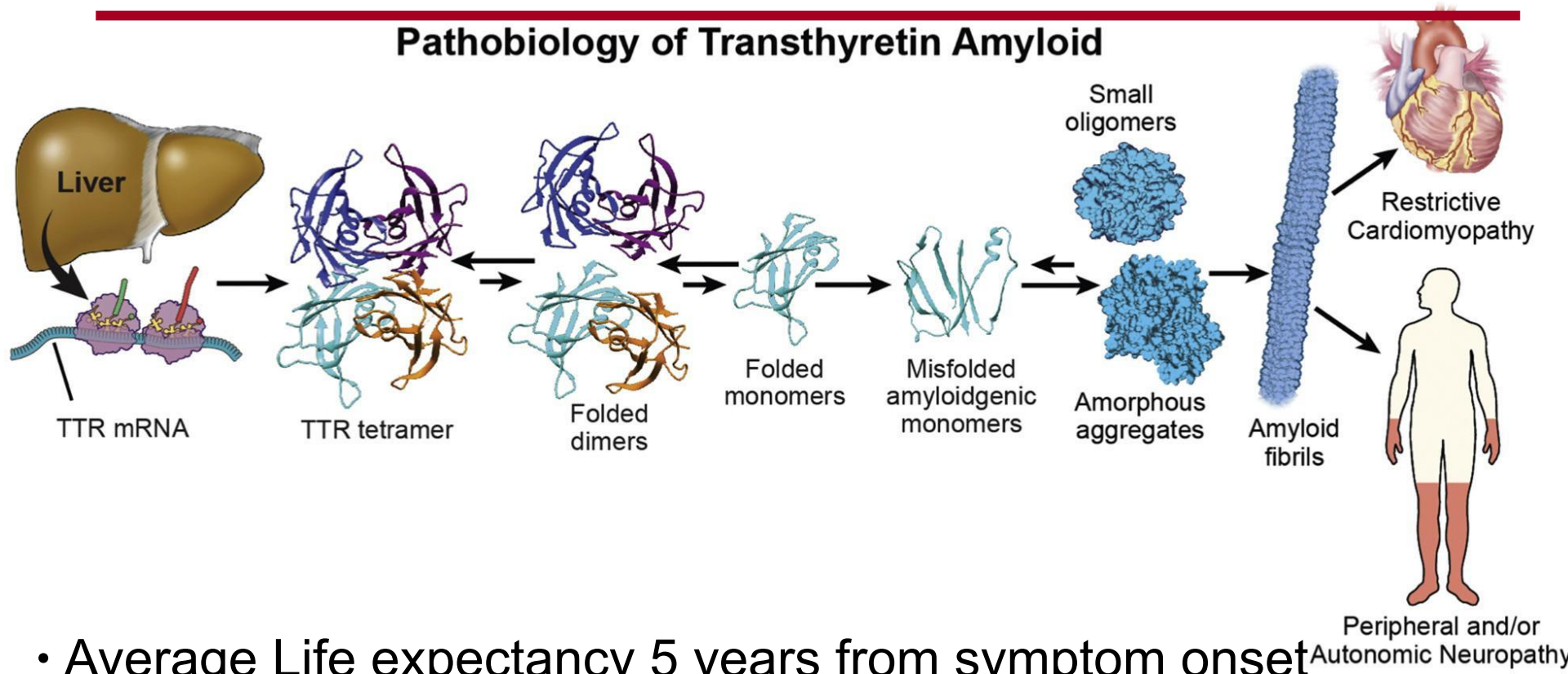
Akinc, A. et al Nature Biotech 2008



- **No protection/deprotection, no solvents, no catalysts, no purification**
  1000's of different lipid like materials synthesize and evaluated

DOTAP

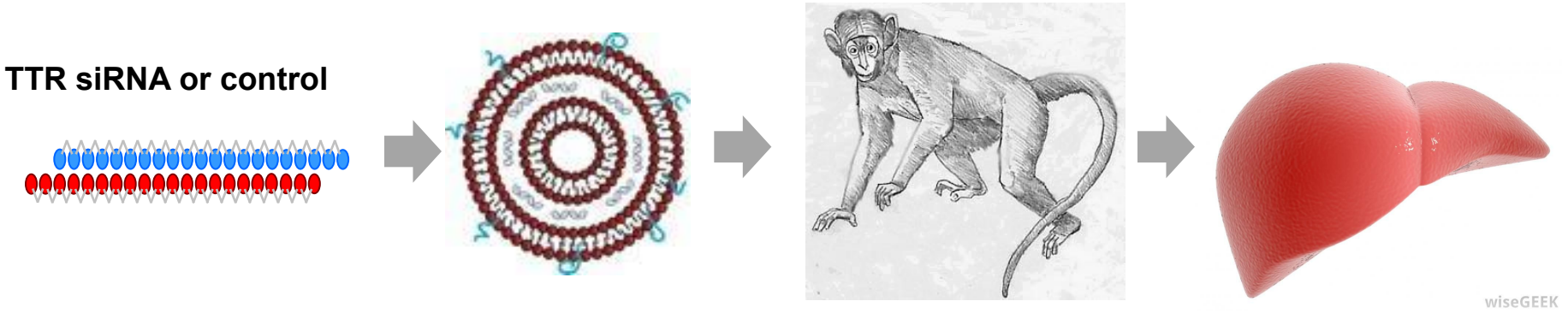
RNA Therapeutics for Liver Example: Transthyretin-mediated amyloidosis



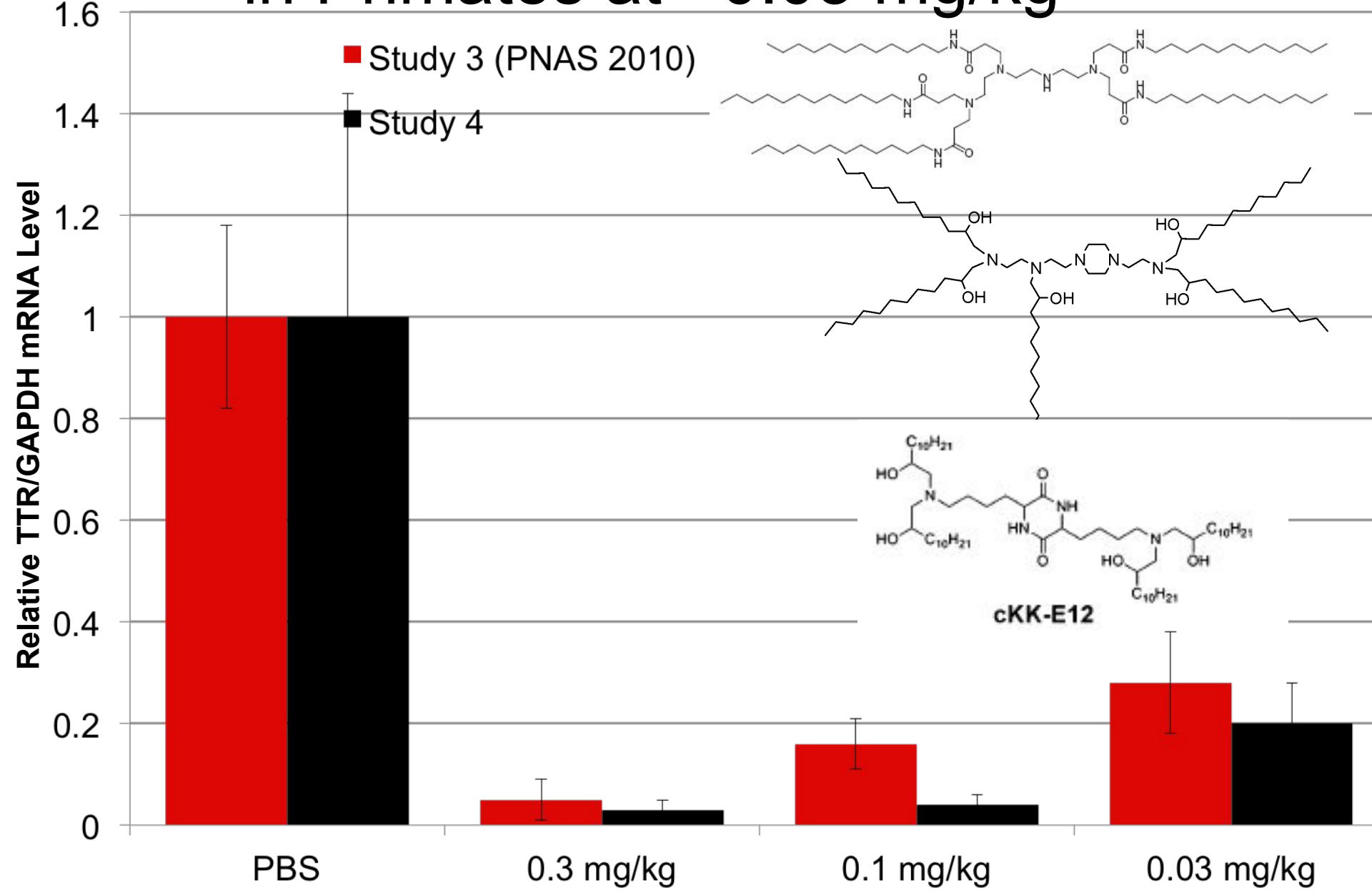
- Average Life expectancy 5 years from symptom onset

- Without gene therapy -- only treatment option in the US was liver transplantation

Example: Lipid-siRNA-Nanoformulations targeting Transthyretin (TTR) in the liver of primates



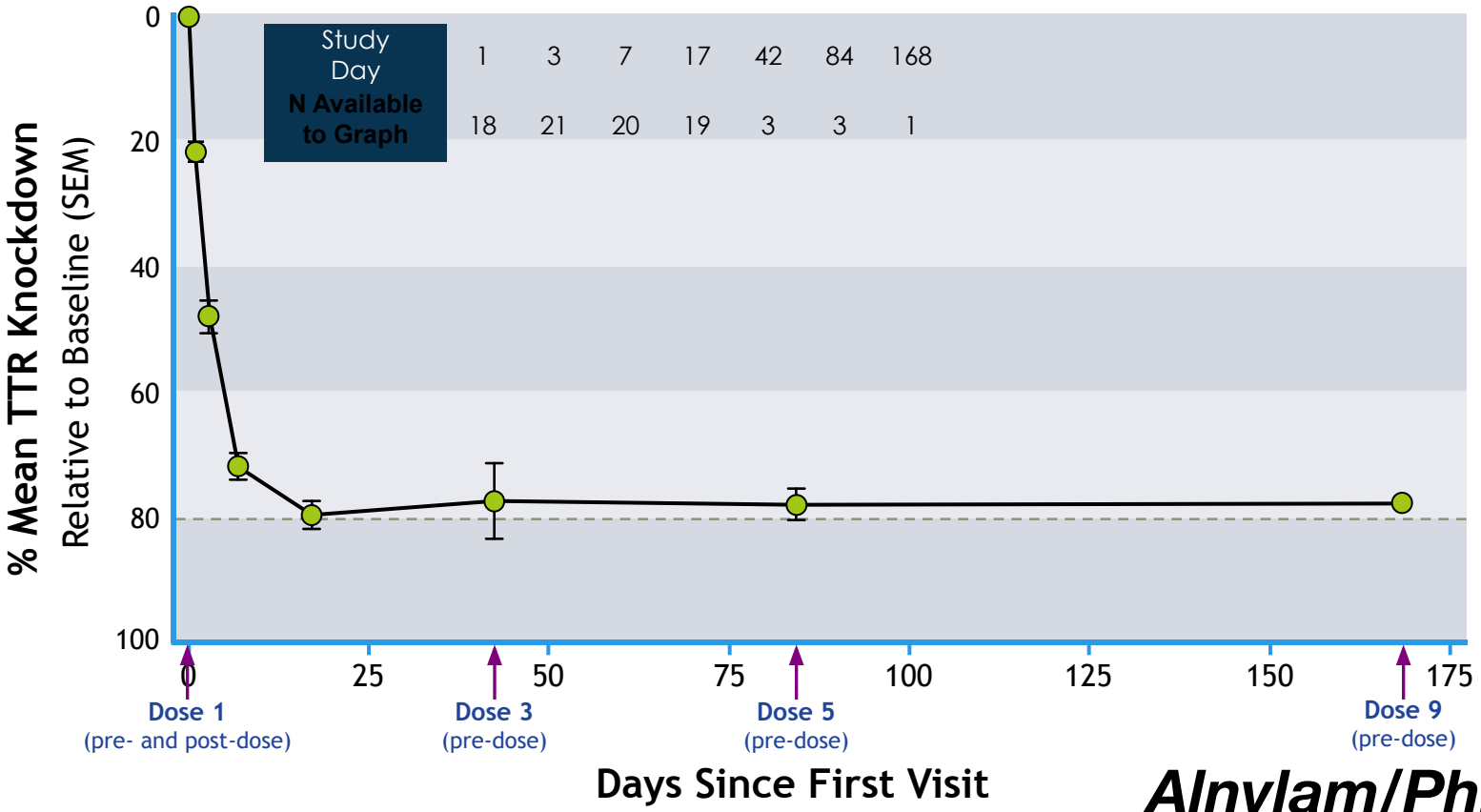
Single Dose knockdown of Target in Primates at <0.03 mg/kg



Delivery of siRNA to human liver

Patisiran (ALN-TTR02) open label

Sustained knockdown of TTR in ATTR patients



Alnylam/Phil Sharp

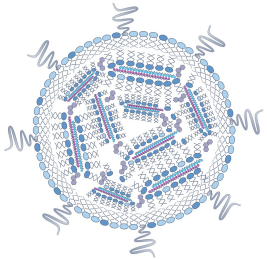
[1]Suhr, *Int'l Symp Amyloidosis*, Apr. 2014 ; **Preliminary results as of April 3, 2014*

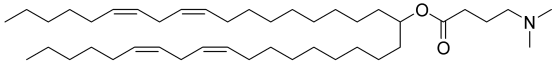
First siRNA Lipid Nanoparticle Approved in August, 2018

Compositional Similarities between Approved mRNA and siRNA Lipid Nanoparticles

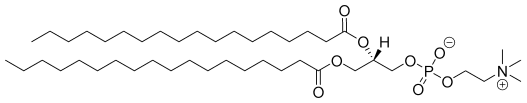
Alnylam PHARMACEUTICALS

onpattro (patisiran) lipid complex injection

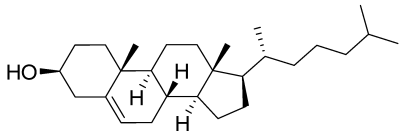




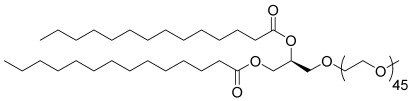
DLin-MC3-DMA



DSPC

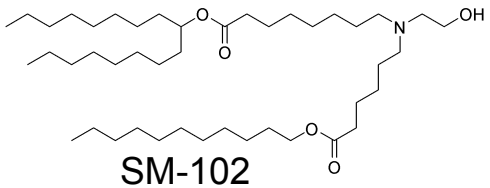


Cholesterol

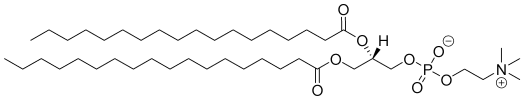


PEG2000-DMG

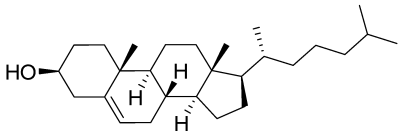
moderna



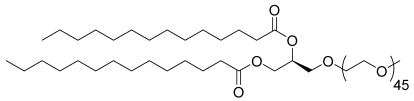
SM-102



DSPC

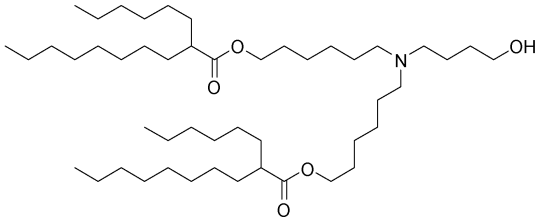


Cholesterol

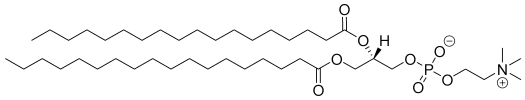


PEG2000-DMG

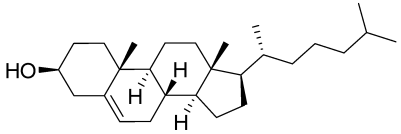
Pfizer BIONTECH



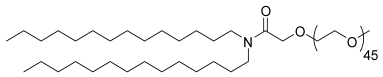
ALC-0315



DSPC

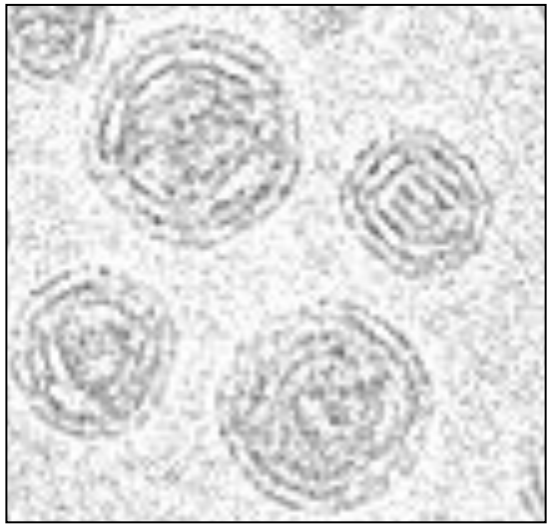


Cholesterol



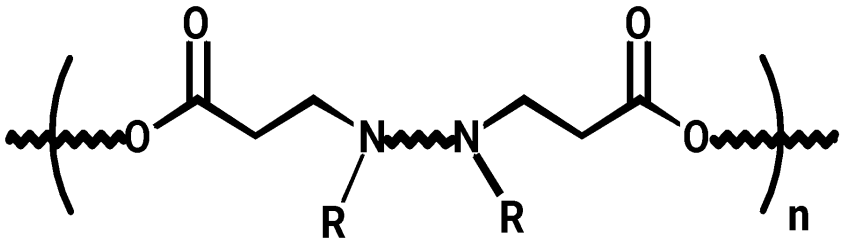
ALC-0159

Nanoparticle-mediated RNA Delivery is NOT limited to LNPs, vaccines or hepatocytes: Polymer NanoParticles (PNPs)

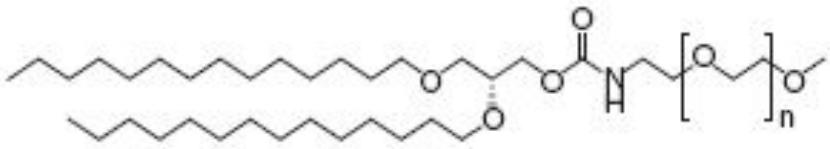


- **Variable: surface charge**
- **Small size particle 30 --- 100 nm**

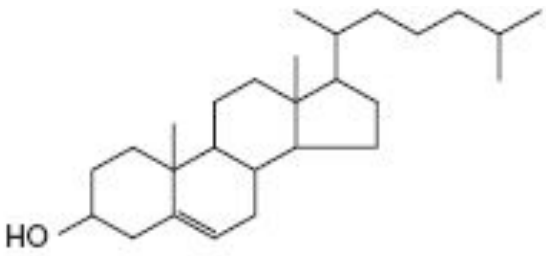
1) AMINE containing Polymer – Degradable
AMINE containing Low MW Polymer -- Preferably Degradable



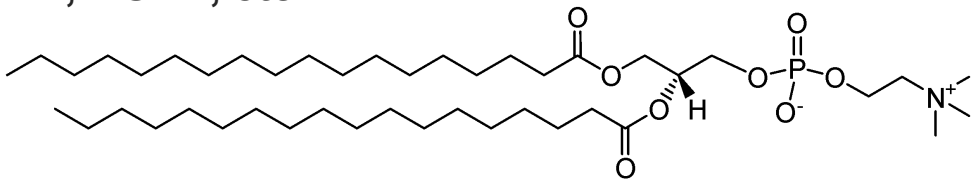
2) Optional:"PEG –lipid"
$mPEG_{2000}$-C14 glyceride



4) Optional: Cholesterol



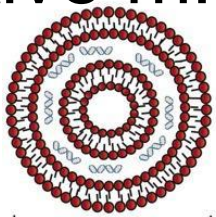
3) Optional: "HELPER lipids"
DSPC, DOPE, etc



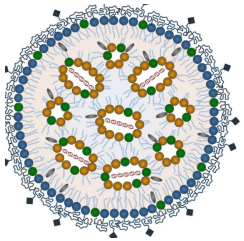
Tissue Selective mRNA delivery via IV injection

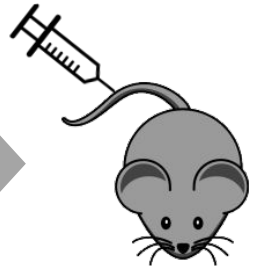
mRNA encoding Luciferase

PBAE

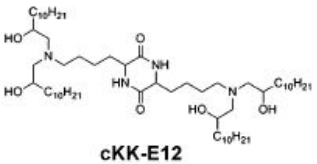


or



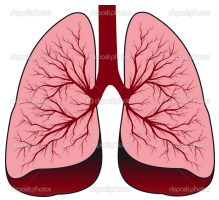


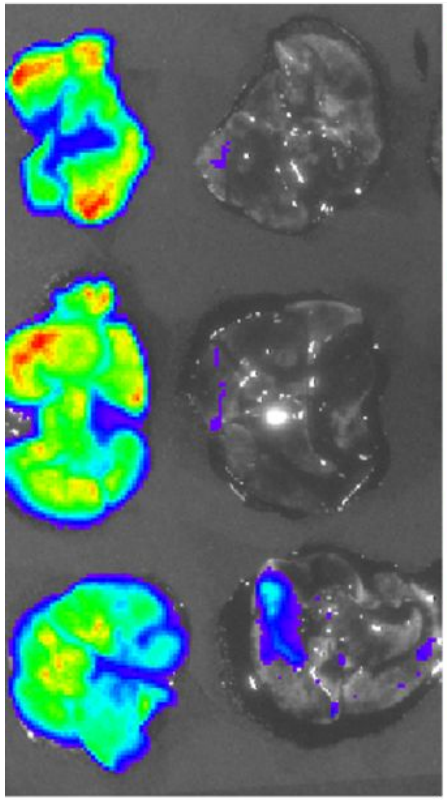
BL/6 Mice
0.5 mg mRNA/kg
mouse
24 hrs.

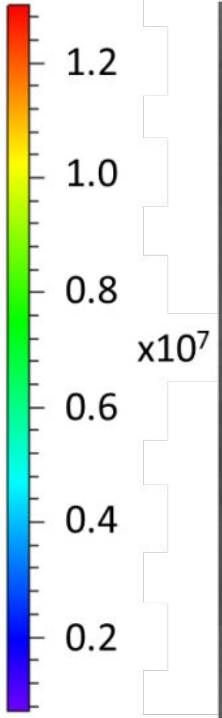


cKK-E12

Lungs

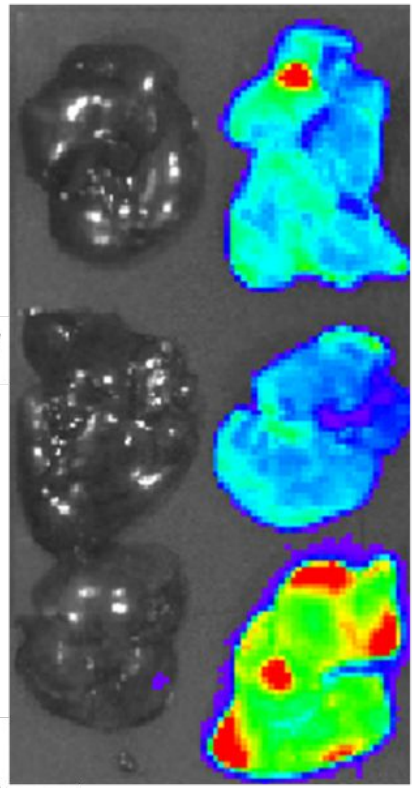


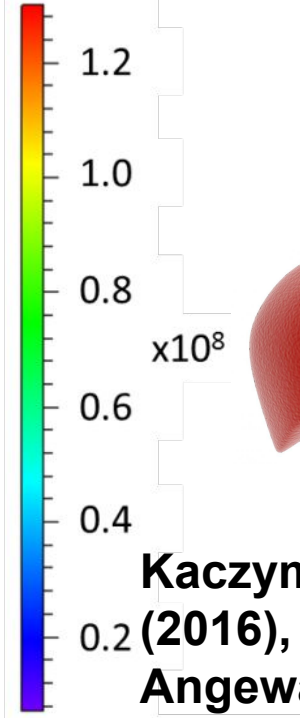




PBAE MD1

Liver

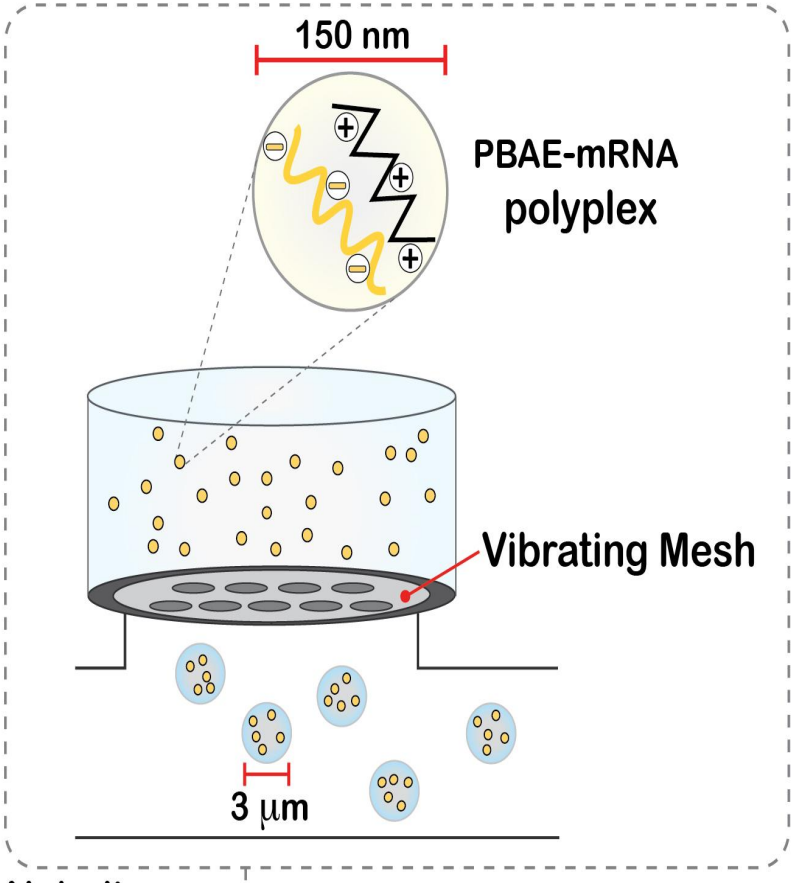


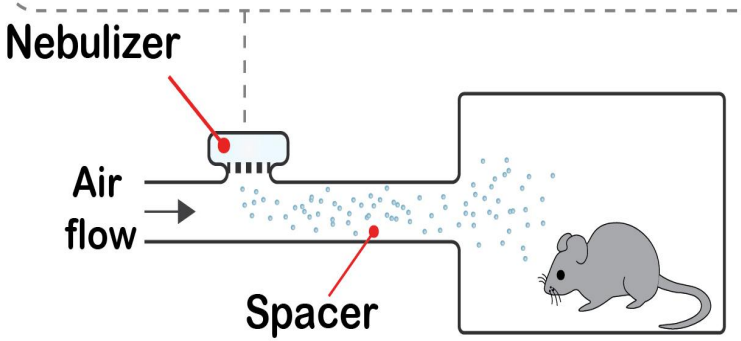


PBAE MD1

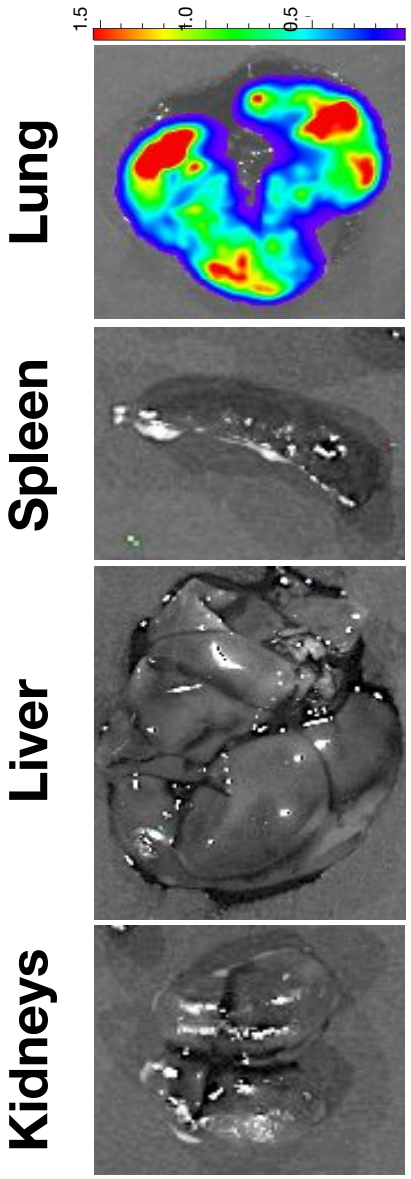
Kaczymarek et al, (2016), Angewandte

mRNA delivery to the lung via nebulization with PNPs



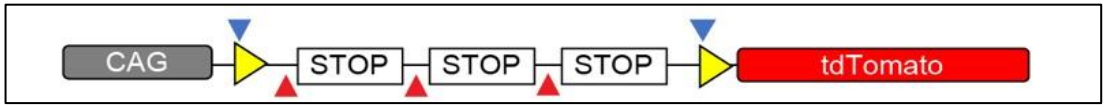


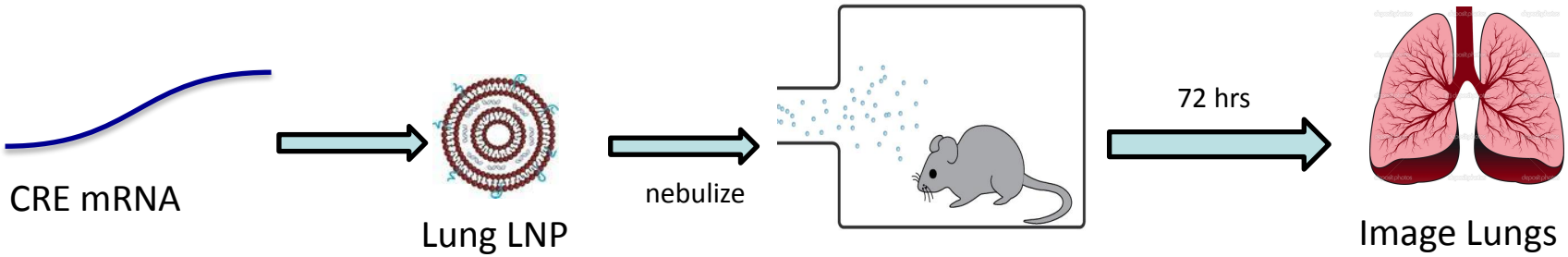
Patel, A., et al., Advanced Materials 2019

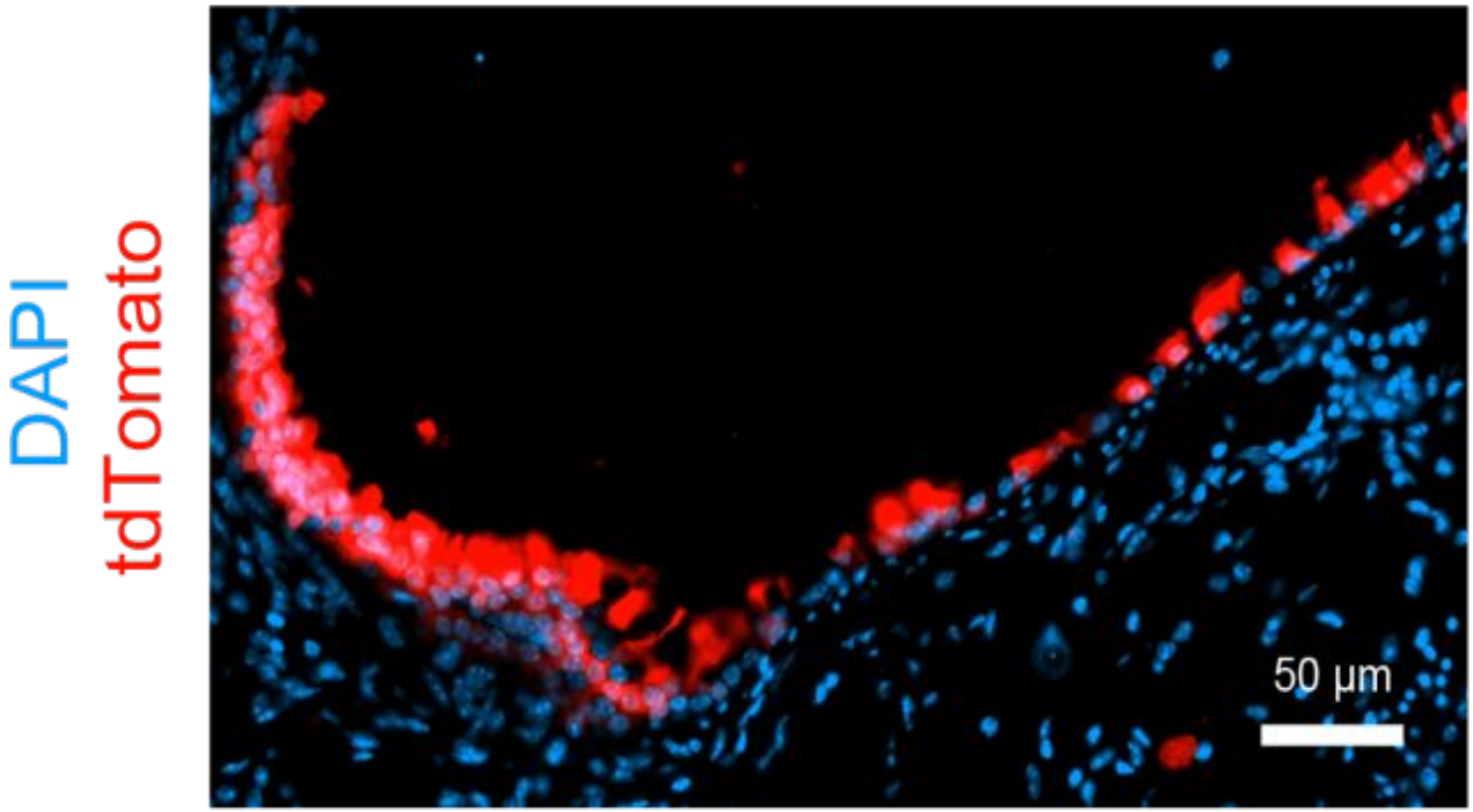


Nebulized Biodegradable LNPs of luciferase mRNA

Ai9 mouse

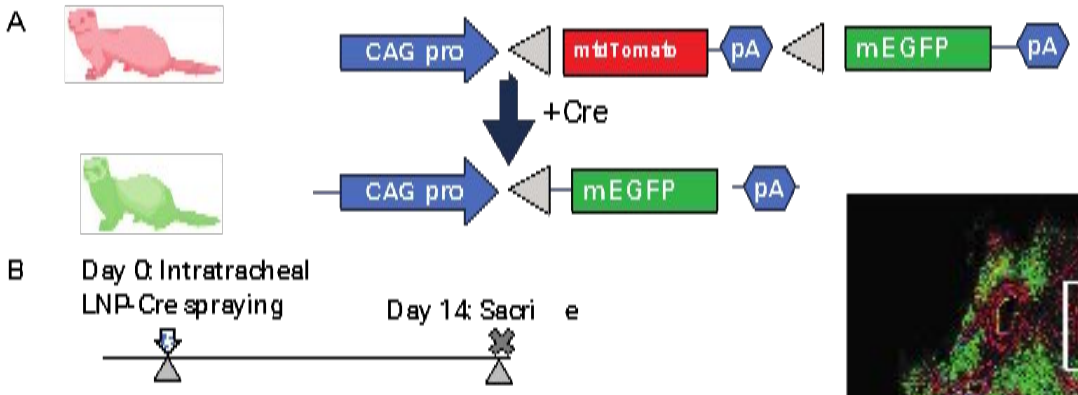




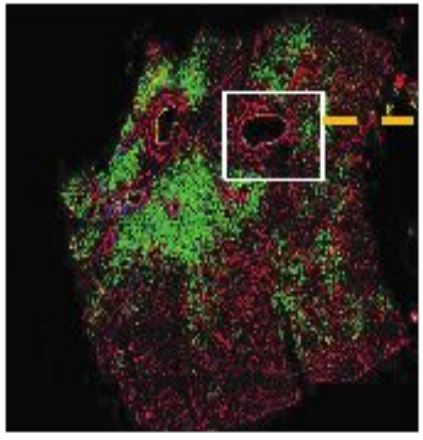


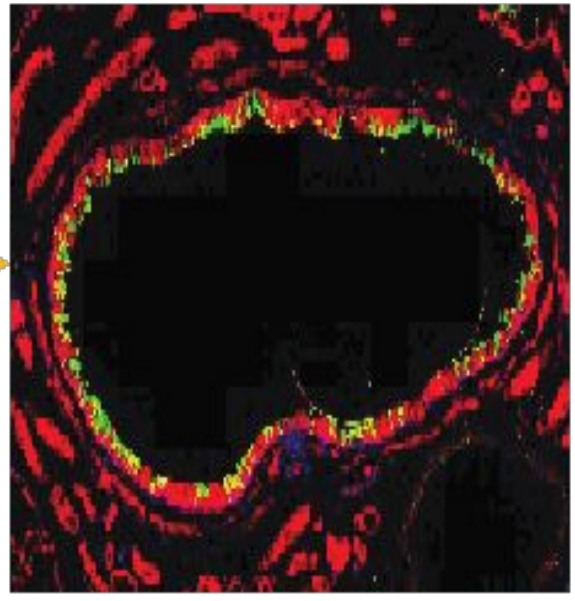
Jiang, Witten, Raji, et al., *Nature Nanotechnology (2023)*

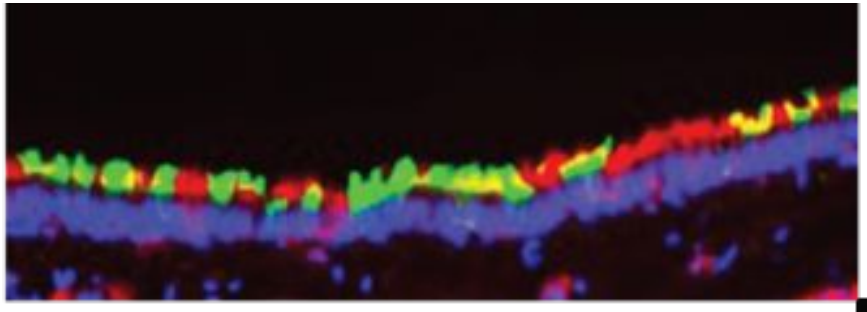
mRNA delivery to ferret respiratory and conducting airways



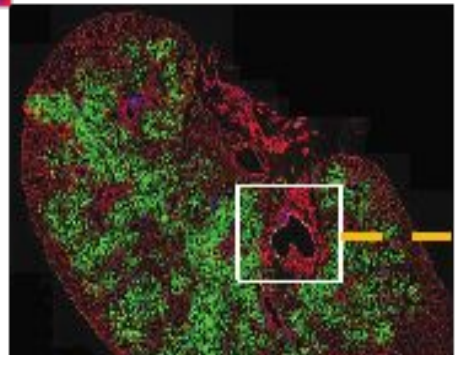
- Diffuse green is efficient alveolar transfection

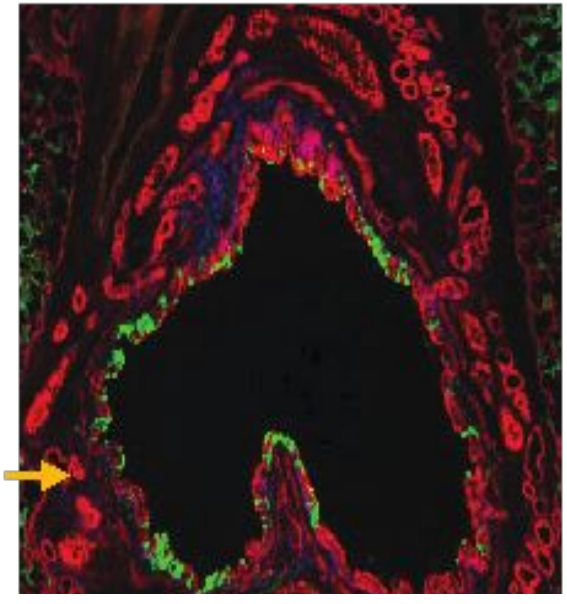






Close-up of trachea transfection



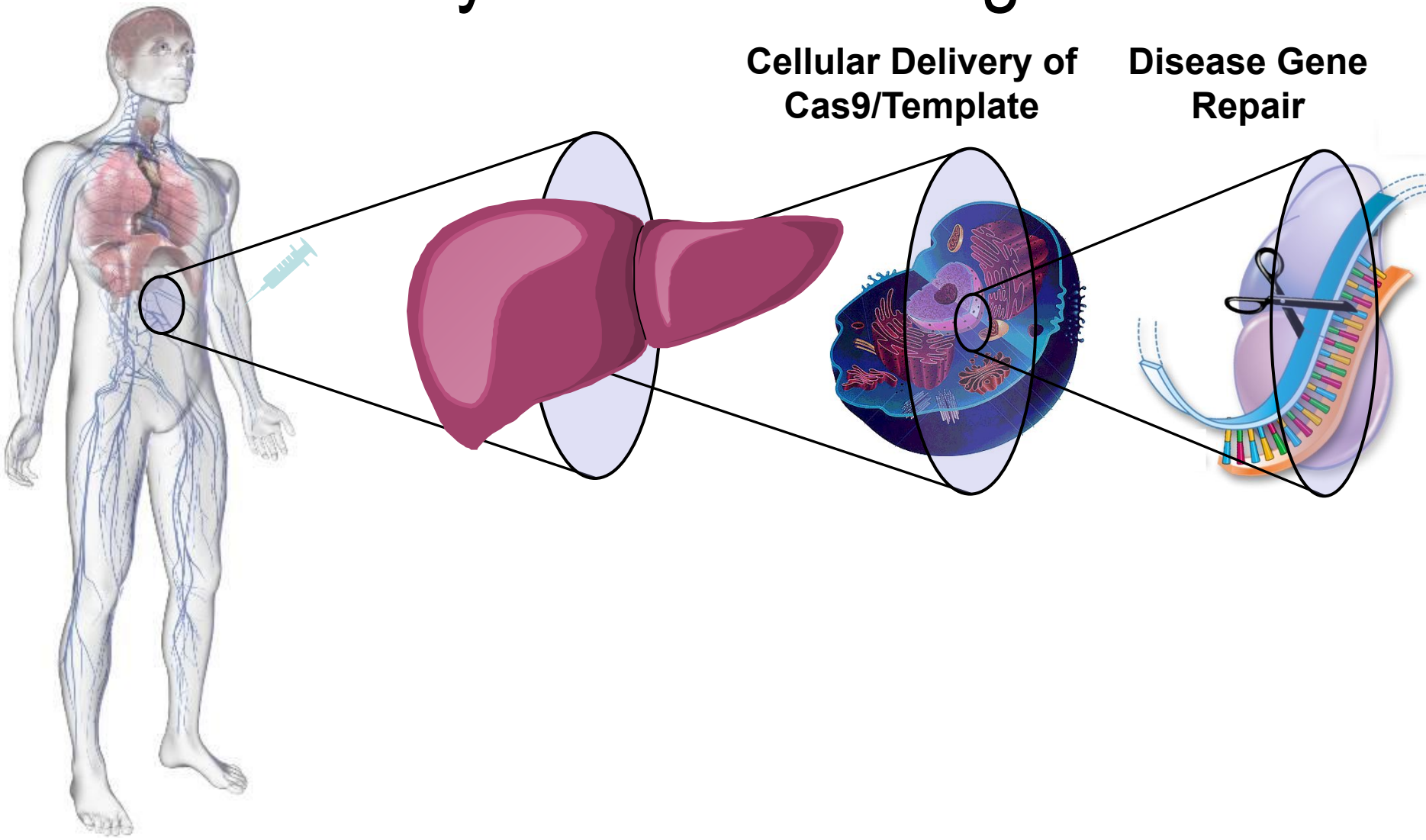


Witten*, Raji*, Manan*, *et al.*,
by *Nature Biotechnology (2024)*

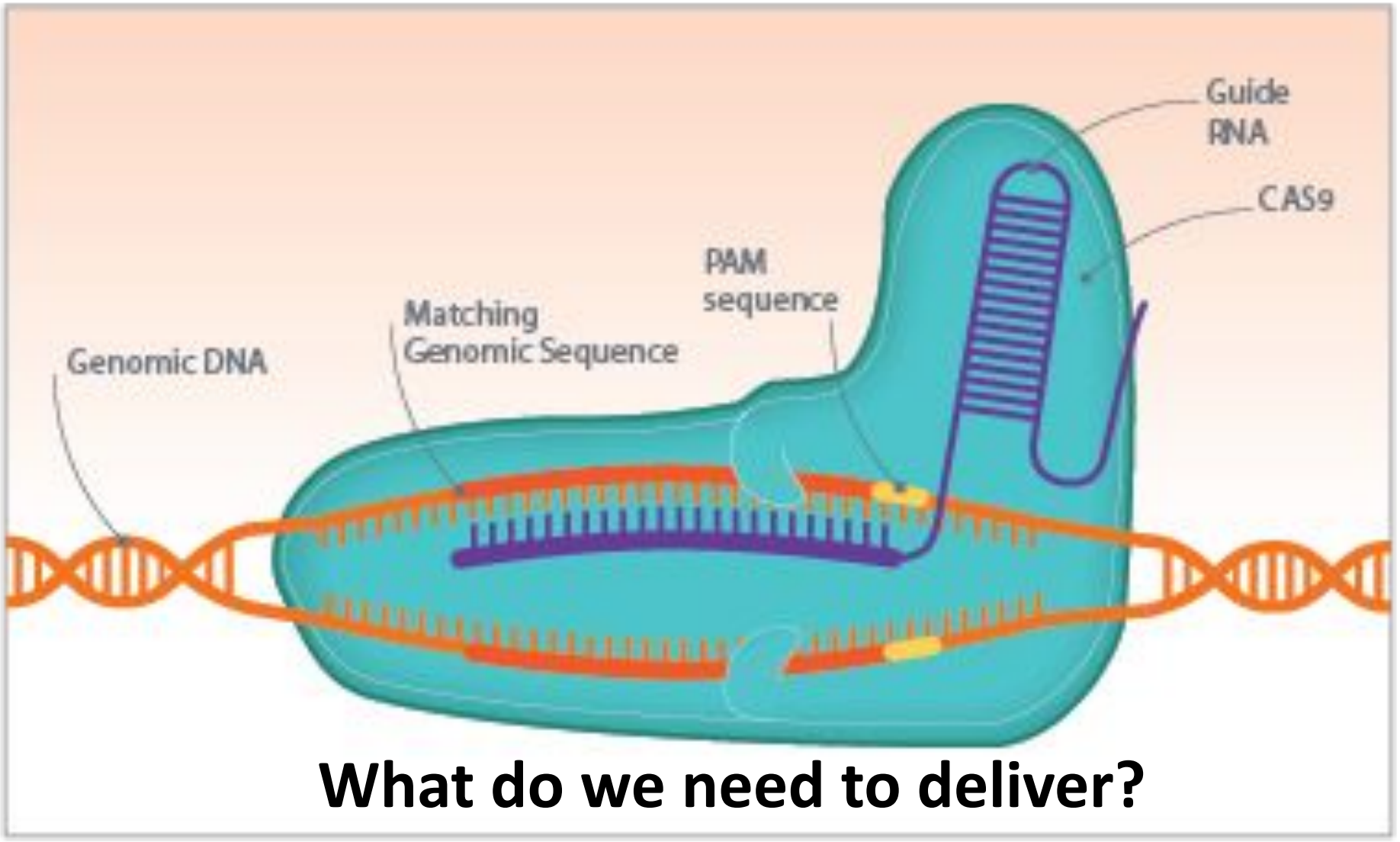
Dry Powder mRNA for Lung Delivery

Gordon, A., et al. Advanced Healthcare Materials, 2024

Can we make drugs that repair your DNA while you are still using it?

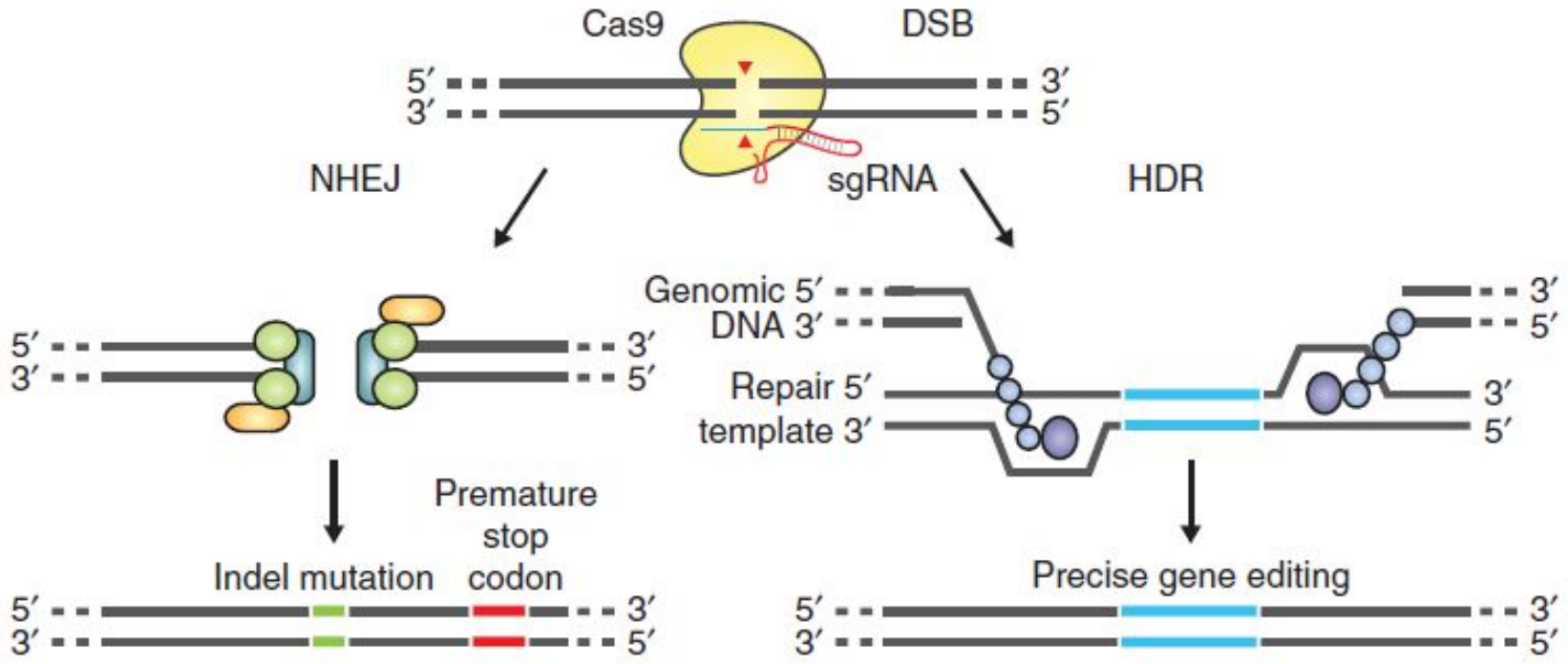


Can we build a CRISPR Nanoparticle?



What do we need to deliver?

Can We build a Cas9-Nanoparticle that Permanently Turns Genes Off, **in vivo**?



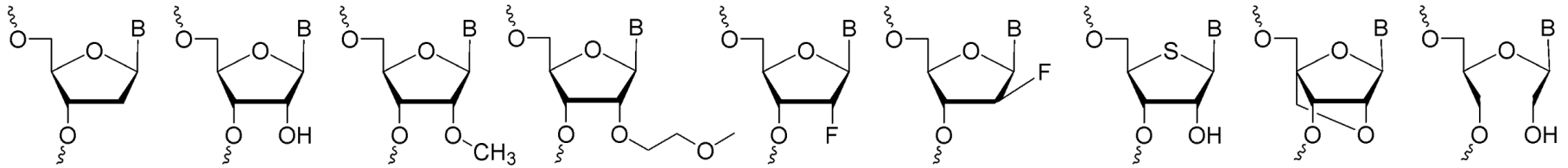
NATURE PROTOCOLS | VOL.8 NO.11 | 2013 | 2281

- Inside of nucleus of target cells we need:

-Protein (Cas9) -- mRNA encoding Cas9
-RNA (Guide RNA) -- ? ?

Chemically Modified RNA for improved therapeutic delivery

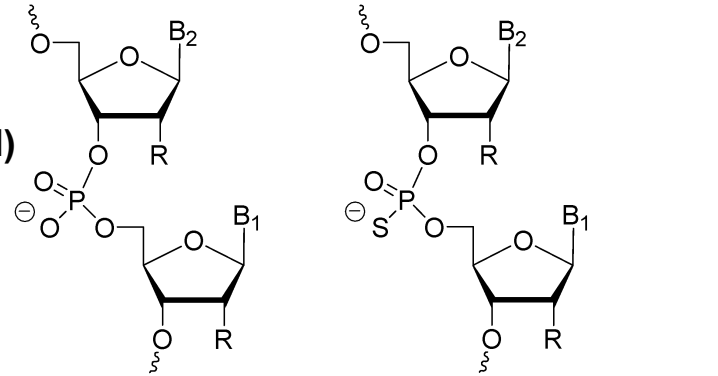
Sugar



2'-Deoxy | Ribo | 2'-*O*-Me | 2'-*O*-MOE | 2'-F | 2'-*ara*F (FANA) | 4'-Thio | LNA | UNA

Backbone

(R = H, OH or 2'-modified)

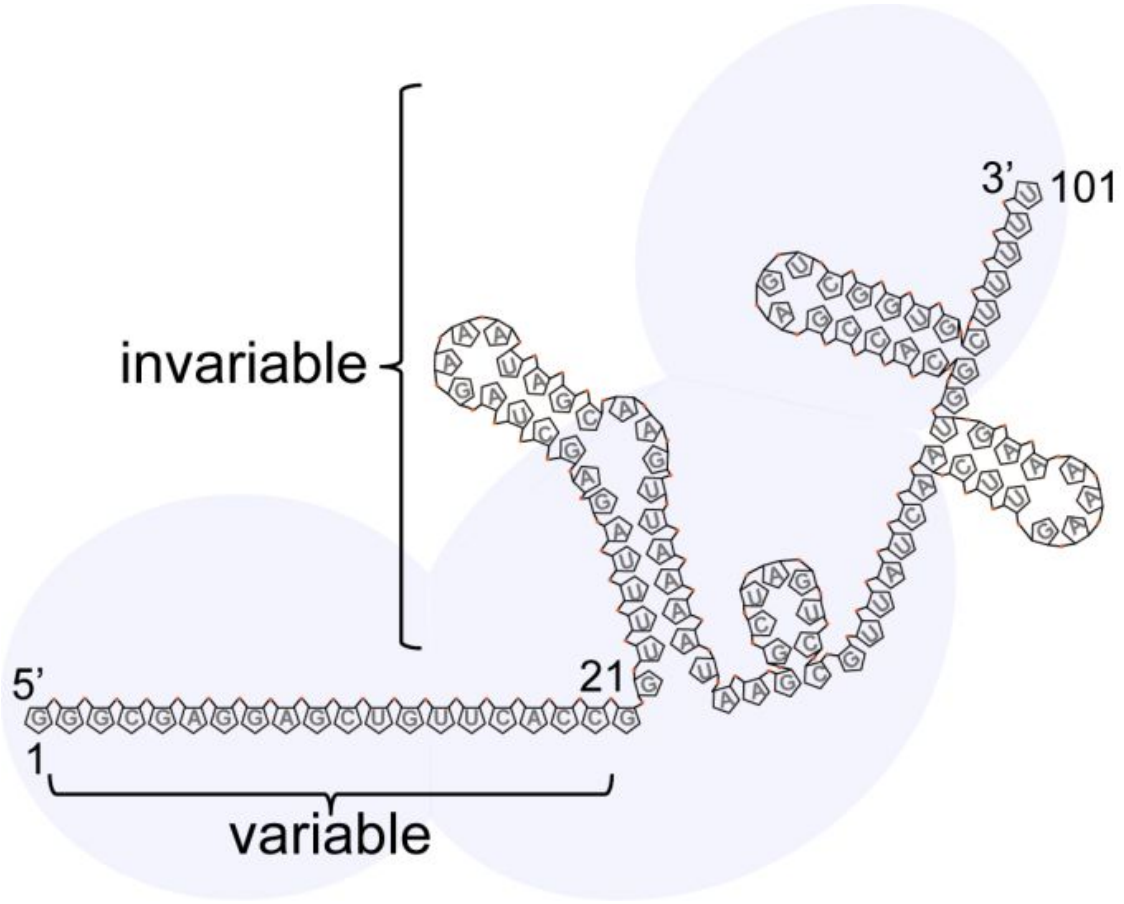


Phosphate (P=O)

Phosphorothioate (P=S or PTO)

Nature Chemical Biology **2, 211, 2006**

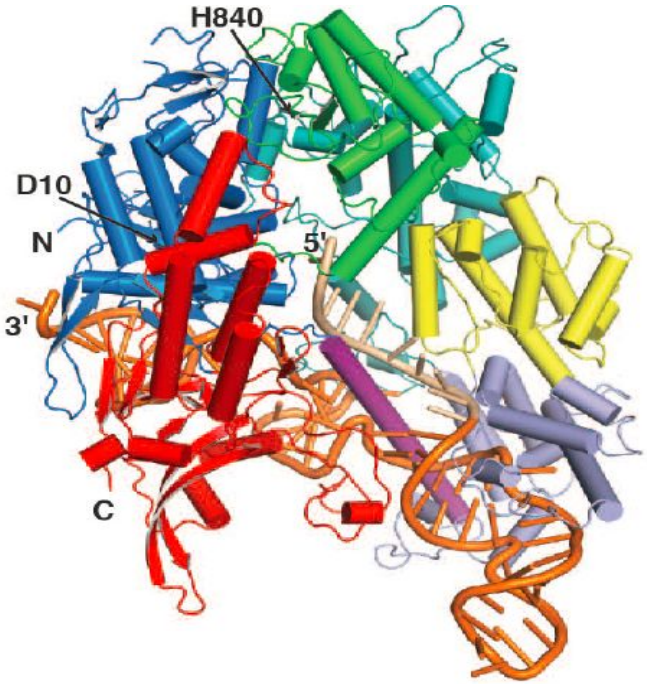
Can Chemical Modification of Guide RNA improve Genome Editing *in vivo*?



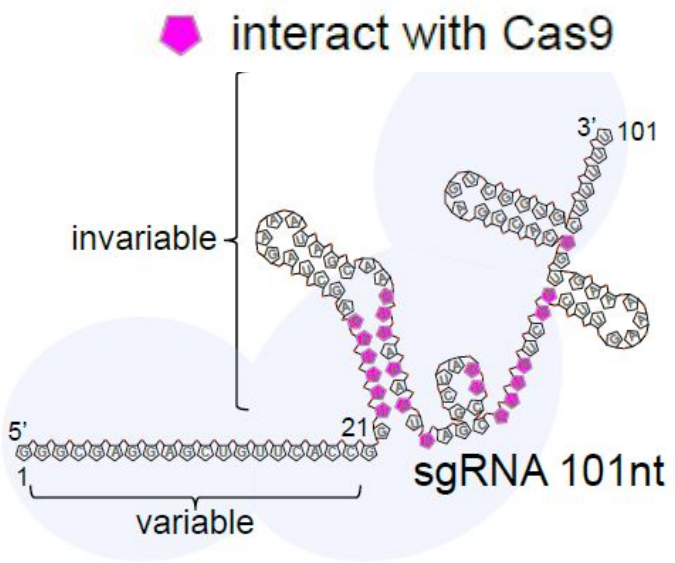
Problem– too many possibilities:

Structure-guided Chemical Modification of Guide RNA

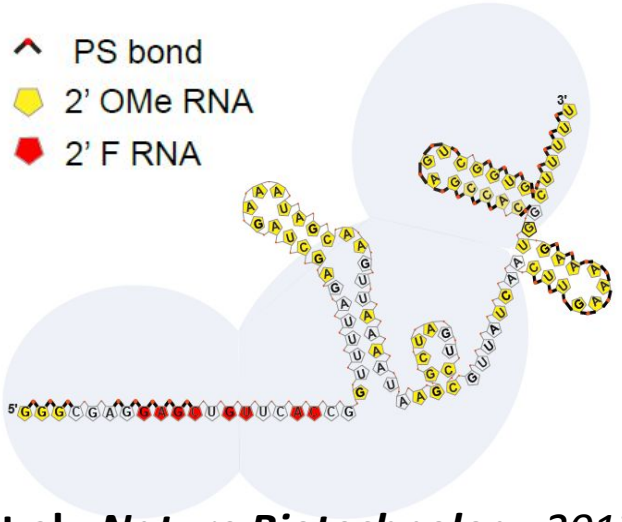
Cas9-sgRNA complex



Doudna lab *Science* 2015; Zhang lab *Cell 2014*

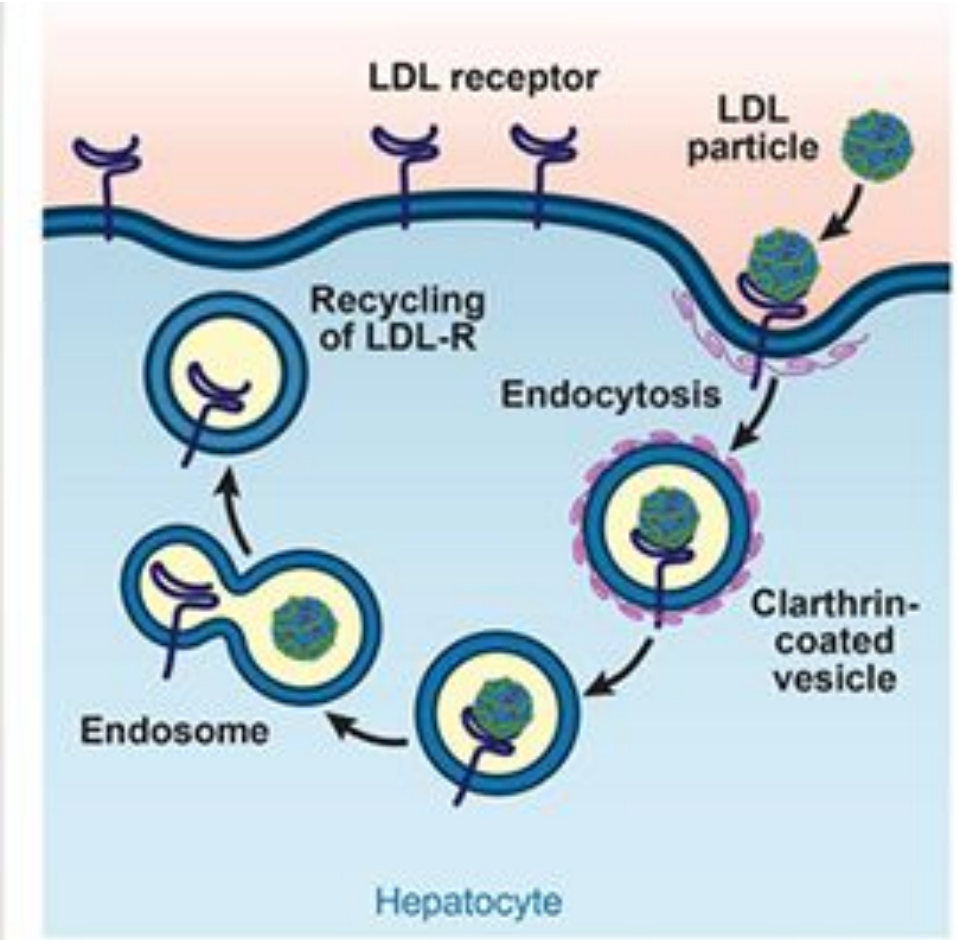


enhanced sgRNA (e-sgRNA)

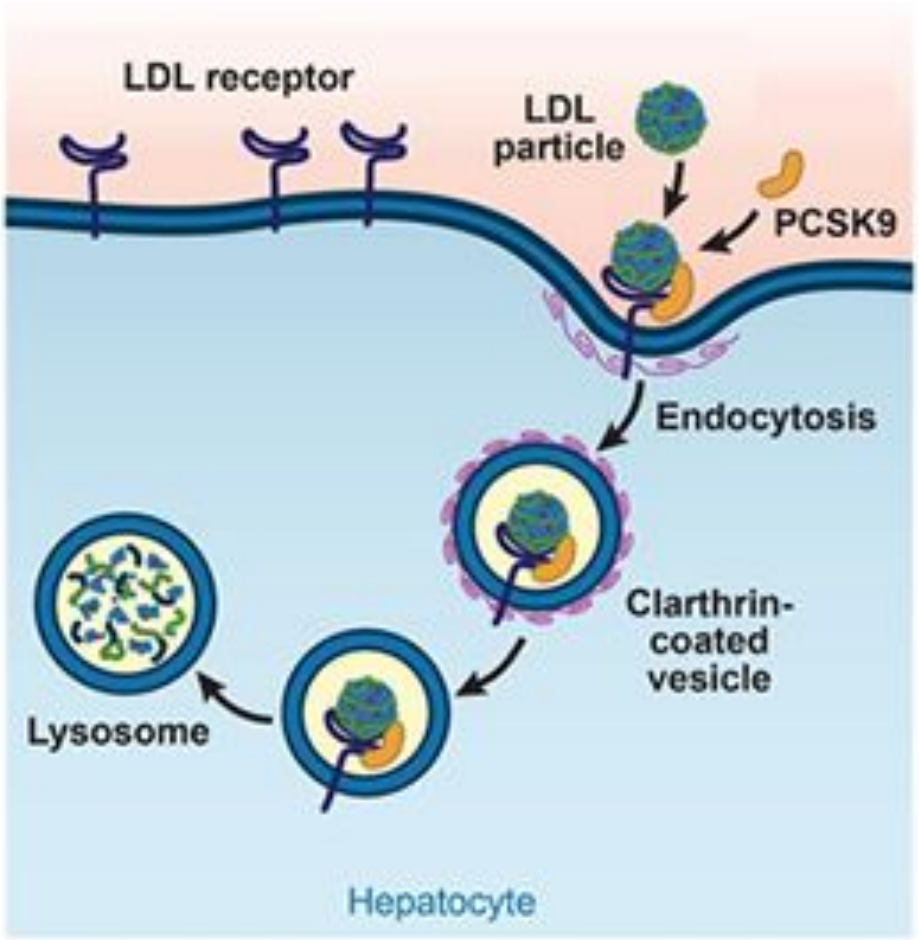


Yin et al., *Nature Biotechnology 2017*

Can A CRISPR PCSK9 nanoformulation Permanently treat hyper-cholesterolemia?



LDL degradation and recycling of LDLR

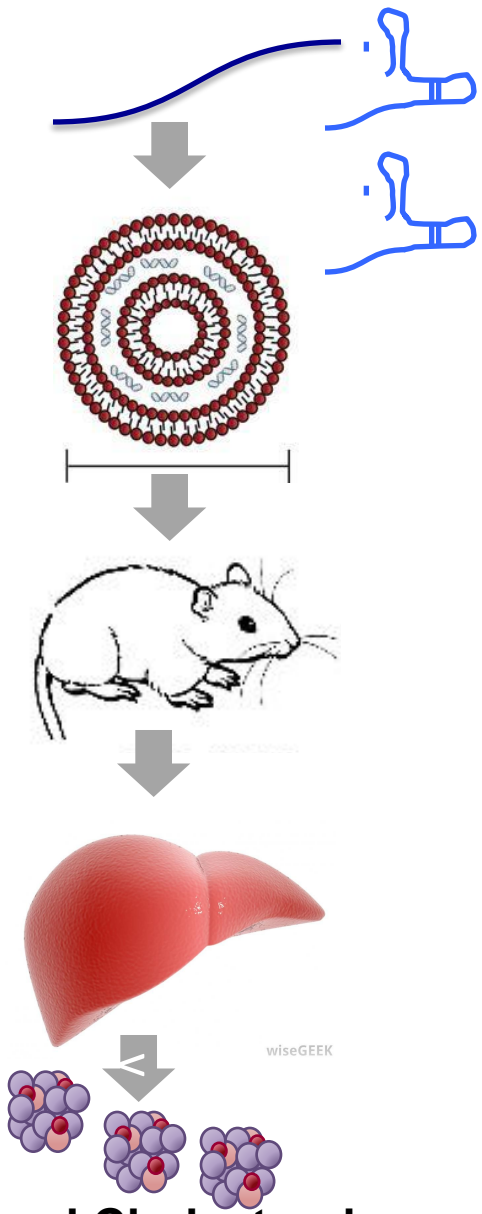


PCSK9-mediated degradation of LDLR

Lambert G, et al. *J. Lipid Res.* 2012;53:2515-2524.[6]

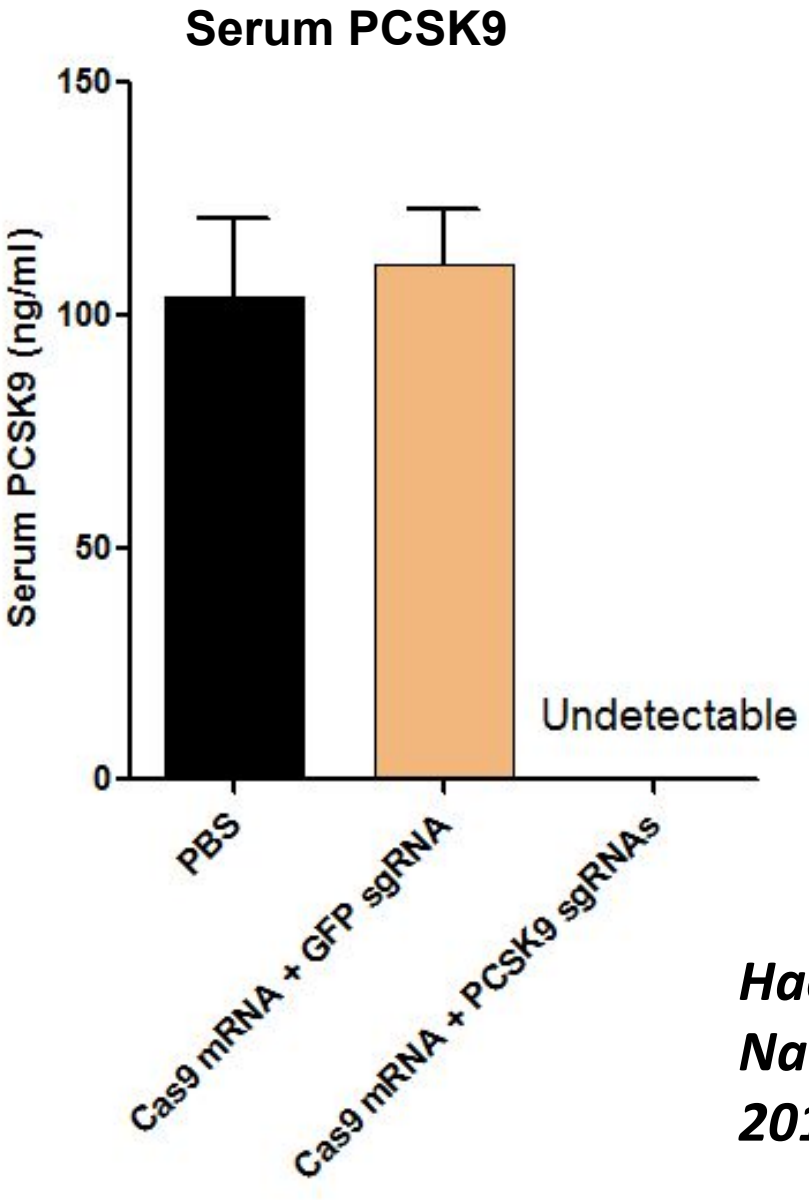
In vivo CRISPR Genome Editing

mRNA Encoding Cas 9+
2 modified synthetic guides



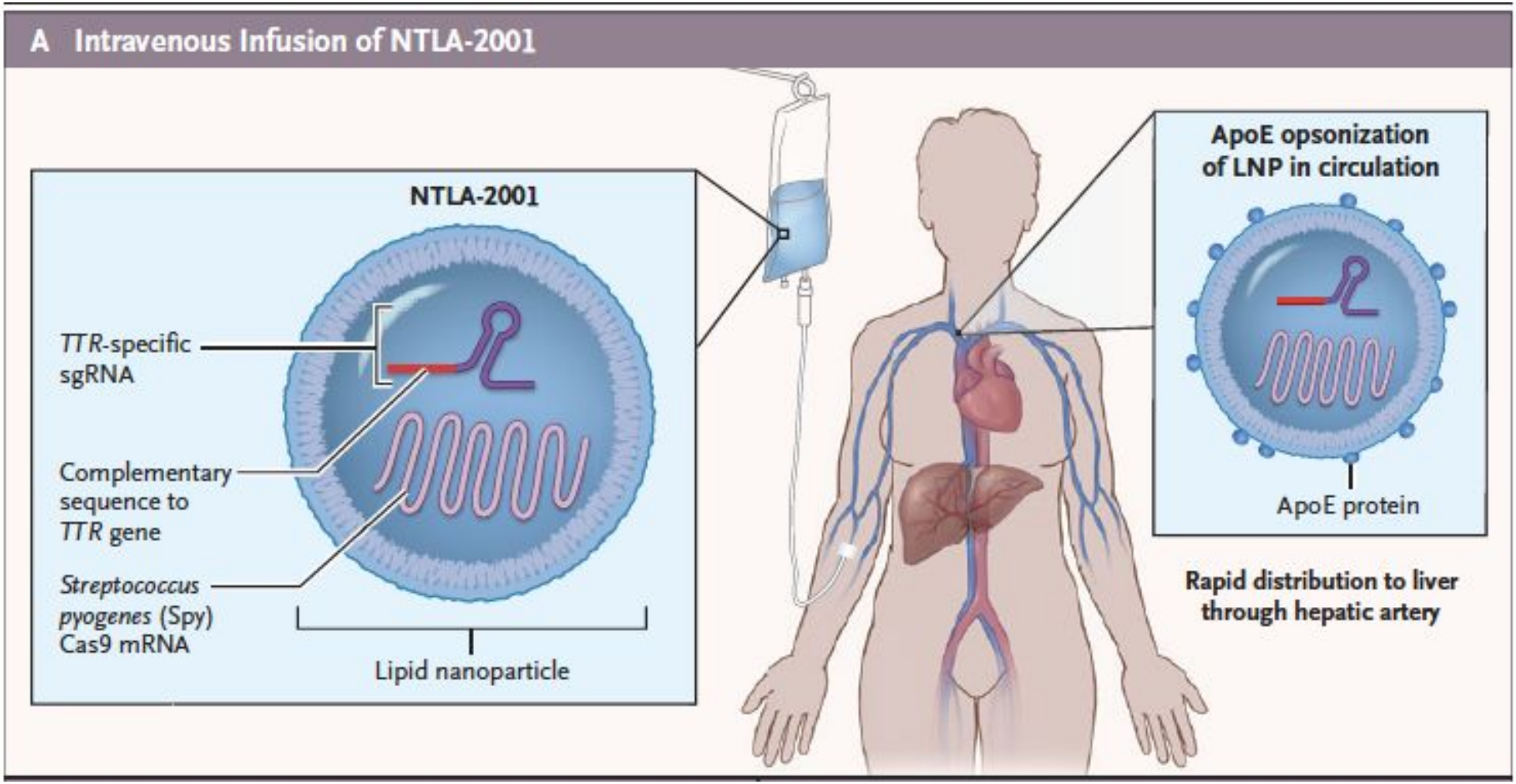
Blood Cholesterol
Liver PCSK9 Analysis

>80% of all liver PCSK9 genes inactivate



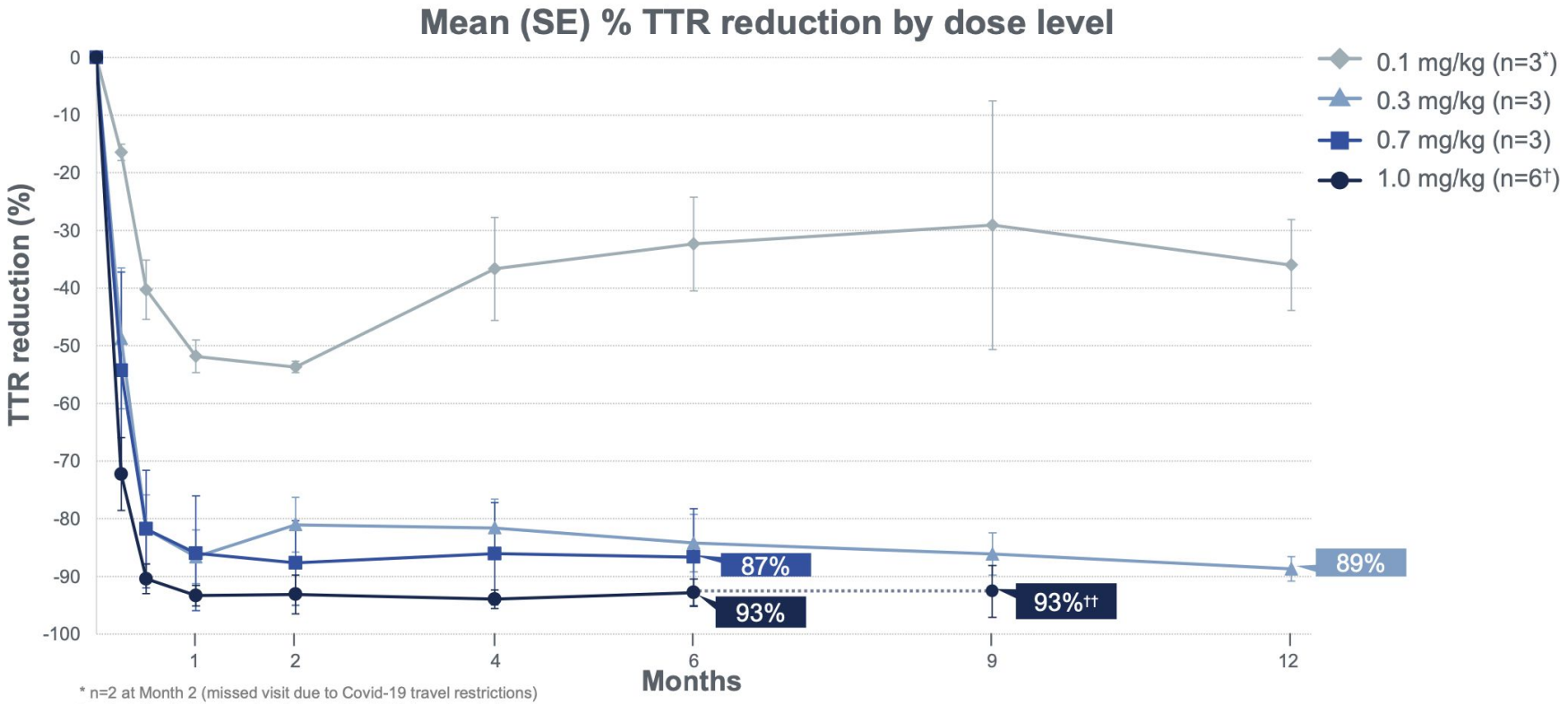
Hao Yin, et al.,
Nature Biotechnology
2017

Can a Editing Nanoparticle Permanently lower TTR?



DOI: 10.1056/NEJMoa2107454

ATTRv-PN Arm: Dose-Responsive Rapid and Deep Serum TTR Reduction Sustained Through 6-12 Months



* n=2 at Month 2 (missed visit due to Covid-19 travel restrictions)
† n=5 at Month 2 (missed visit due to Covid-19 travel restrictions)
†† n=3 have reached Month 9 follow-up
Data disclosed: June 24, 2022

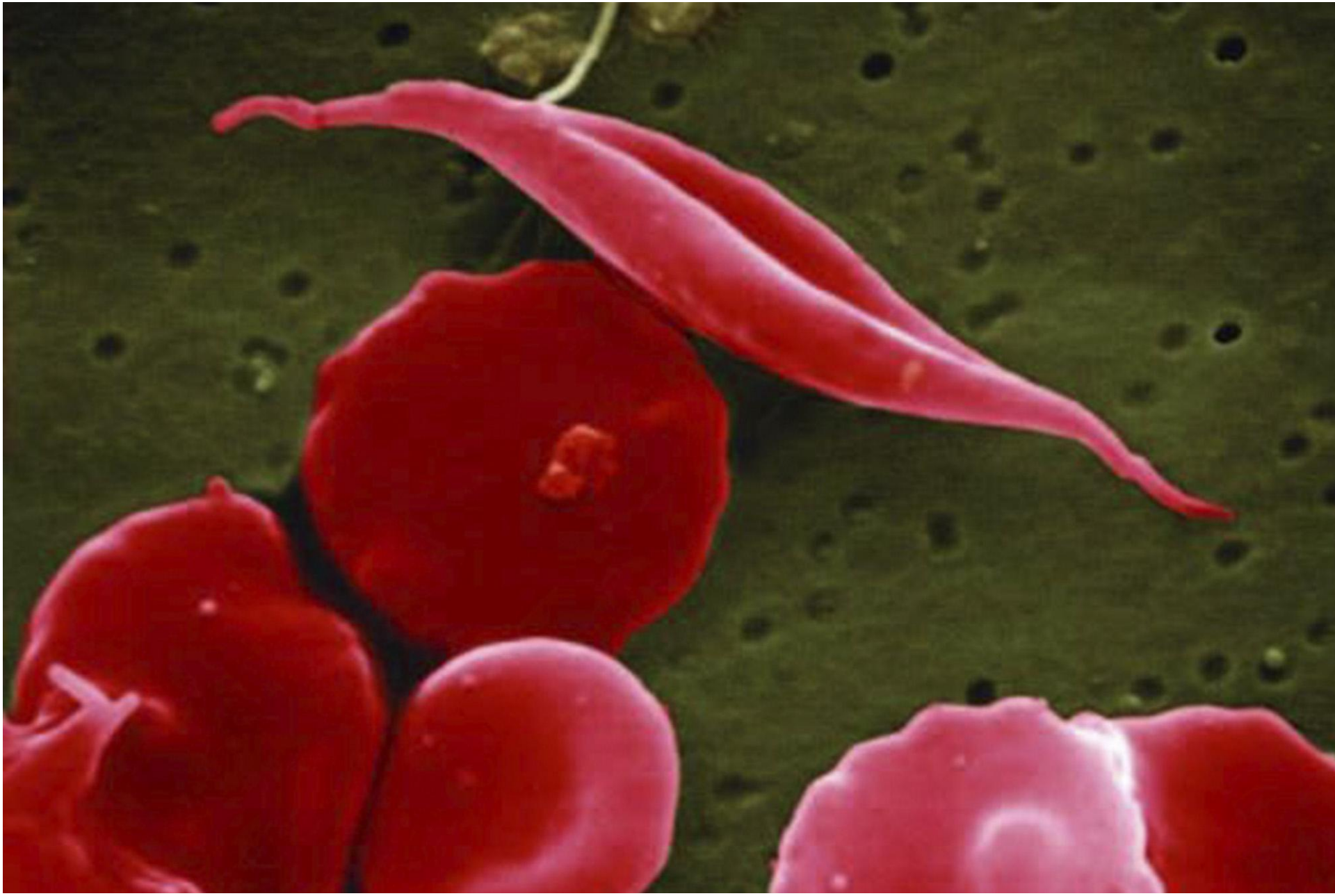
26 This slide includes data for investigational products not yet approved by regulatory authorities.
SE: standard error; **TTR**: transthyretin; **ATTRv-PN**: hereditary ATTR amyloidosis with polyneuropathy

REGENERON Intellia THERAPEUTICS

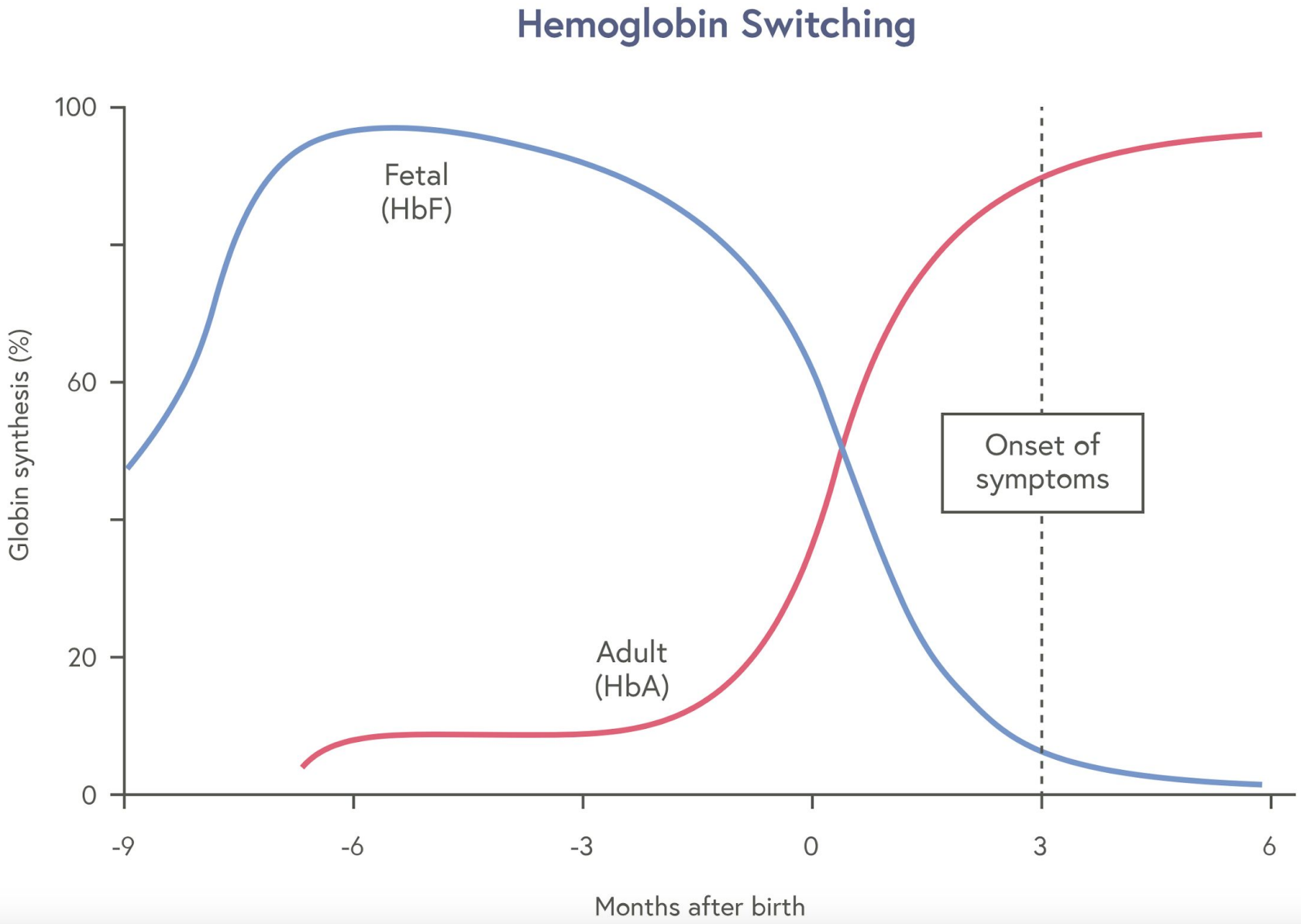
DOI: 10.1056/NEJMoa2107454

How else might in vivo genome editing help patients?

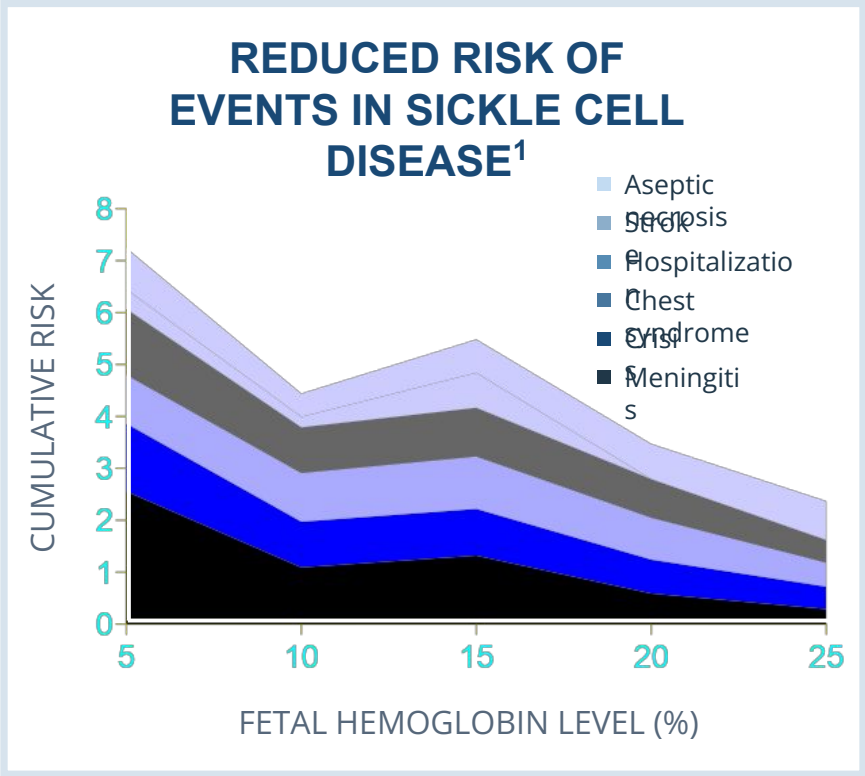
SICKLE CELL DISEASE

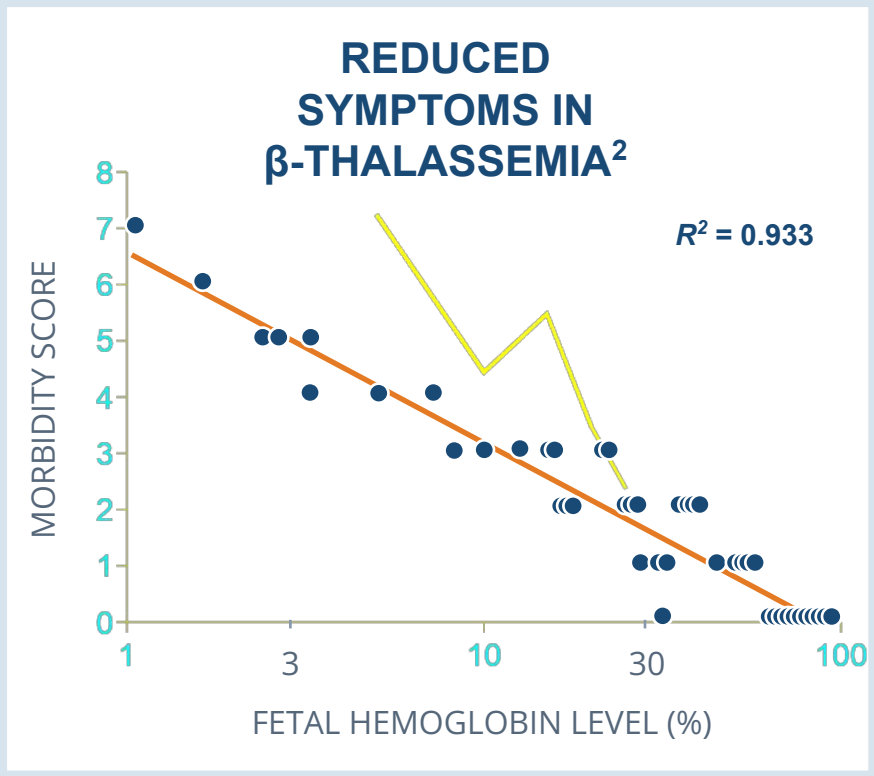


Fetal Hemoglobin is replaced with Adult Hemoglobin



Human Genetic Variants with higher Fetal Hemoglobin reduce or eliminate disease

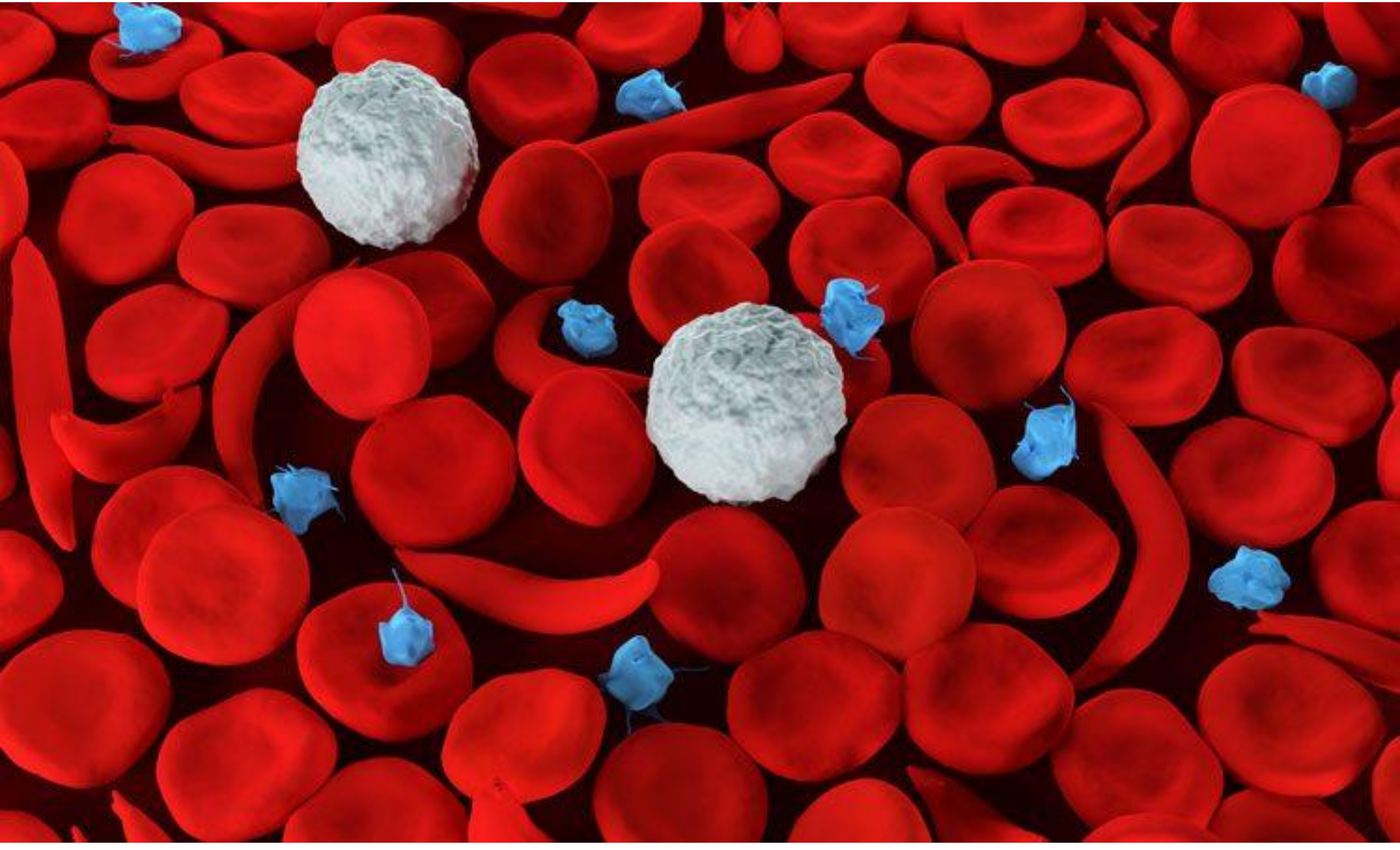




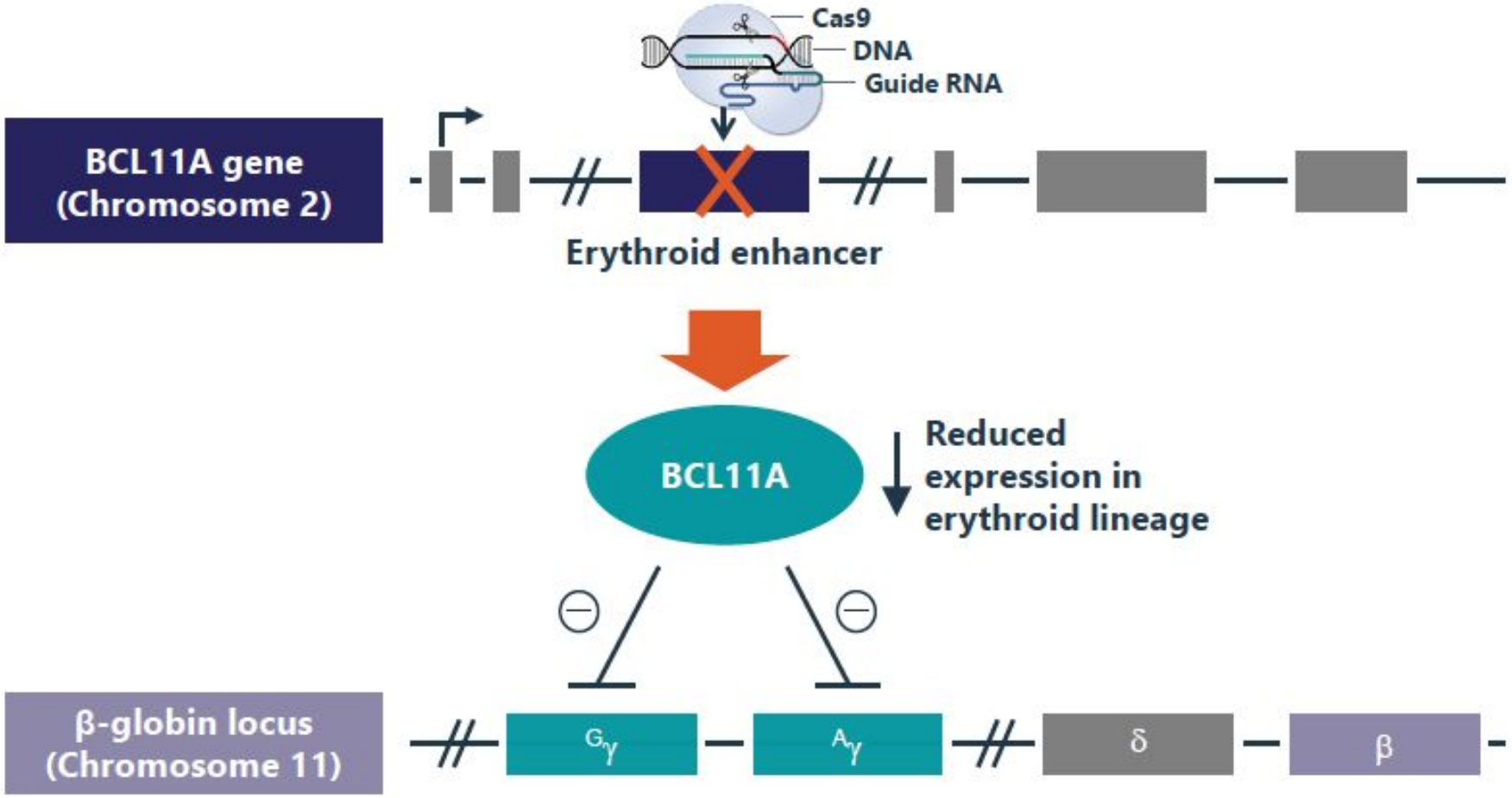
Can Non-Viral Genome Editing Upregulate Fetal Hemoglobin?

1. Powars, *et al*. Blood 1984; 2. Musallam, *et al*. Blood 2012

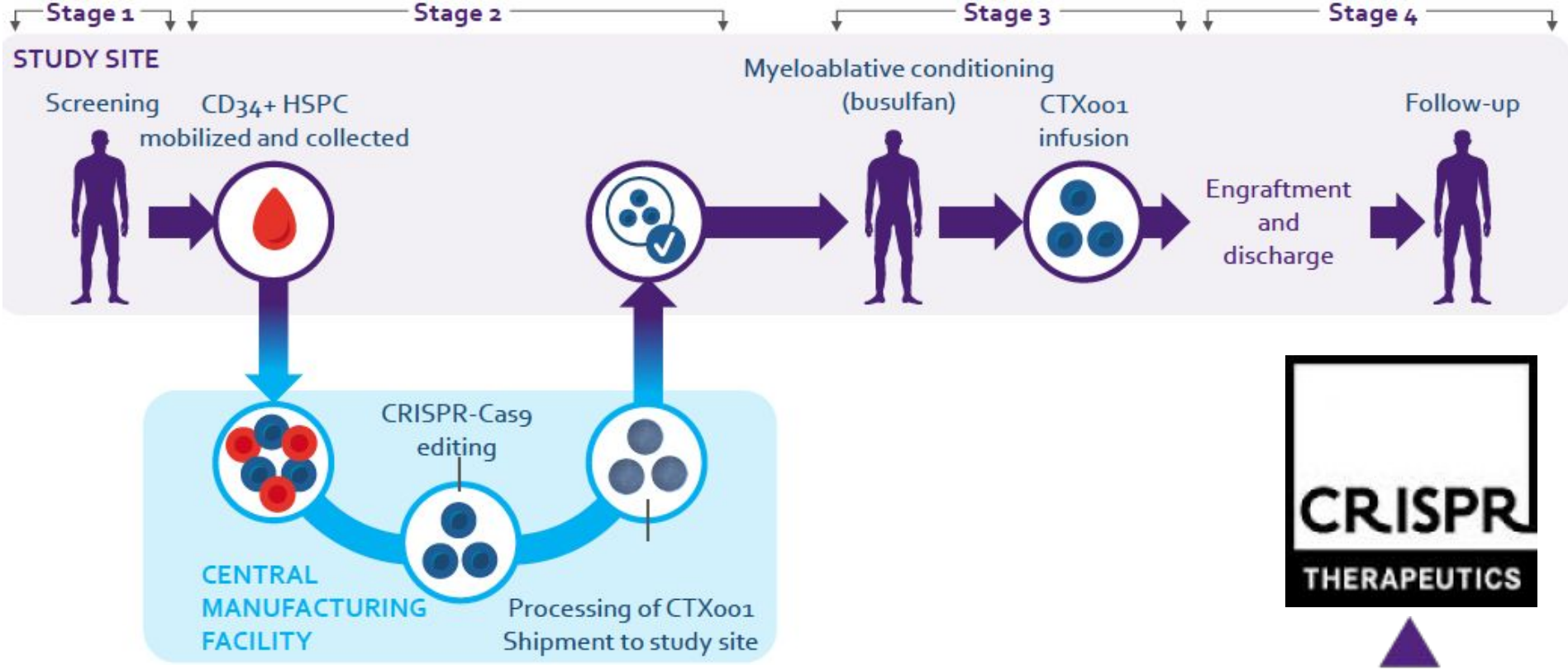
Target: Bone Marrow Hematopoietic Stem Cells



Ex Vivo delivery of Cas9/Guide to CD34+ HSPC's can specifically inactivate BCL11A and induce upregulation of Fetal Hemoglobin



Genome-Edited Stem Cell Therapy for Sickle Cell Anemia



CRISPR THERAPEUTICS

VERTEX

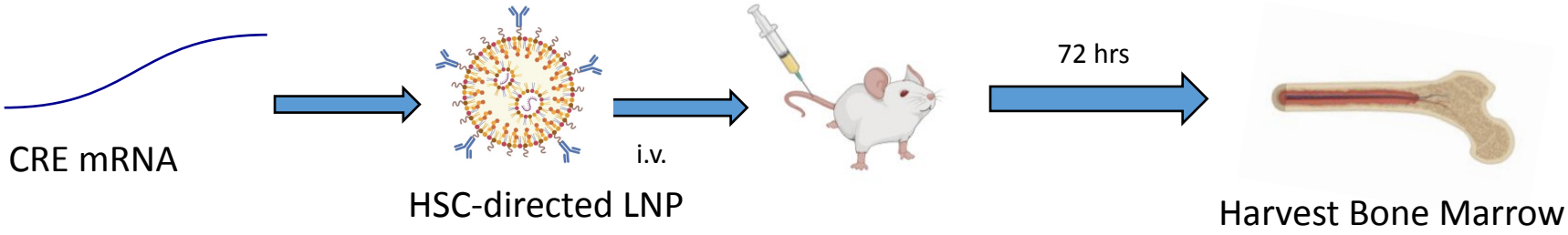
Dec 2023 – First FDA Approved CRISPR Therapy

100% of people evaluated (30 out of 30 people) were not hospitalized for a severe VOC for 12 months in a row after receiving CASGEVY†

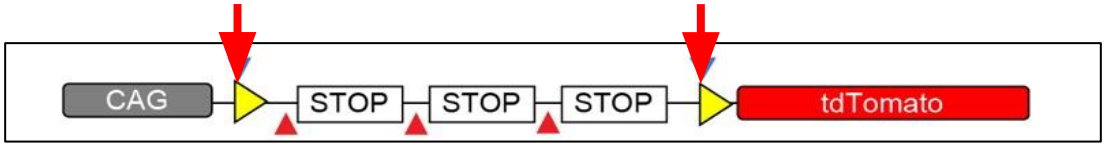
casgevy™ (exagamglogene autotemcel) suspension for IV infusion

Can RNA Nanoparticles Edit your Genome In vivo?

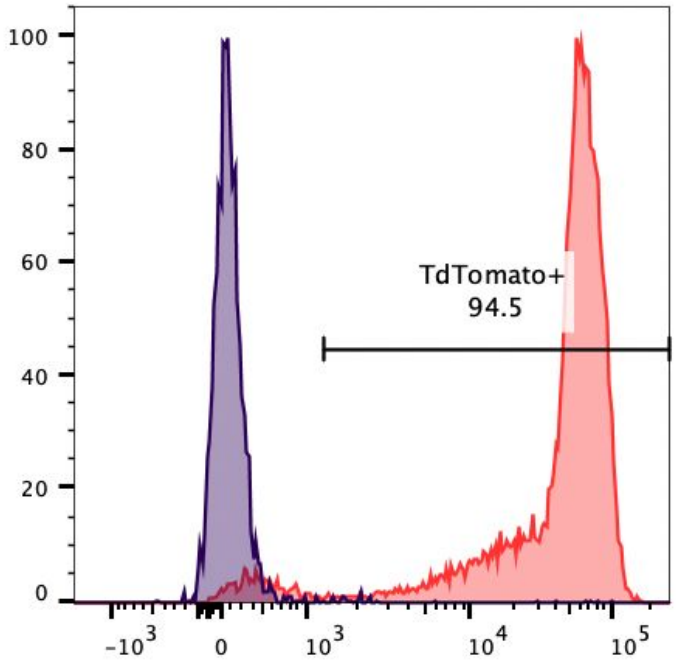
CD117-Targeted mRNA delivery to HSC's in vivo



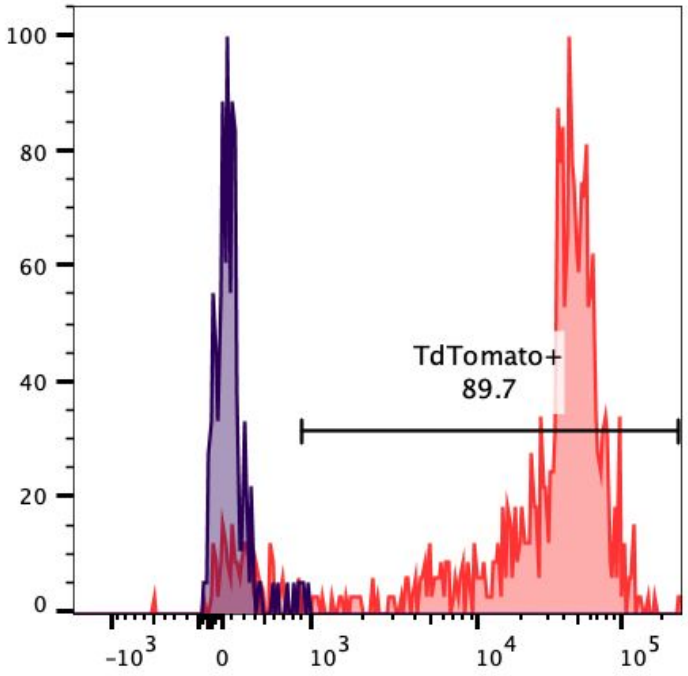
Ai9 mouse



HSPC: Lin- Sca1+ cKit+

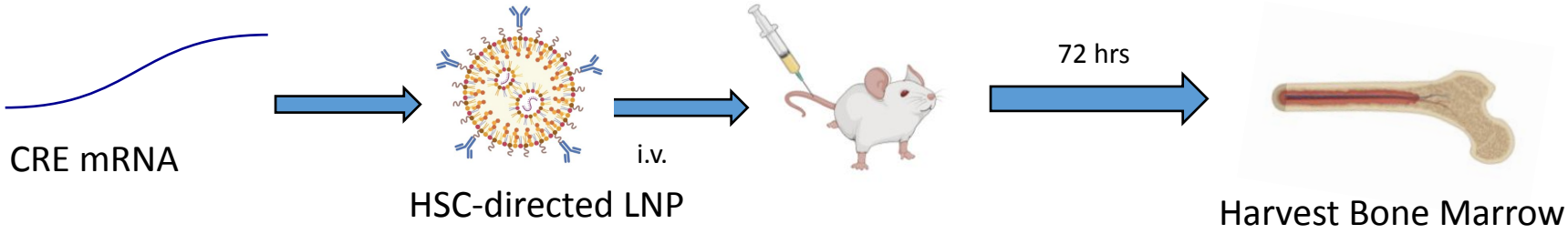


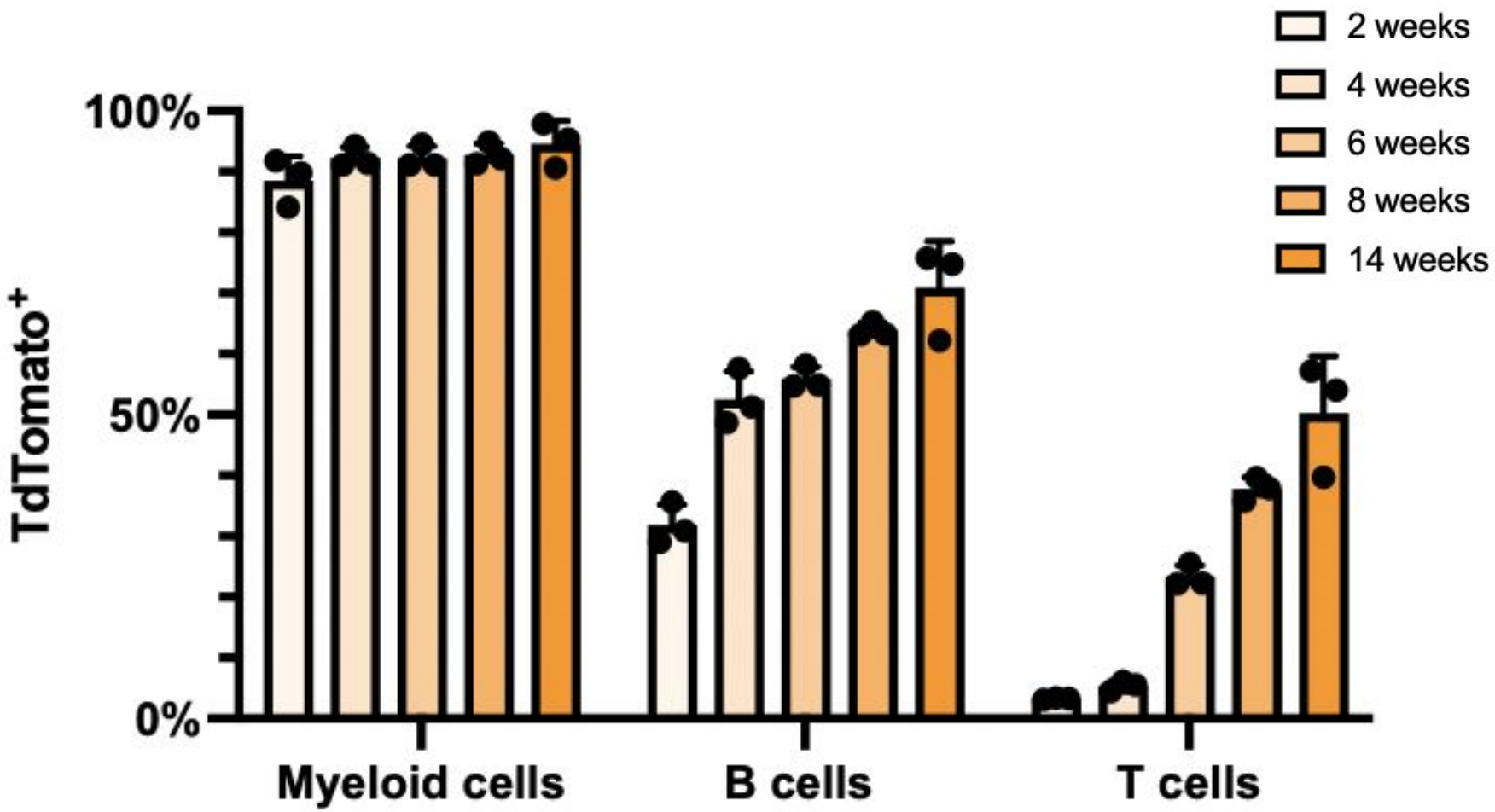
LT-HSC: Lin$^-$ Sca1$^+$ cKit$^+$ CD34$^-$ Flk2$^-$



Shi, D. et al., Nanoletters 2023

Delivery of Cre mRNA with CD117-LNP results in TdTomato+ cells

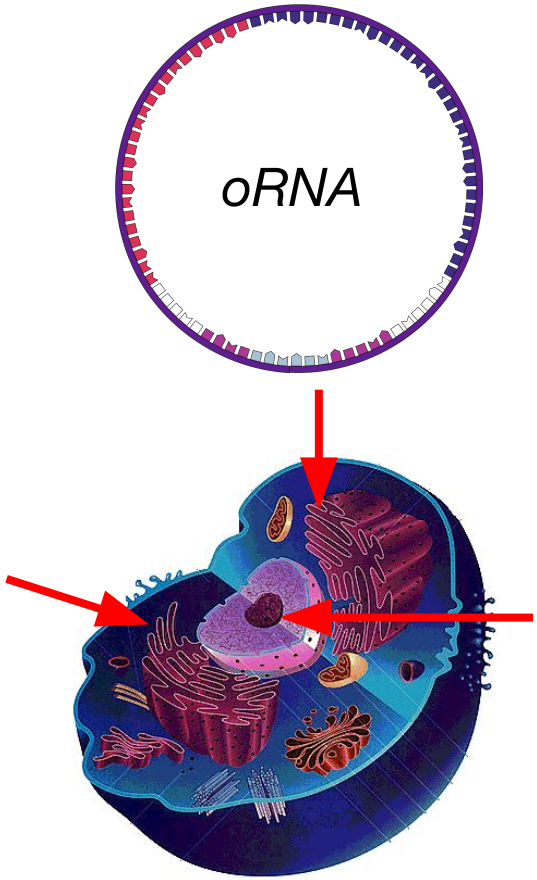


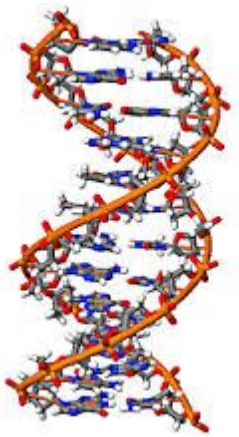


Shi, D. et al., Nanoletters 2023

Intracellular Delivery of Nucleic Acids
will Revolutionize Medicine

mRNA

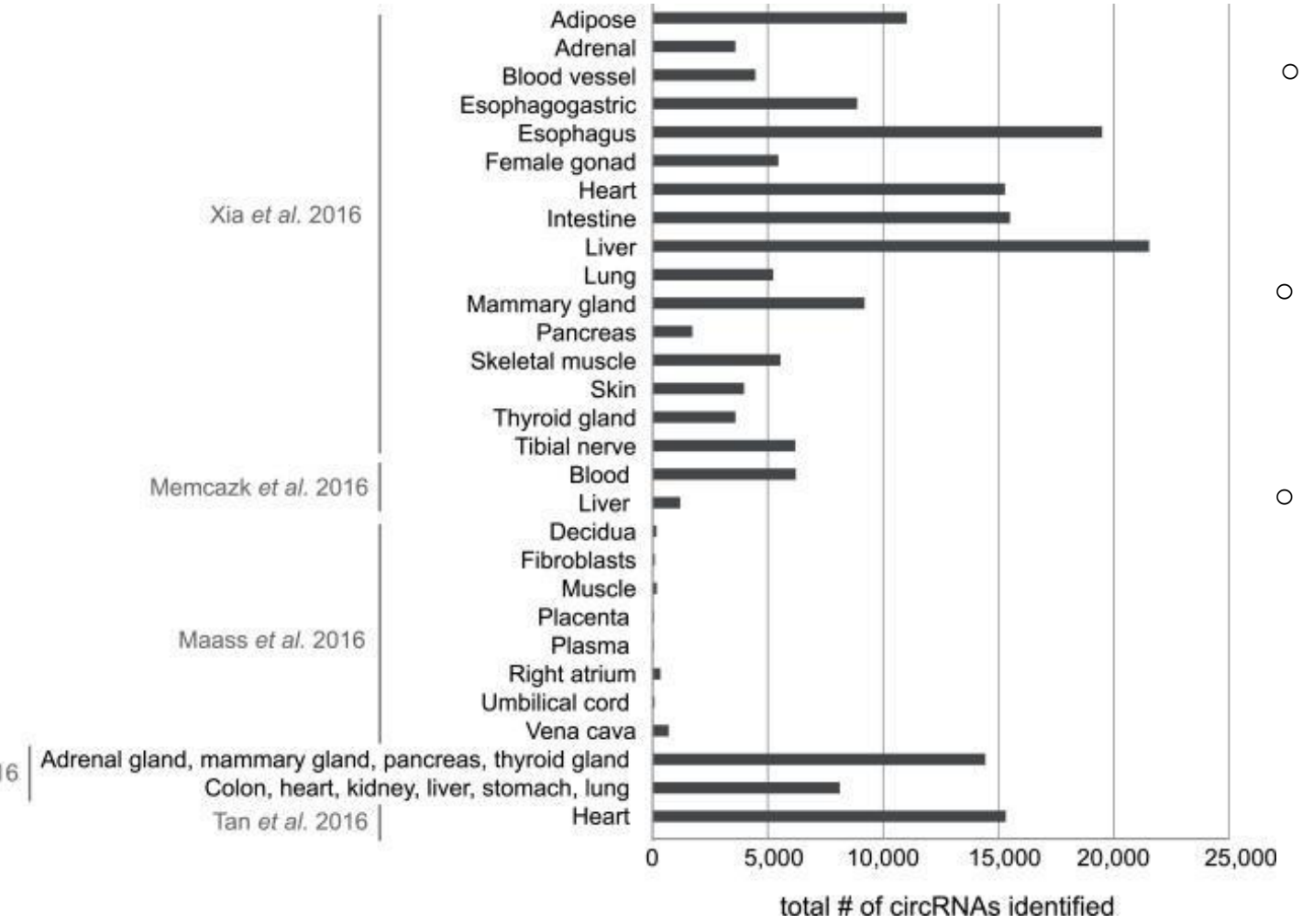




DNA

Many promising forms – what about circles?

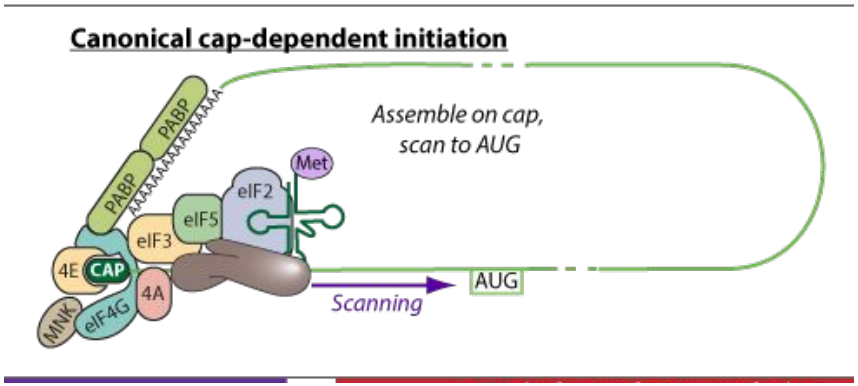
Circular RNA's are present in human cells



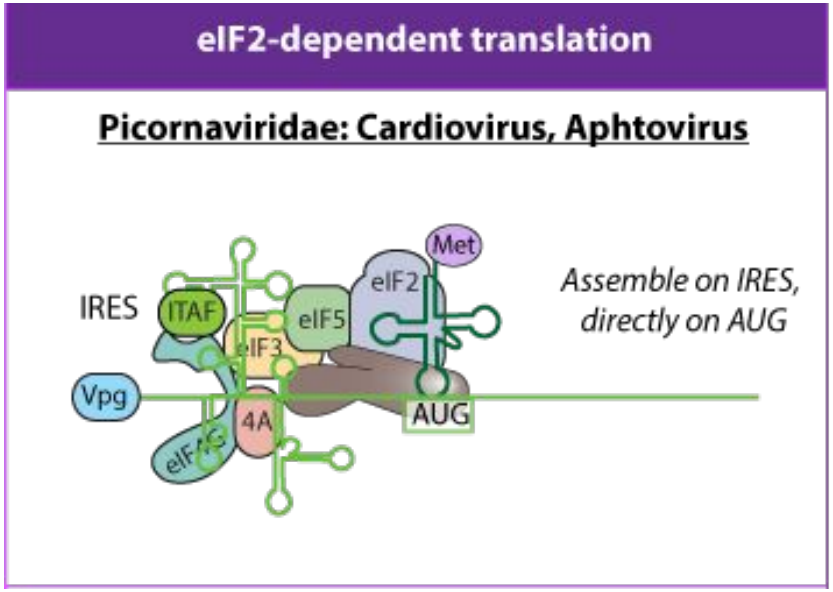
- Circular RNAs have been found at varying levels in a broad range of mammalian tissues
- Exon backsplicing is the most common known source of circular RNA in mammalian tissues
- Natural circular RNAs have hypothesized functions including translation into peptides and microRNA sequestration

Sekar and Liang, 2019

Circular RNA is NOT mRNA

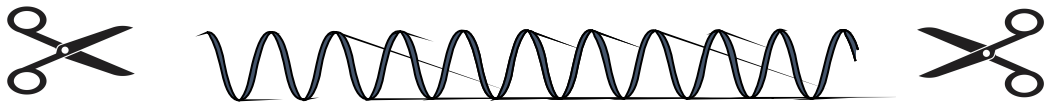


Viralzone

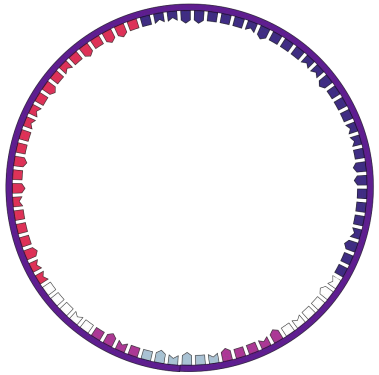


- oRNA is not susceptible to decapping
- mRNA is subject to exonuclease-mediated degradation, while oRNA is not

- Translatable oRNAs require an internal ribosome entry site (IRES) as they do not have a cap
- An IRES engages different host factors from a cap, and different IRESs engage different factors

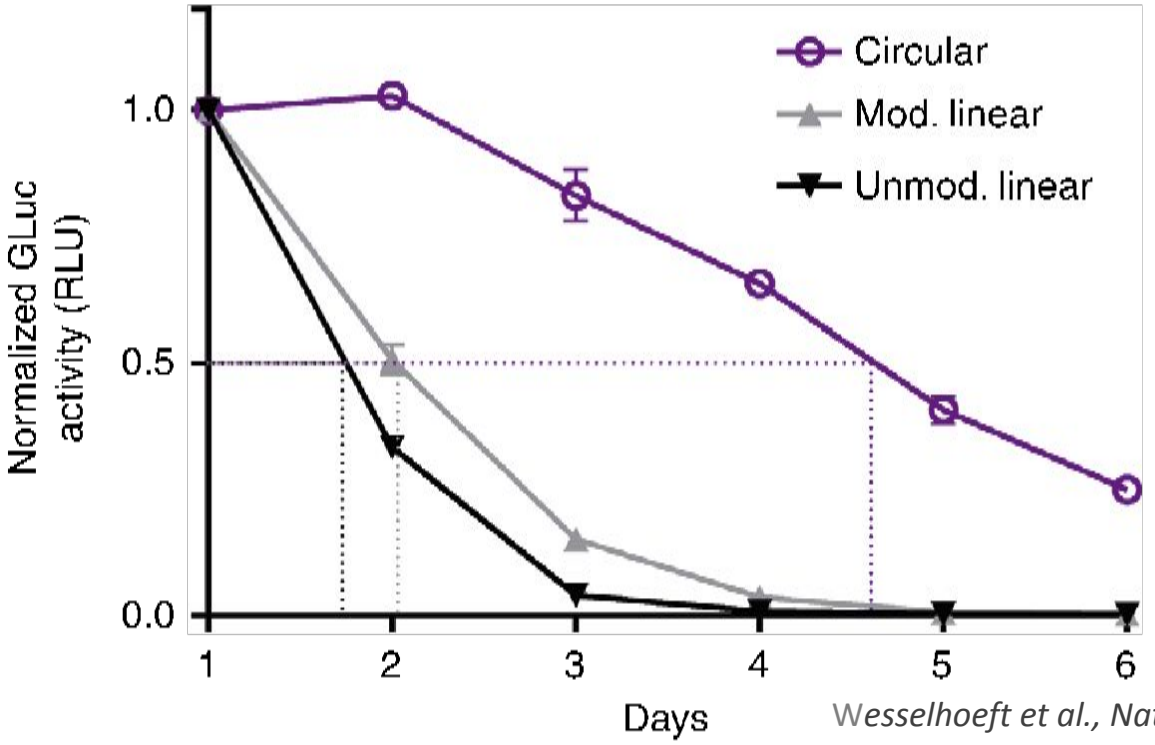


mRNA



oRNA

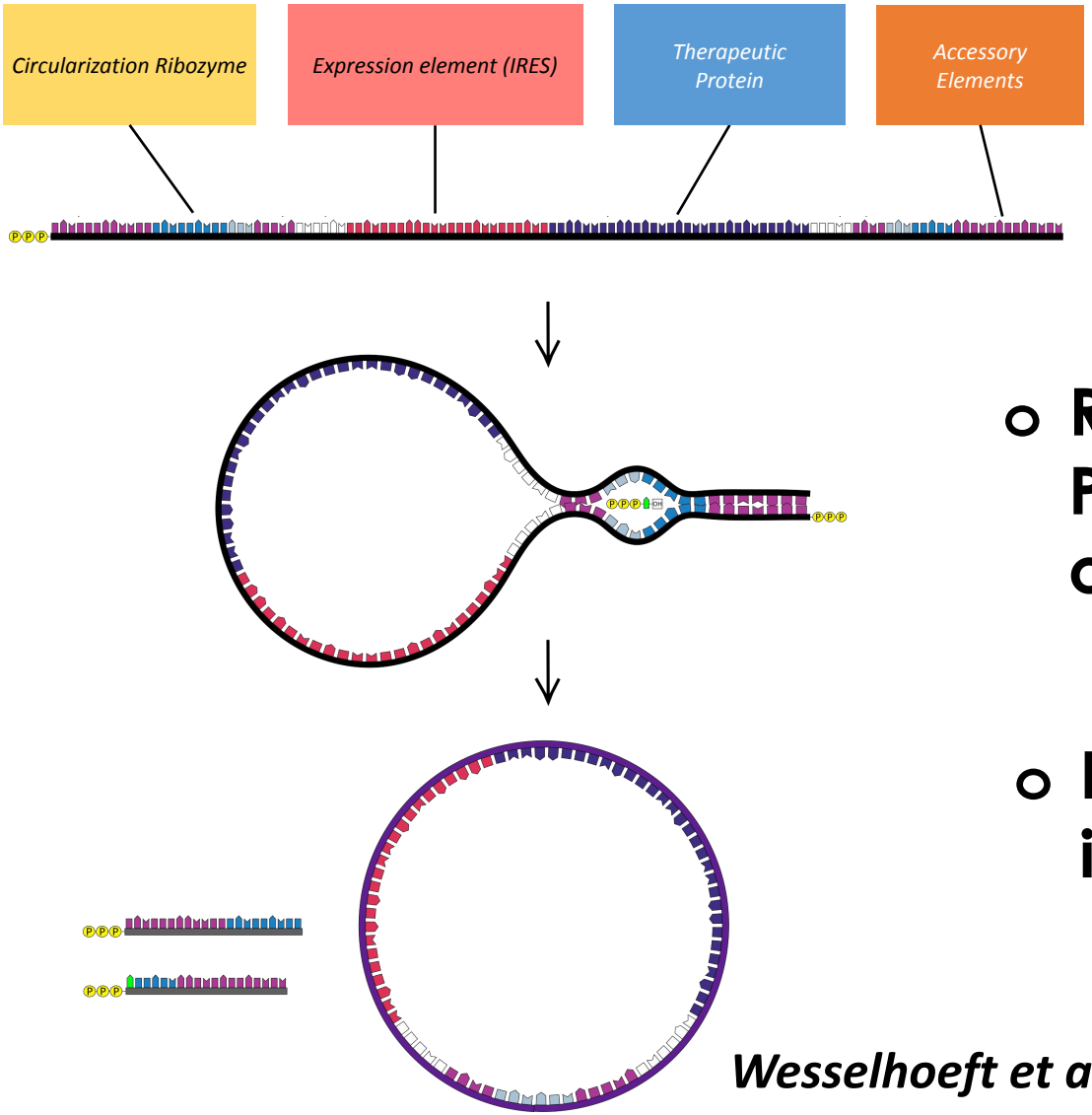
oRNA induces durable expression in vitro (Hela)



Wesselhoeft et al., Nature Communications, 2018;

Wesselhoeft et al Mol. Cell, 2019

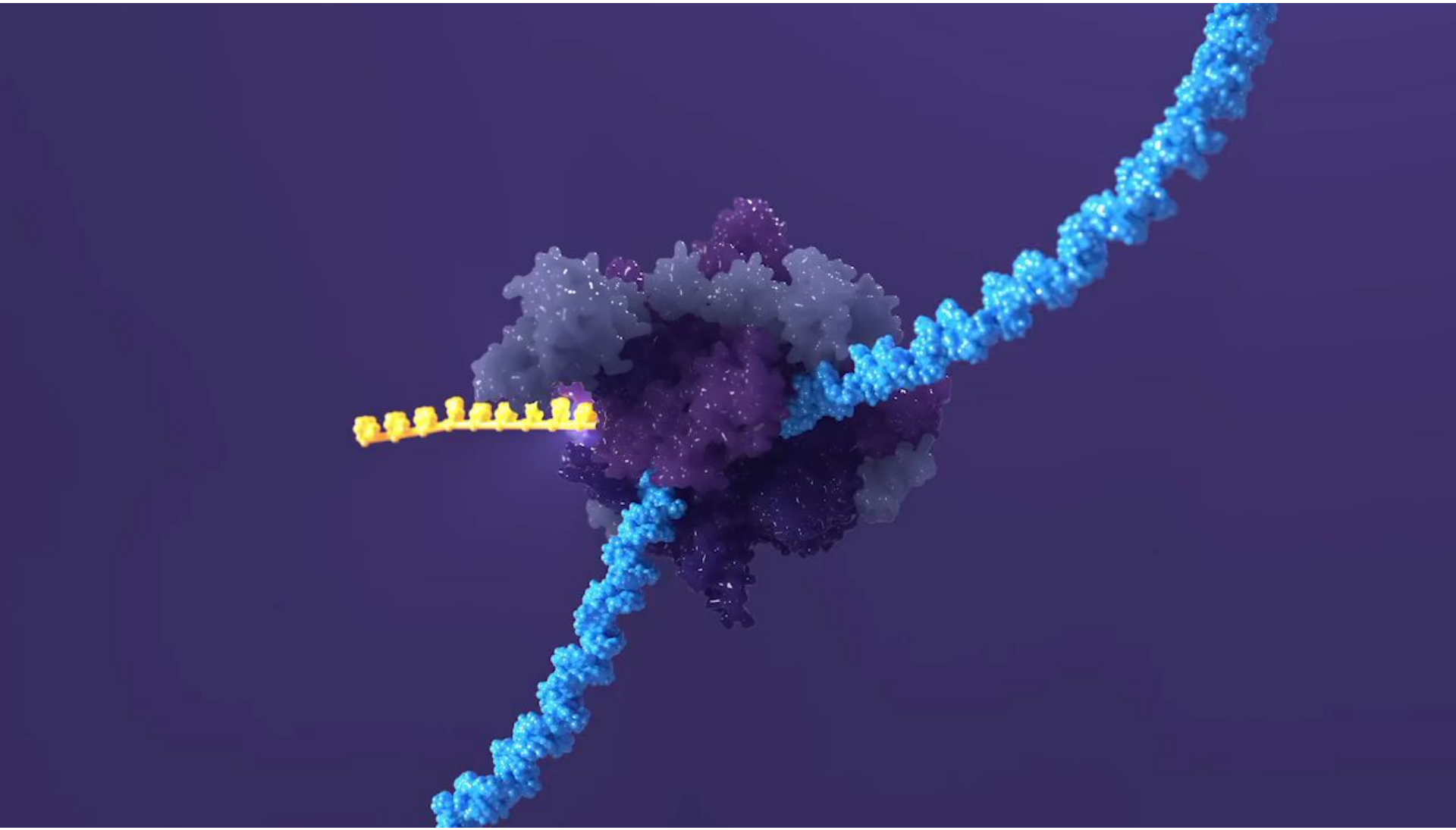
Rapid Production of synthetic circular RNA through self-splicing



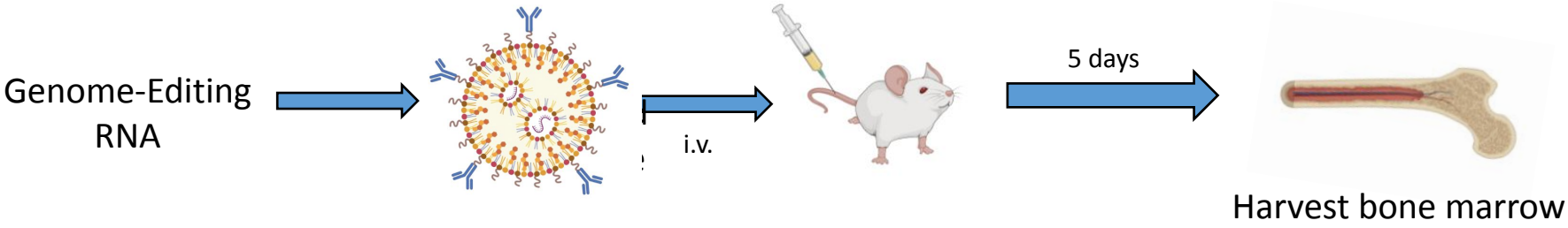
- Rapid and Efficient Production of RNA's over 10kb
- Purified oRNA is not immunostimulatory

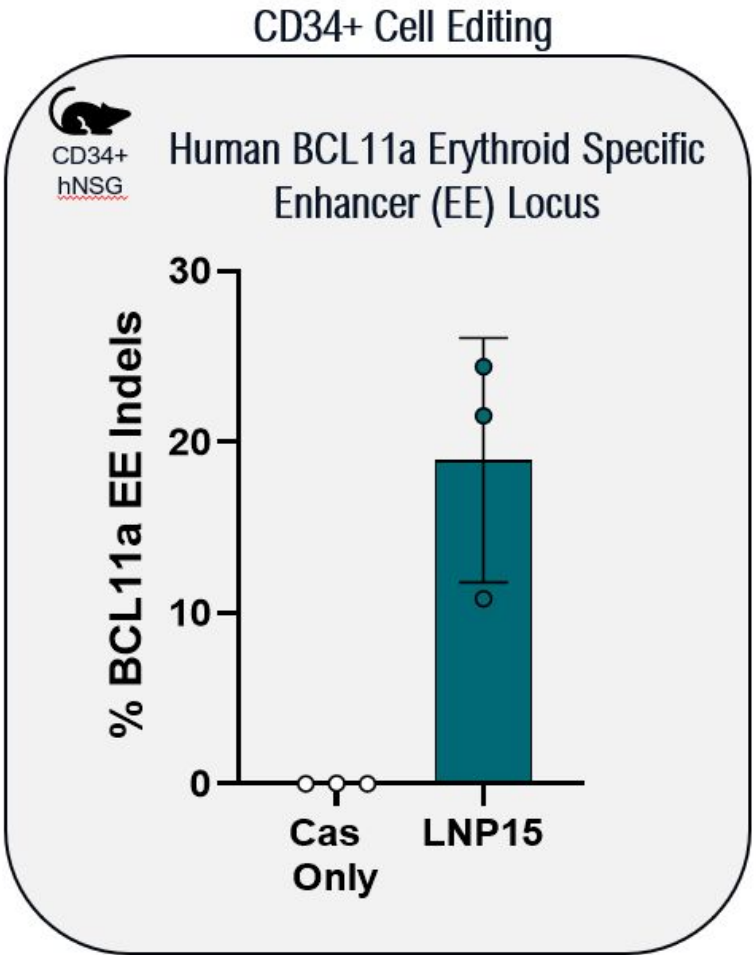
Wesselhoeft et al., Nature Communications, 2018;

Wesselhoeft et al Mol. Cell, 2019

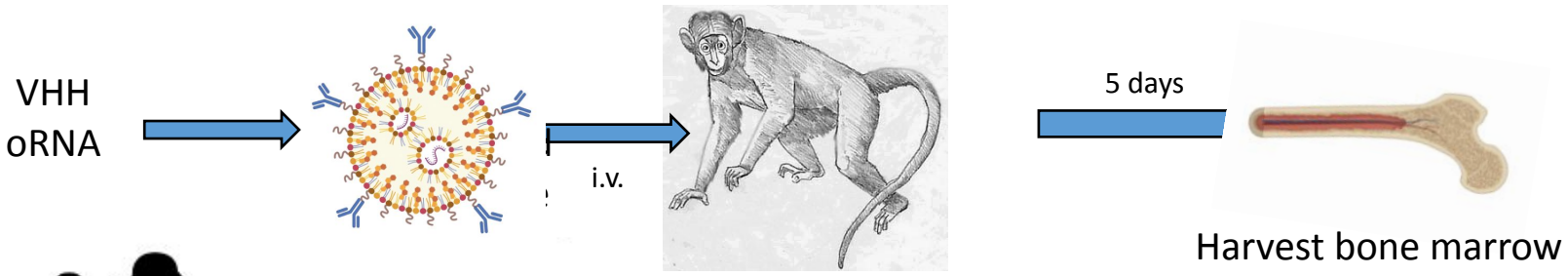


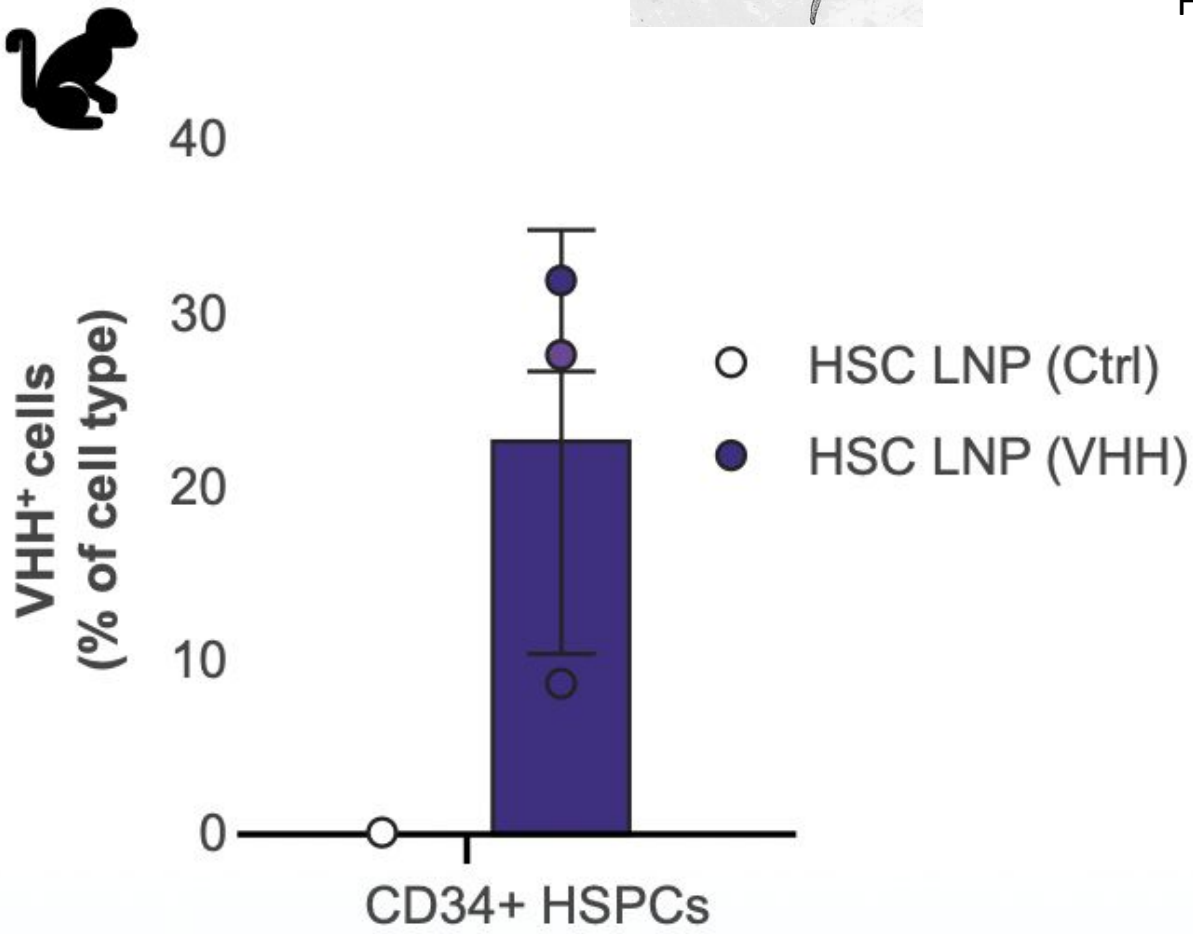
Targeted Genome Editing of Mice with Human Immune Systems,





Non Human Primate Delivery to HSPCs achieved



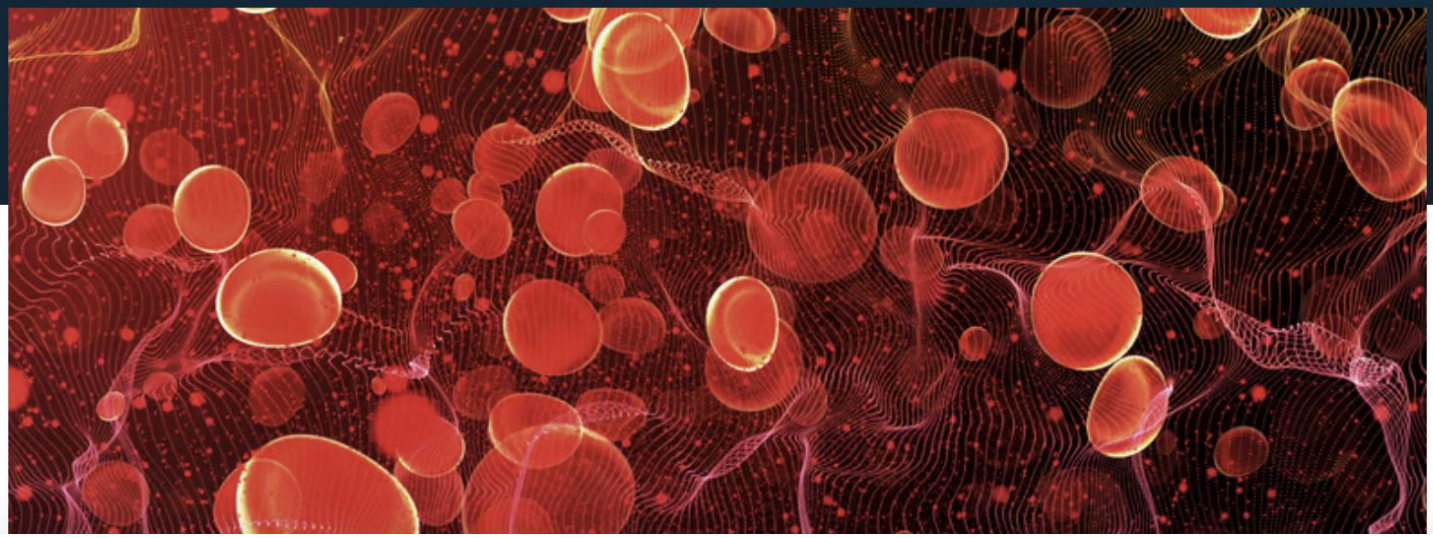


BIOTECH

Vertex pays Orna $65M to work on next-gen gene therapies for blood disorders

By **Gabrielle Masson** · Jan 7, 2025 7:00am

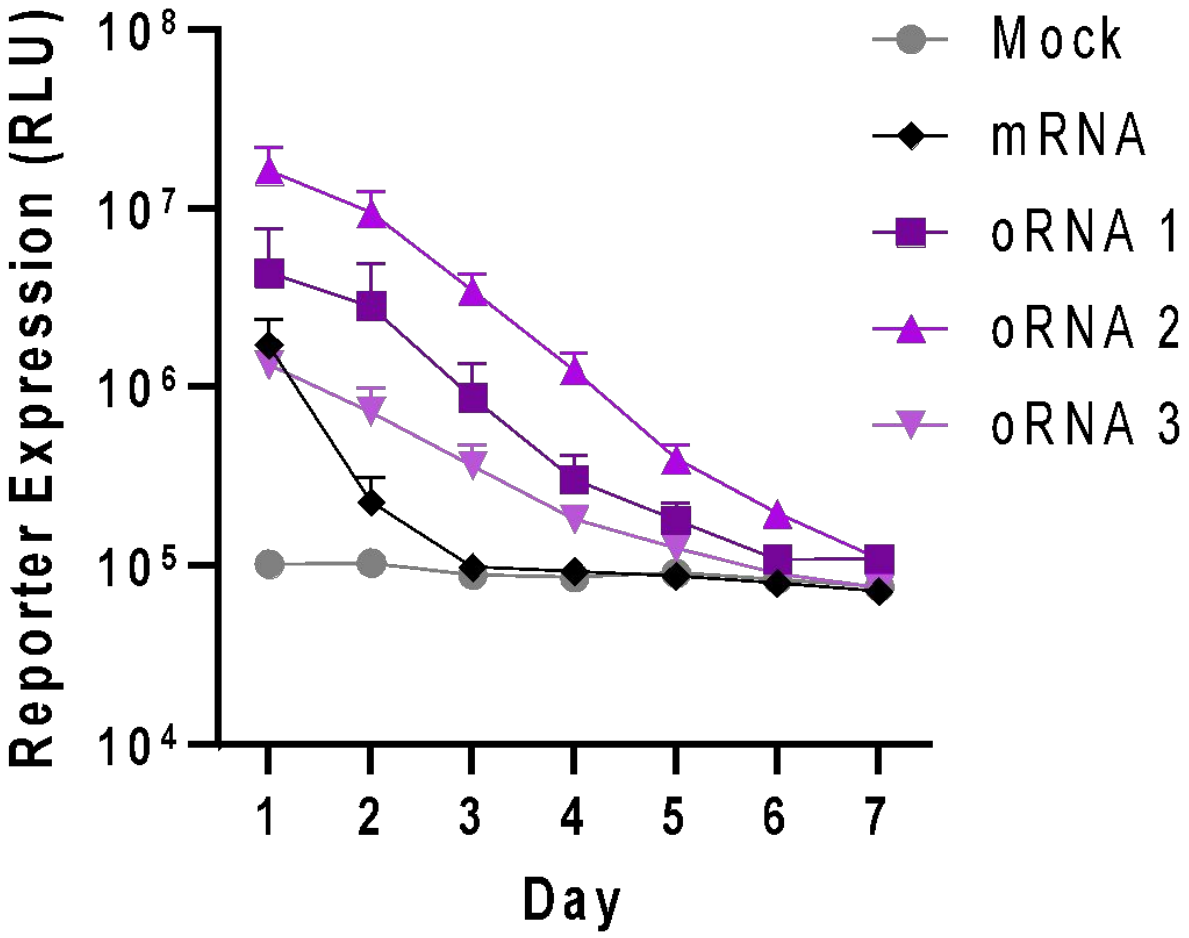
Orna Therapeutics | Vertex Pharmaceuticals | ReNAgade Therapeutics | Moderna



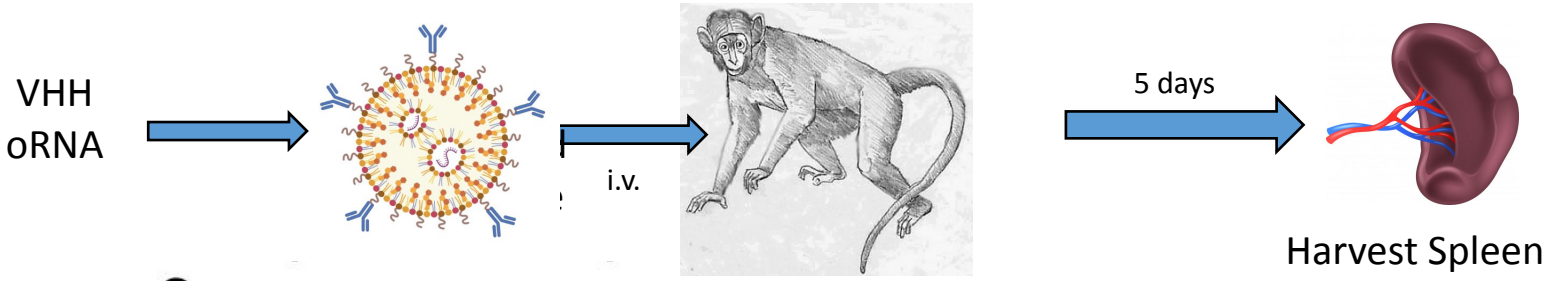
oRNA expression in human T cells

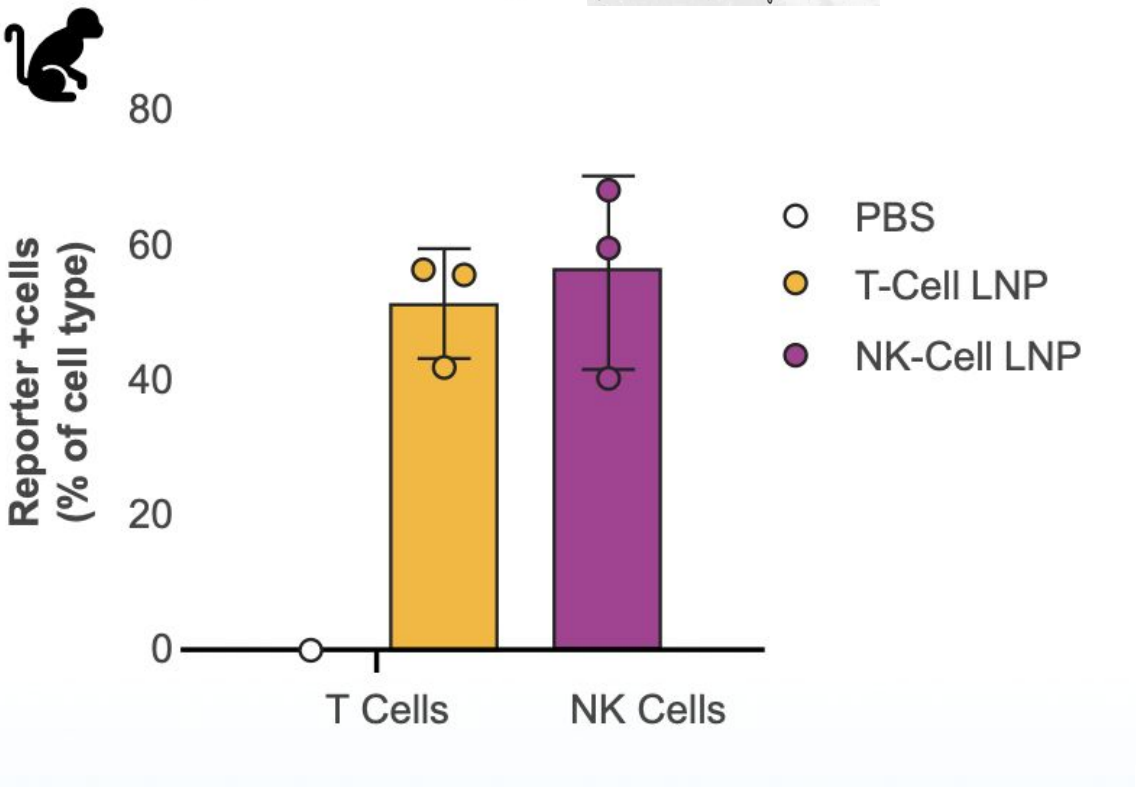
- IRES-driven oRNA expression in proliferating human T cells outperforms mRNA in C_{max}, AUC, and $t_{½}$
- Orna's proprietary immune IRESs provide a new level of functional tuning

IRES increases expression



Immunotropic RNA delivery in Primates to T-Cells & NK Cells





ORNA

RNA platform generated oRNA encoded CAR with immunotropic LNP delivery

Novel Binders

scFv

Dozens evaluated

CD19
BCMA

IRES Optimized

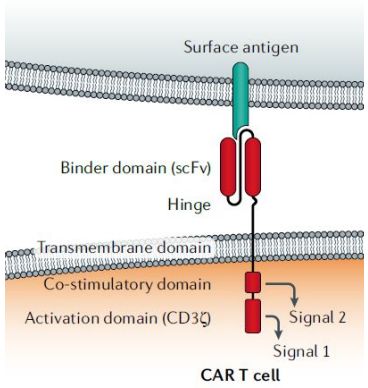
Tens of thousands tested

Cross-species Translation

Immunotropic LNPs

>300 individual CAR constructs screened

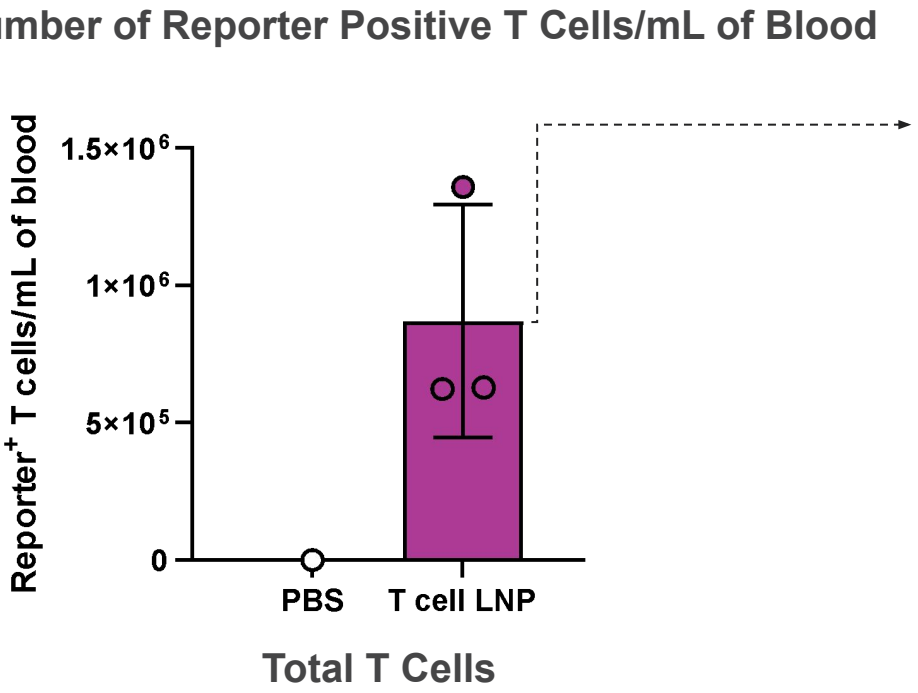
Top NHP/Human cross-reactive binders



ORNA

Robust delivery to peripheral blood T cells in NHP

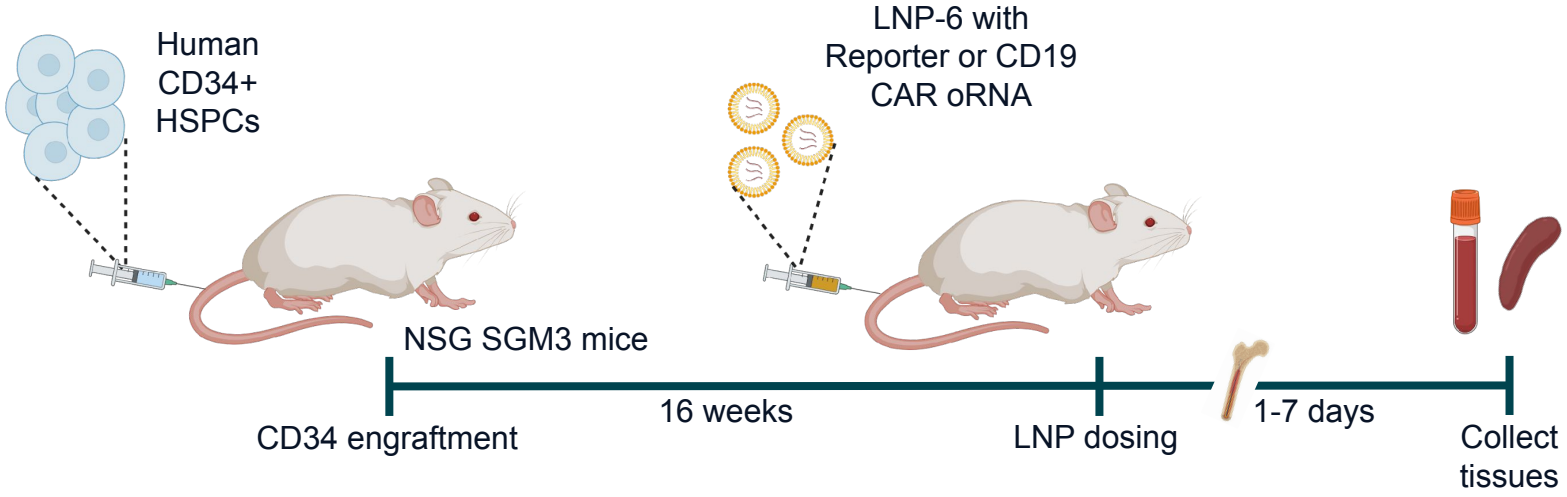
Achieved ex vivo CAR T cell dose levels with a single in vivo transfection



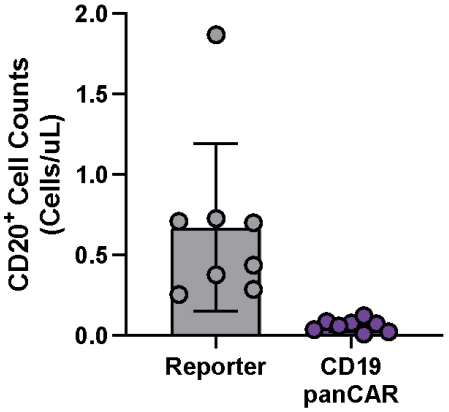
1 - Assuming a 3 kg NHP: Total Blood = 150 mL: Take these numbers x 150 to get absolute count.

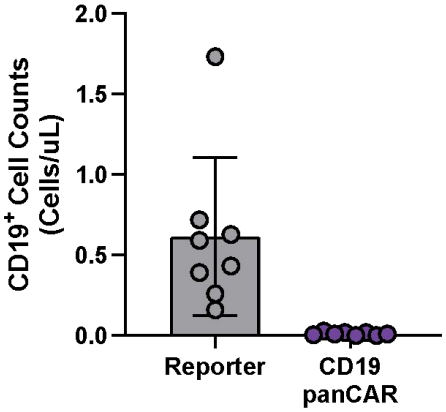
ORNA

Single dose of CD19 PanCAR™ depletes peripheral B cells in humanized mice

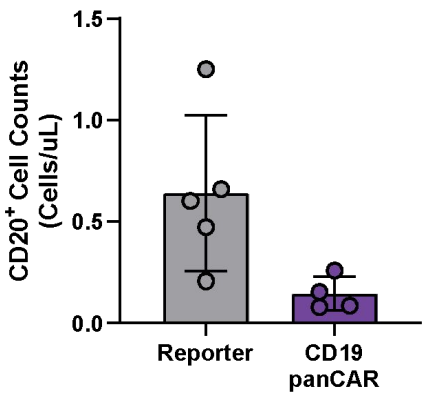


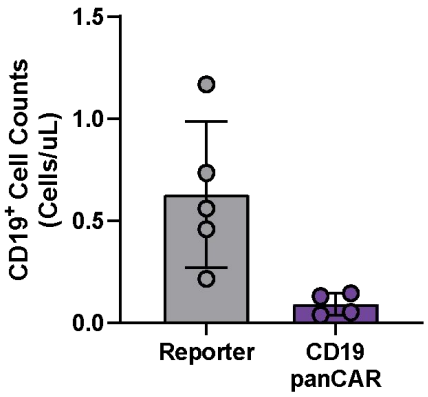
Reduction in Peripheral Blood B cell counts Day 1





Reduction in Peripheral Blood B cell counts Day 7

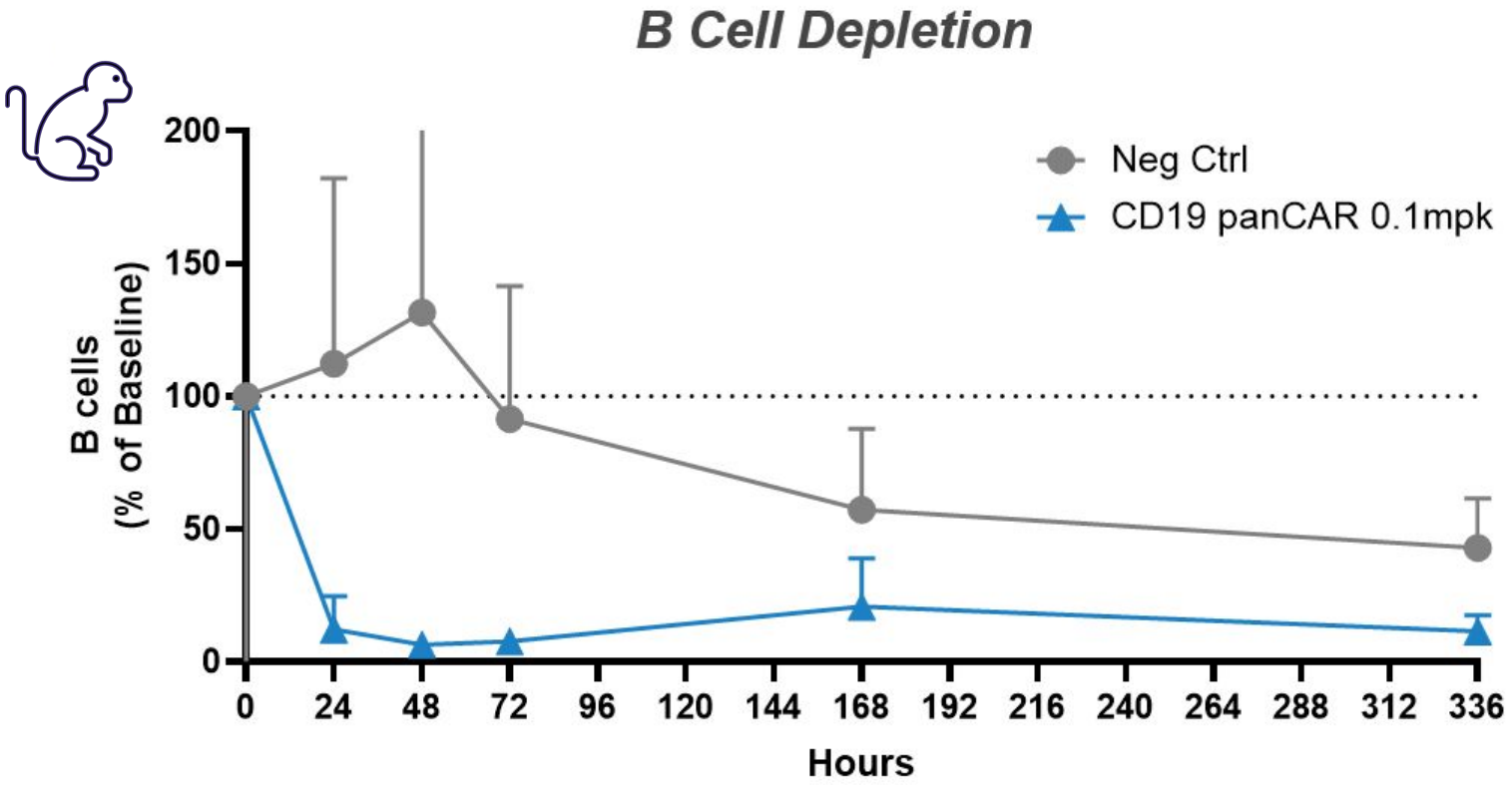




CAR, chimeric antigen receptor; HSPC, hematopoietic stem and progenitor cells; NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice; LNP, lipid nanoparticle

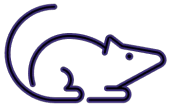
ORNA

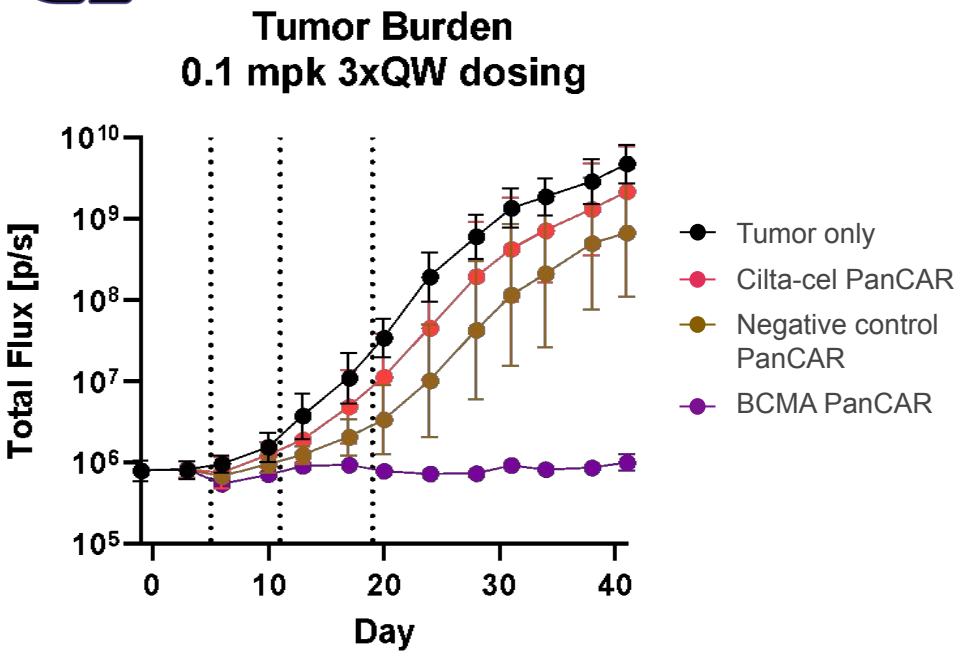
Single dose of CD19 PanCAR™ significantly reduces peripheral B cells in NHP

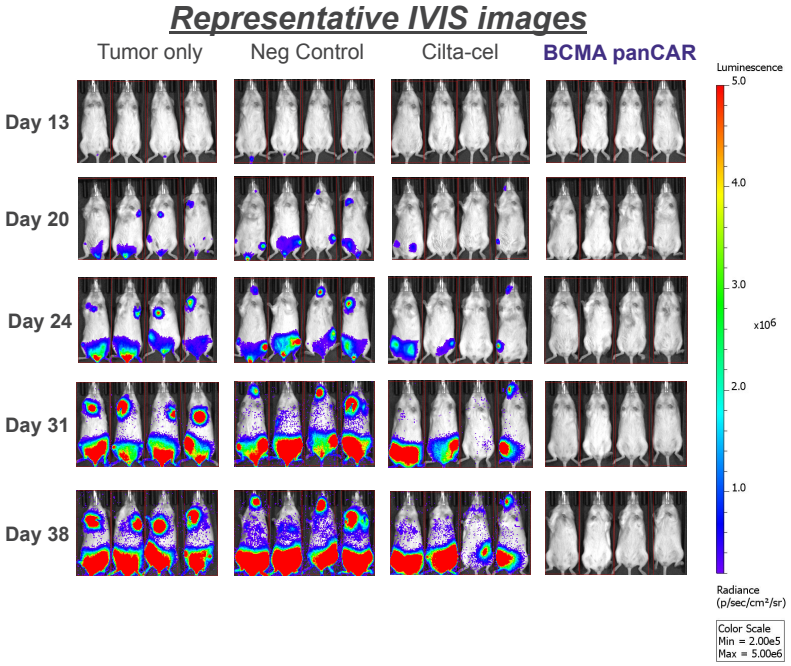


ORNA

BCMA *in vivo* PanCAR™ eradicates tumor at low dose

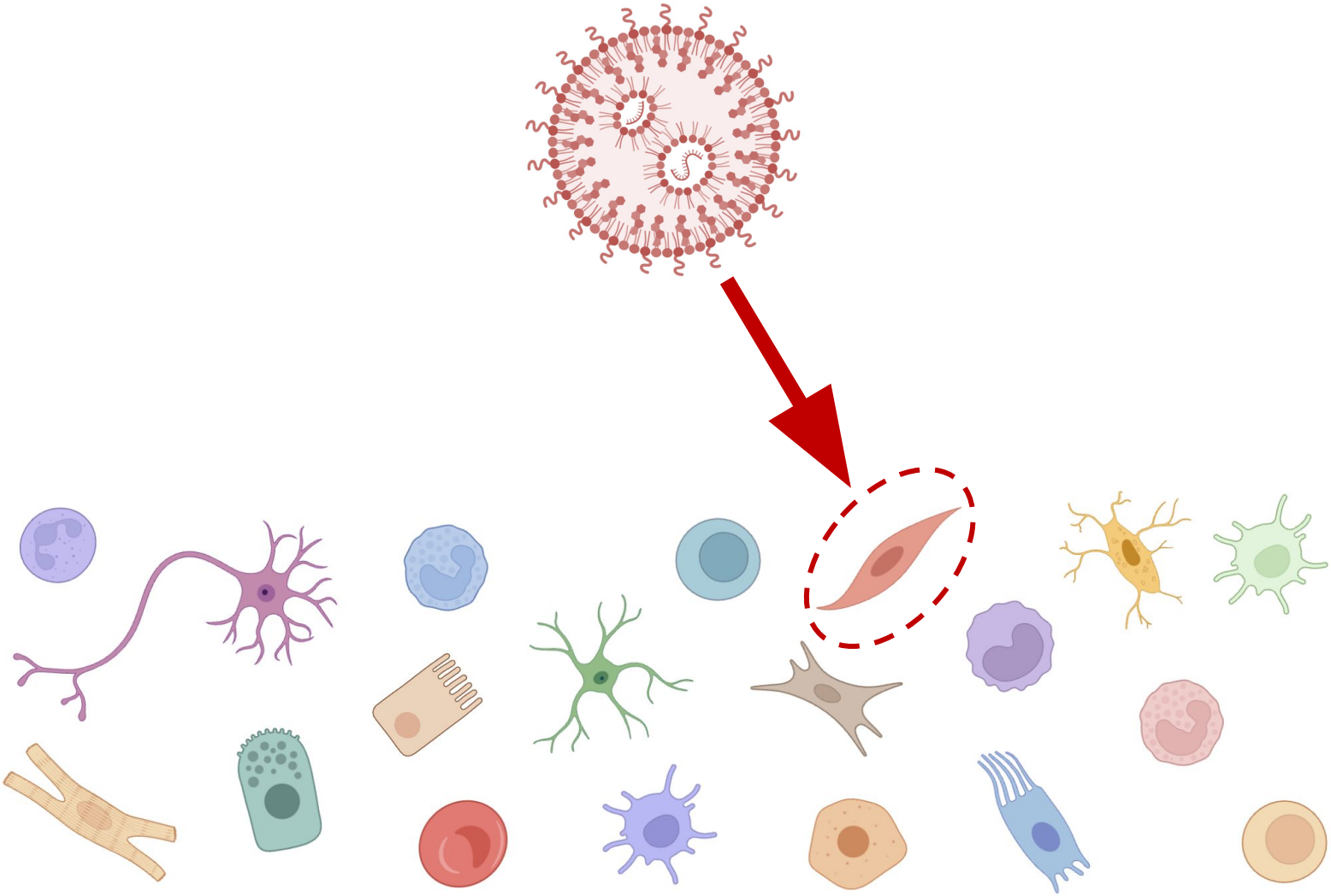






ORNA

Targeted RNA Delivery will Enable Genetic Therapy and Editing



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