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Lipid Nanoparticles Are Enabling Gene Therapies

Alternative Title: A 25 Year Journey to Enable Gene Therapies That Led to the Pfizer/BioNTech Covid 19 Vaccine

Conflicts of Interest
Precision NanoSystems: Co-Founder
Acuitas Therapeutics: Co-Founder
NanoVation Therapeutics: Co-Founder & Chairman

Gene Therapy

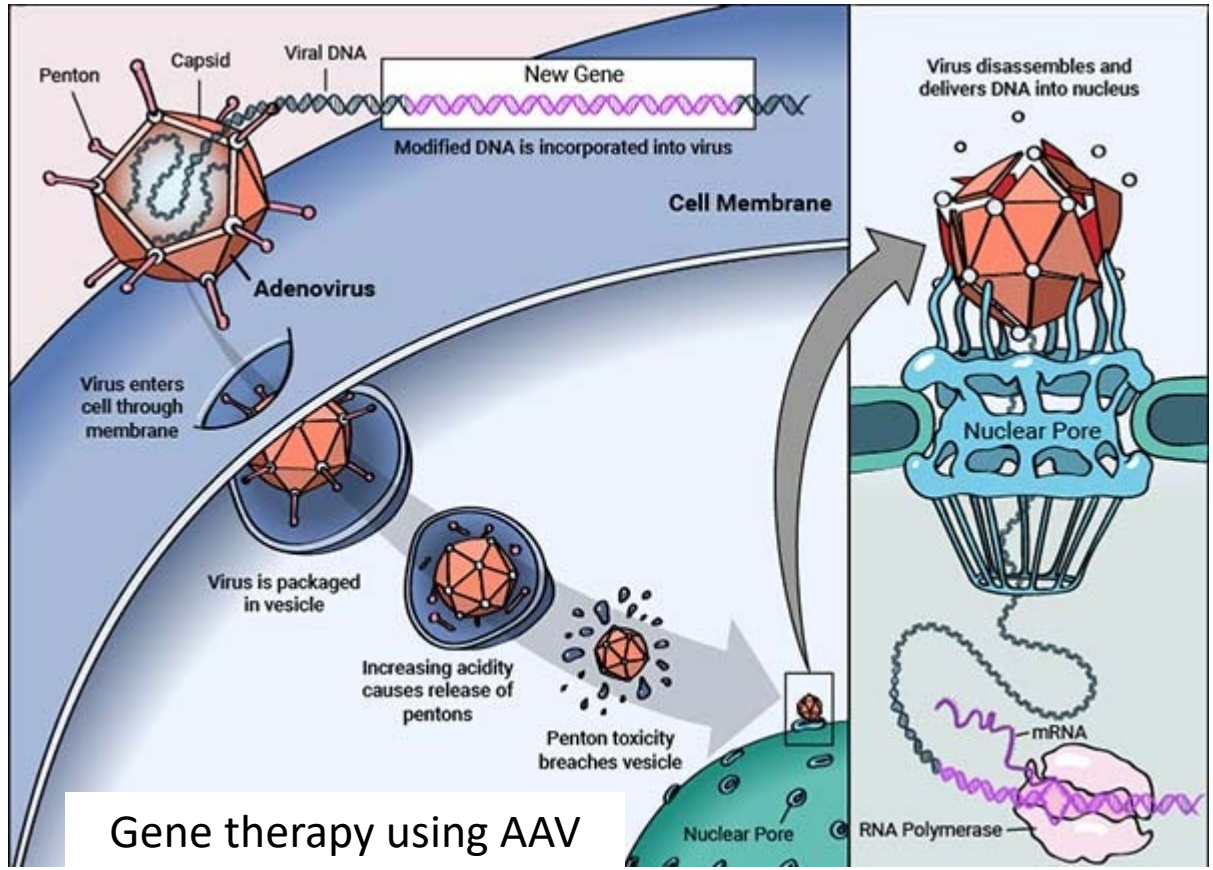
Wikipedia: gene therapy is the therapeutic delivery of nucleic acid into a patient's cells as a drug to treat disease

Gene therapy requires a delivery system to deliver the nucleic acid polymer into a patient's cells:

- Unmodified nucleic acid polymers are rapidly degraded in biological fluids
- Nucleic acid polymers do not preferentially accumulate in target tissue
- Nucleic acid polymers cannot penetrate into target cells even if they get to target tissue

Gene Therapy

Most gene therapy efforts have used viruses to deliver the nucleic acid

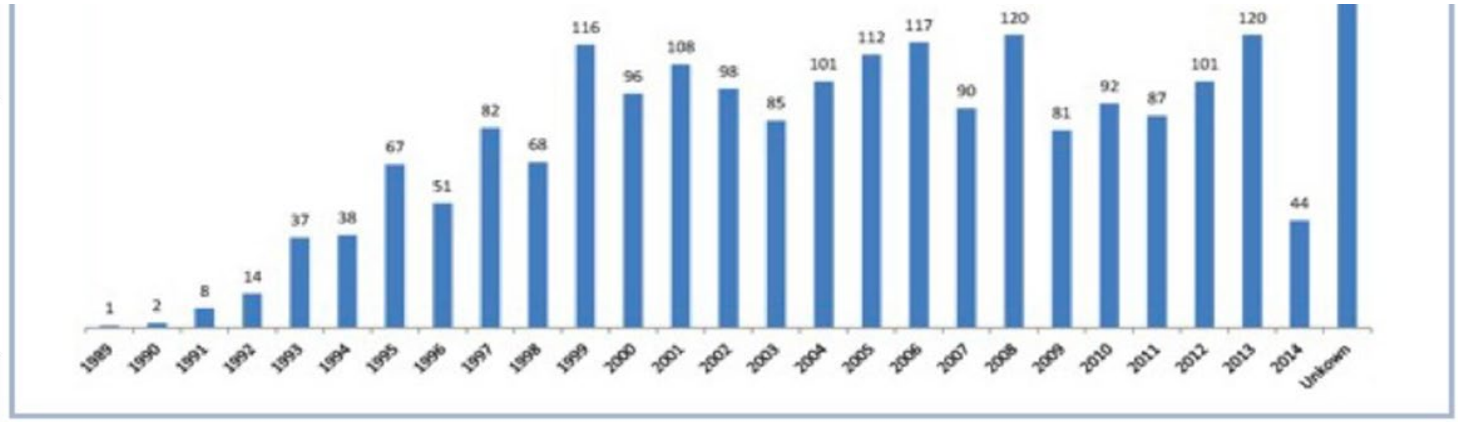


Gene therapy using AAV

Gene Therapy

Area of intense interest, potentially any disease can be treated using gene therapy approaches

Over 2000 gene therapy clinical trials conducted over the last 30 years, most used viral delivery systems, only six drugs approved by FDA



Drug	Date	Delivery system	Indication
Kymriah	2017	AAV (ex vivo)	CAR-T cell therapy for cancer
Luxturna	2017	AAV (intraocular)	Blindness
Zolgensma	2019	AAV (i.v.)	Spinal muscular atrophy
Onpattro	2018	LNP (i.v.)	Transthyretin induced amyloidosis
Pfizer/BioNTech BNT162b2	2020	LNP (i.m.)	COVID-19 vaccine
Moderna vaccine	2020	LNP (i.m.)	COVID-19 vaccine

Gene Therapy: Current Status

Why are we finally making some progress?

Viral vectors have proven very problematic delivery systems:

- Limited genetic capacity
- Difficult to engineer and manufacture
- Stimulate an immune response (can give only one dose)
- Potential for insertional mutagenesis, viral transmission
- Are extremely expensive (up to $2M per dose)
- Can be toxic

Non-viral vectors potentially have none of the problems associated with viral vectors:

- Historically, non-viral vectors have limited transfection potency and can be toxic in vivo
- **These issues are being overcome using lipid nanoparticle (LNP) delivery systems. How did this happen?**

Lipid Nanoparticles That Enable Gene Therapies

- Design of LNP systems for delivery of nucleic acid polymers (1995-2020)
- The Patisiran (Onpattro) story (1995-2012): development of an siRNA-based LNP drug to treat hereditary amyloid transthyretin (hATTR) amyloidosis
  - ➢ LNP siRNA program: gene silencing in the liver
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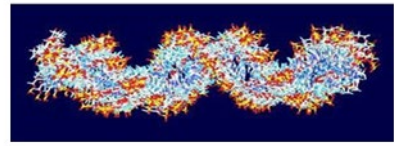
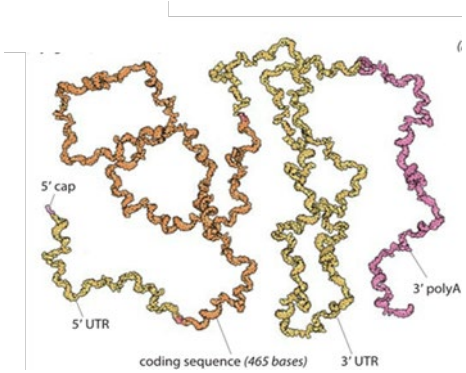
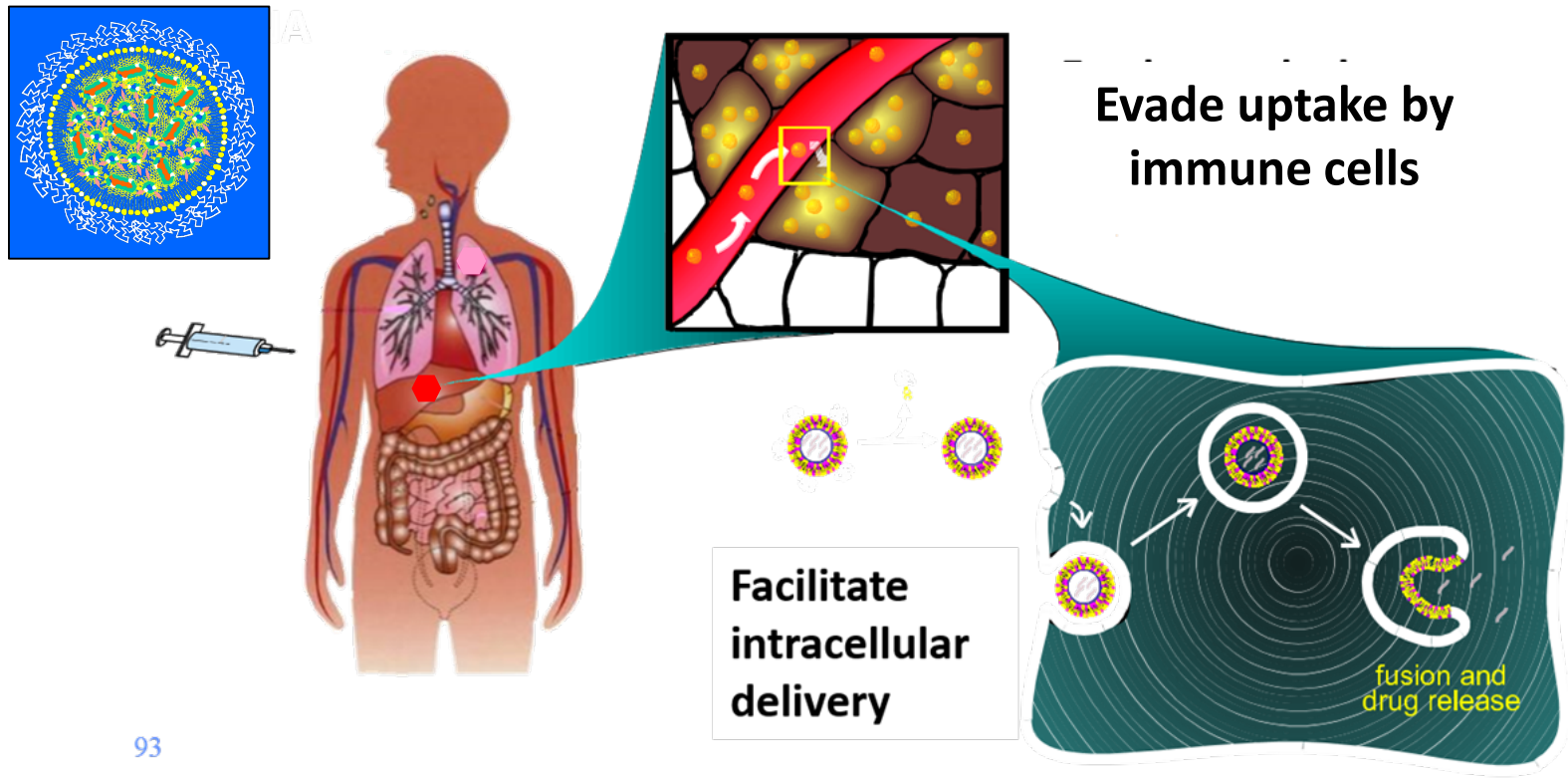




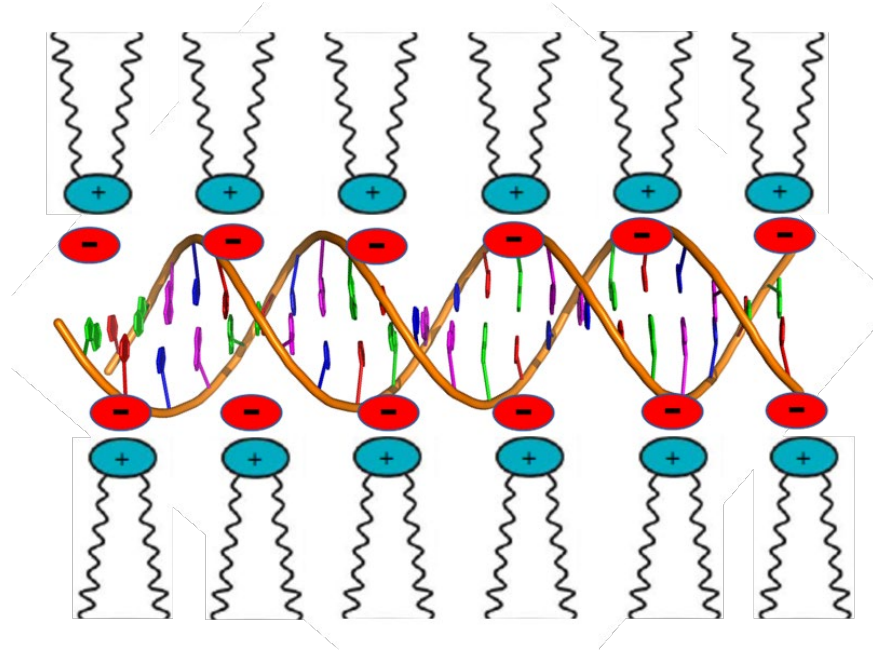
Chose to Develop a Delivery System That Takes Nucleic Acid-Based Drugs to the Liver and Enables Intracellular Delivery into Hepatocytes

Package nucleic acid in LNP



This was the challenge in 1995

Efficient Encapsulation of Nucleic Acid Polymers in LNP Requires Cationic (Positively Charged) Lipids



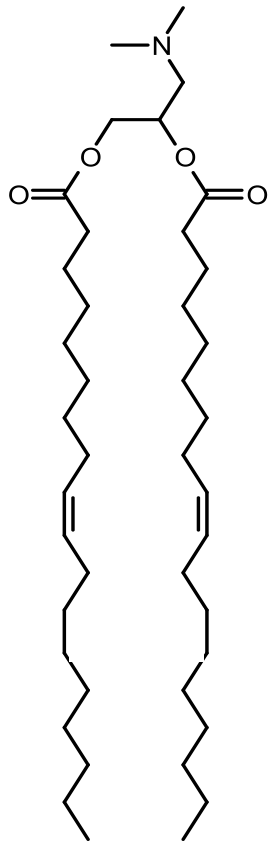
There are no cationic lipids in nature, they are highly toxic.
There are only net neutral lipids or negatively charged lipids

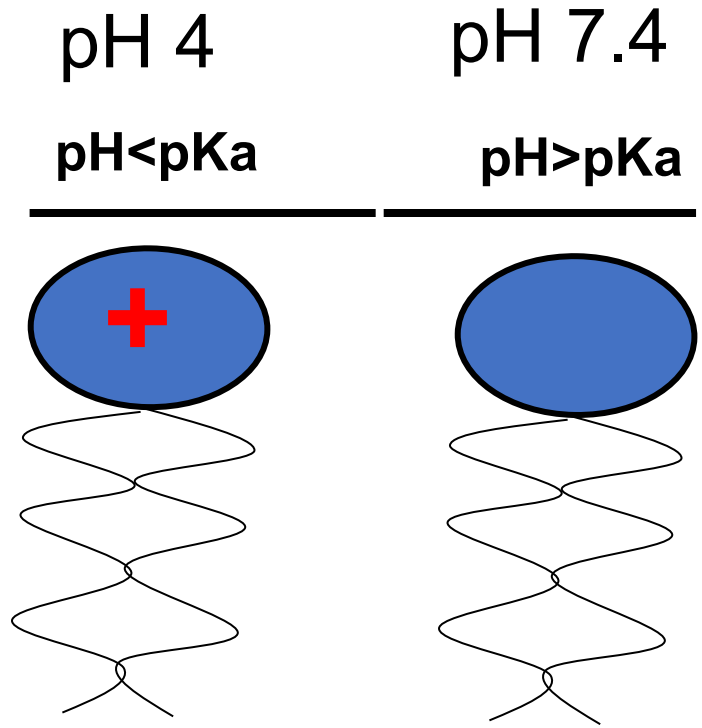
Ionizable Cationic Lipids

In order to avoid the toxicity issues associated with permanently positively charged lipids, we developed **ionizable cationic lipids**:

- pKa ~ 6.5, thus protonated and positively charged at low pH, near neutral at physiological pH
- Found we could load nucleic acid polymers into LNP at low pH (e.g. pH 4) and that contents were retained in LNP when the pH was raised to pH 7.4.
- Much less toxic than lipids that are positively charged at physiological pH
- Turned out to also have important properties for intracellular delivery

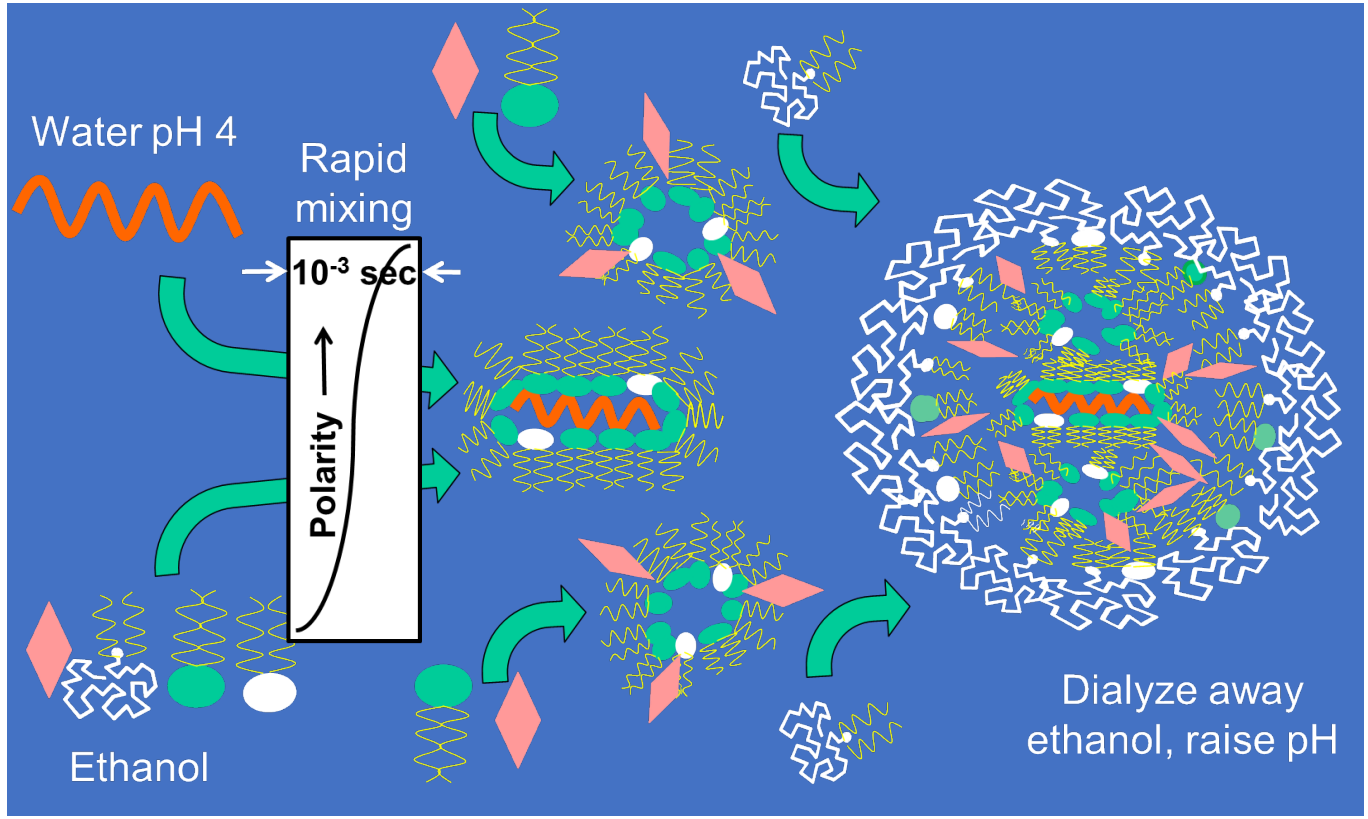
DODAP: First Generation Ionizable Cationic Lipid





DODAP is not charged at physiological pH, does not induce H_{II} phase and is much less toxic

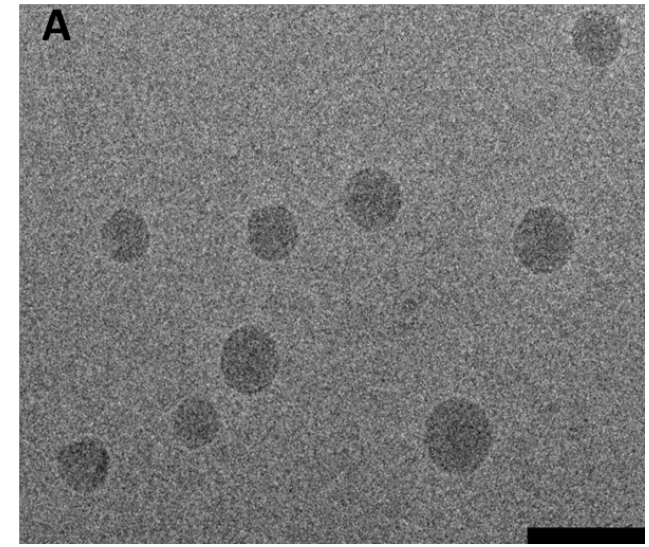
Found We Could Formulate Nucleic Acid Polymers Into Lipid Nanoparticles Containing AL1 Using a Rapid Mixing Procedure

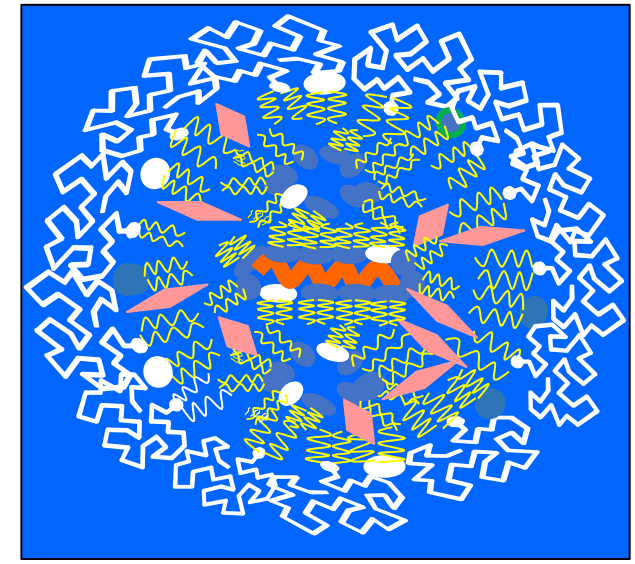


Dissolve lipid in ethanol and rapidly mix with oligonucleotide dissolved in H_2O (pH 4), then dialyze away the ethanol and raise pH to 7.4. Achieve >90% encapsulation efficiencies, siRNA is retained at pH 7.4

LNP siRNA Systems Containing Ionizable Cationic Lipids Are a New Class of Lipid Nanoparticles

- Hydrophobic core as opposed to an aqueous core
- Ideally suited to encapsulation of negatively charged macromolecules such as RNA, DNA constructs
- Encapsulation efficiencies of 100% for siRNA, mRNA, plasmids
- Stable, mono-disperse; can adjust diameter 20-100 nm
- Relatively non-toxic
- Scalable
- Reproducible





Lipid Nanoparticles That Enable Gene Therapies

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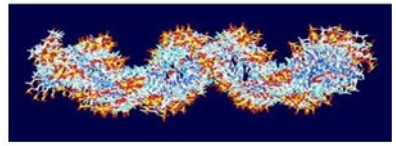
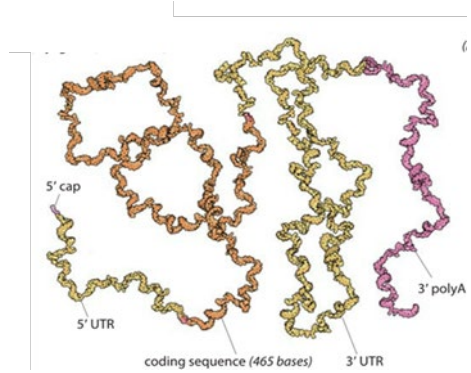
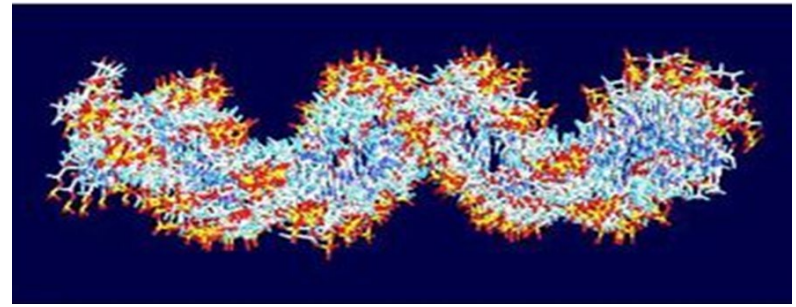




Objective 2005-2012: Develop LNP systems containing siRNA to silence genes in the liver (hepatocytes) following i.v. administration

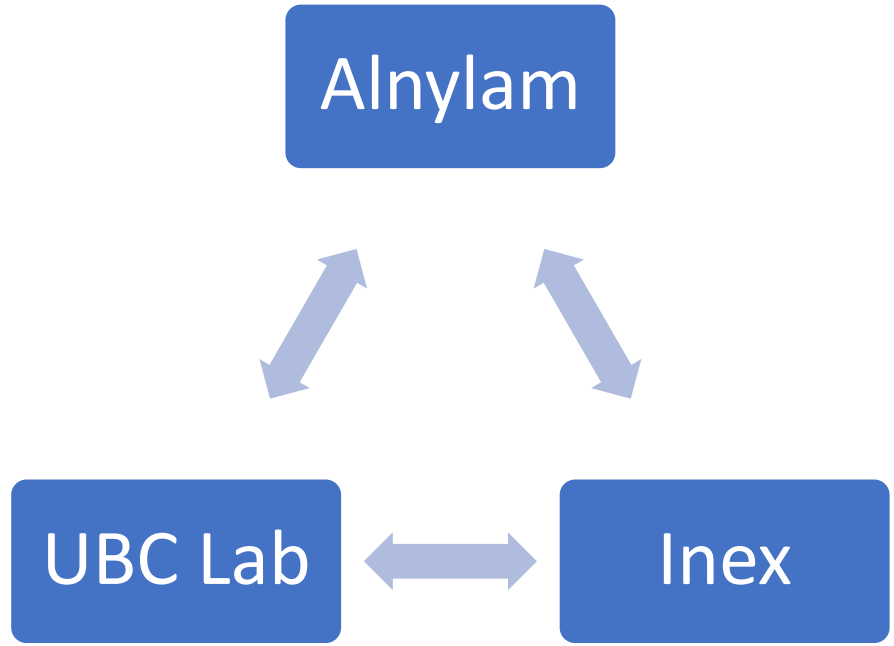
Many diseases can potentially be treated by silencing/expressing/editing genes in liver: blood clotting disorders (e.g. hemophilia A, B), metabolic disorders (e.g. OTC deficiency, hypercholesterolemia, diabetes) liver cancer, hepatitis B & C, etc

After a Conference I Attended in London in 2004 I Was Pursued by Victor Kotelianski, the VP Research for Alnylam Pharmaceuticals

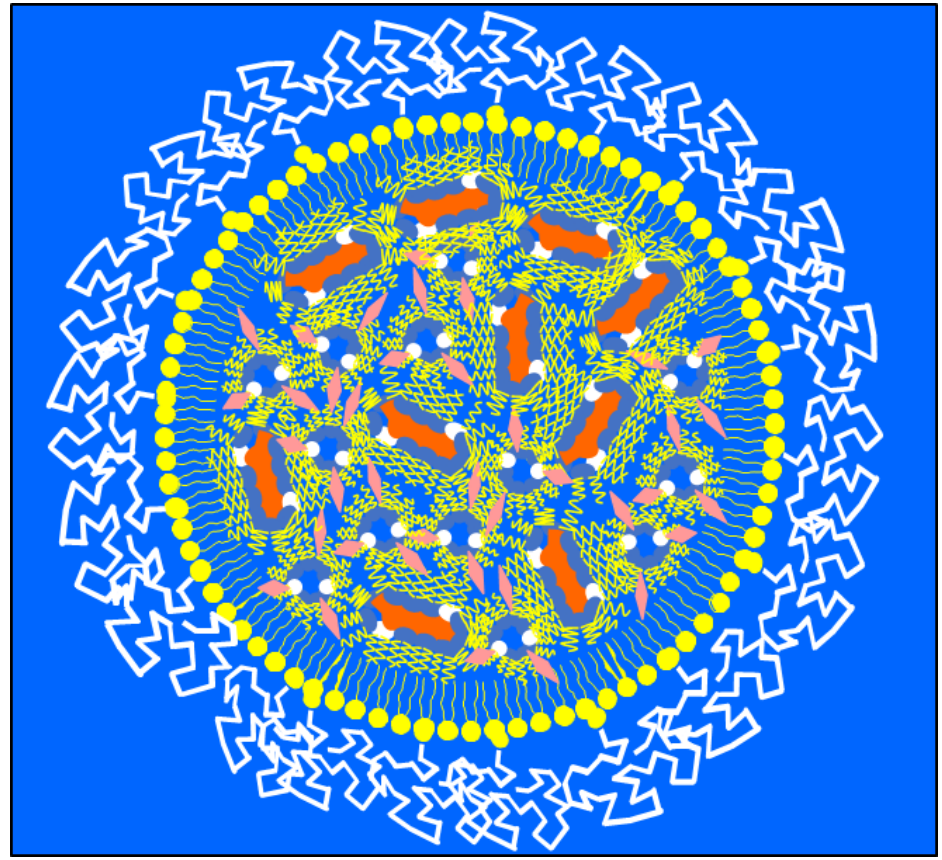


"We have a delivery problem. How do we get our small interfering RNA into hepatocytes in vivo?"

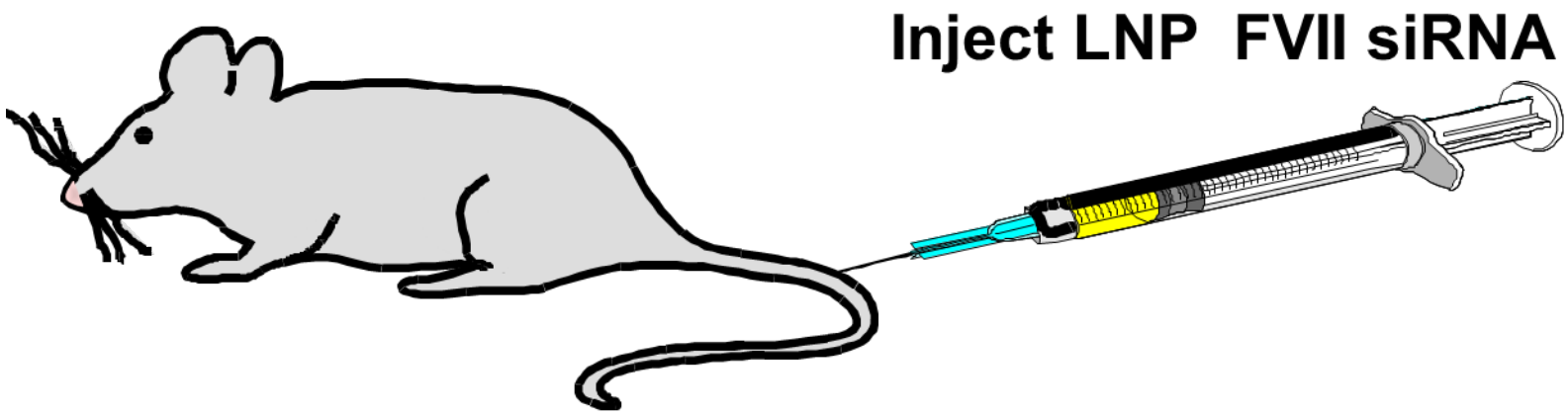
We Set Up A 3-way Collaboration To Develop LNP siRNA Systems To Silence Genes In Liver



Started With the Question: Can These LNP siRNA Systems Containing Ionizable Cationic Lipids Such as AL1 Silence Genes in Hepatocytes?



Assessed In Vivo Potency of LNP siRNA Formulations For Silencing Genes in Hepatocytes Employing a Factor VII Mouse Model

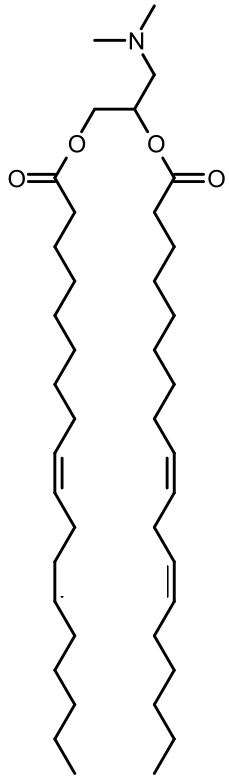


Time 0h	Dose mice with LNP siRNA (range 0.01-10 mg siRNA/kg body weight)
Time 24h	Terminate mice, assay plasma for FVII
Lipid composition	cationic lipid/DSPC/cholesterol/PEG-lipid; usually 40/10/40/10; mol/mol

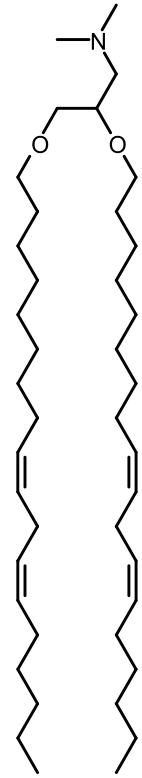
Found That The Potency of LNP siRNA Systems Was Highly Sensitive to the Species of Cationic Lipid Employed

Synthesized and Screened Over 300 Cationic Lipids With Varying pKa and Polymorphic Properties

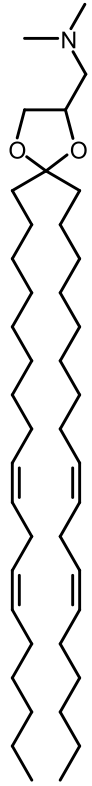
DODAP



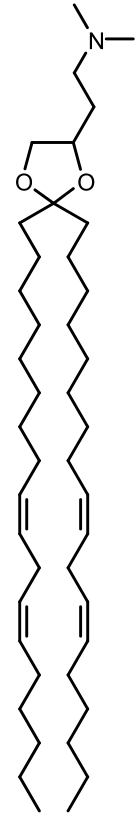
DLinDMA



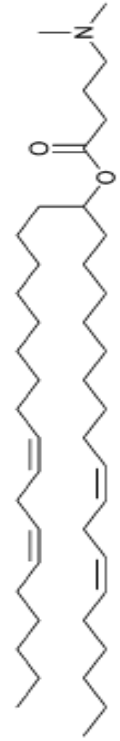
DLinKDMA



DLinKC2DMA

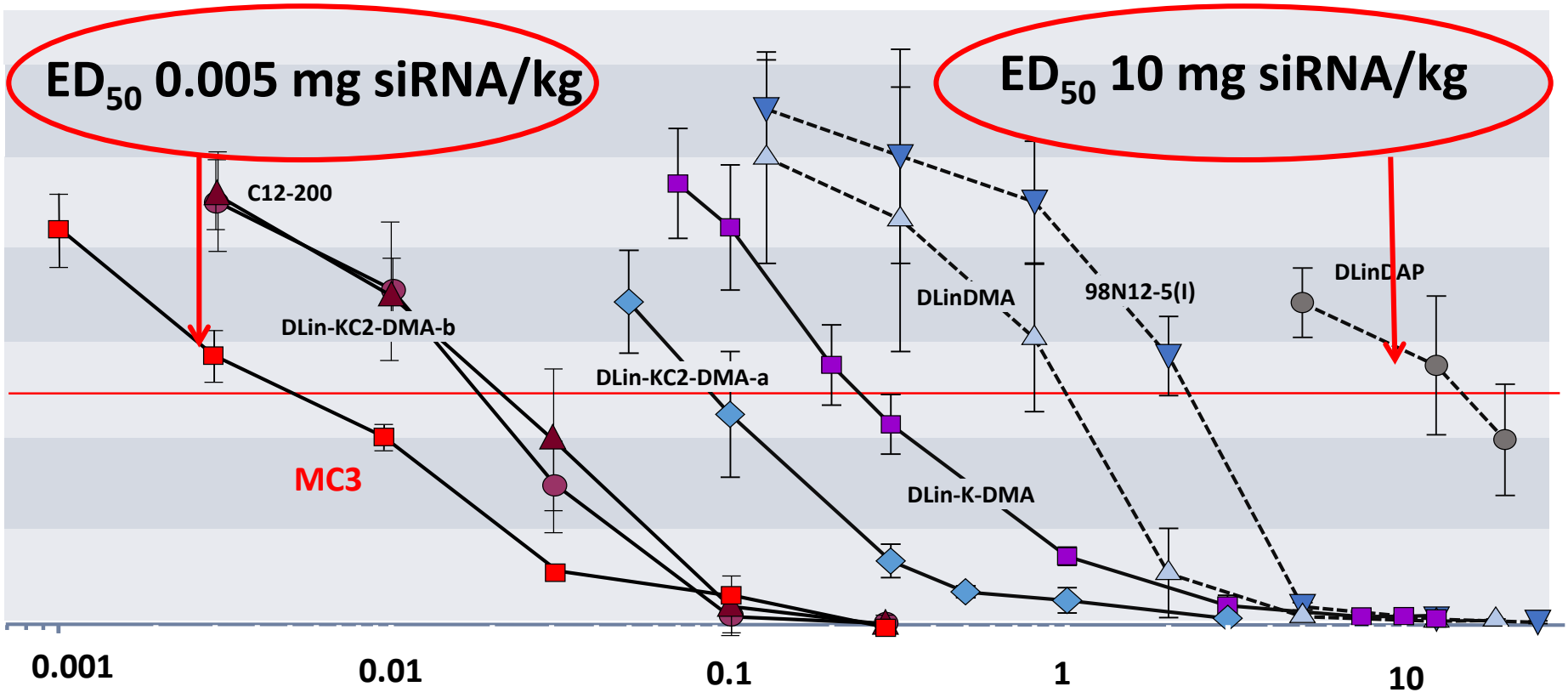


DLinMC3DMA



Optimized Ionizable Cationic Lipids Result In Extremely Potent LNP siRNA Systems for Silencing Genes in Hepatocytes

Therapeutic index > 1000



More than three orders of magnitude increase in potency over the course of the collaboration

Result of LNP siRNA Development Program 1995-2012

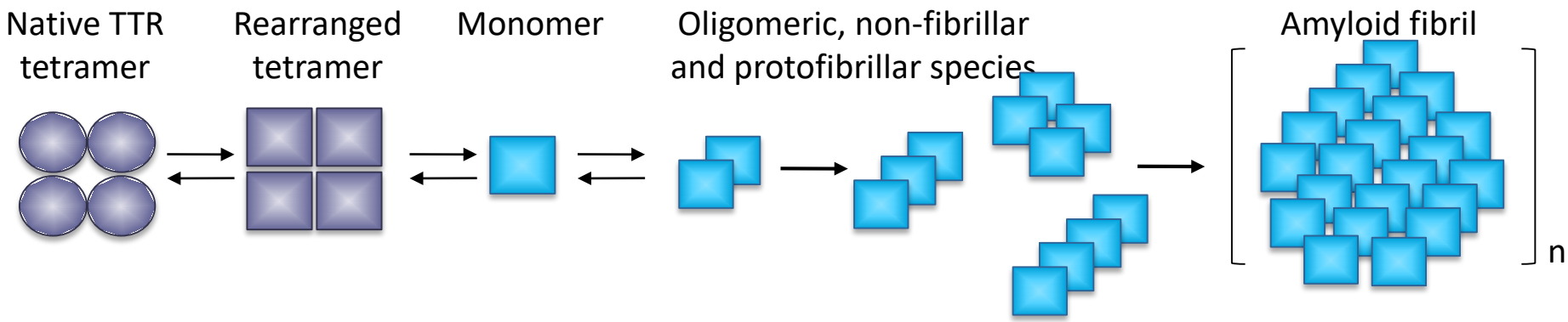
Achieved LNP siRNA systems that can silence FVII gene in hepatocytes (in mice) following i.v. injection with therapeutic indices > 1000

By extension, can silence any gene in hepatocytes. What gene should we silence and will this work clinically?

Chose to develop an LNP siRNA therapeutic to treat the hereditary disease transthyretin-induced amyloidosis

Hereditary Amyloid Transthyretin (hATTR) Amyloidosis

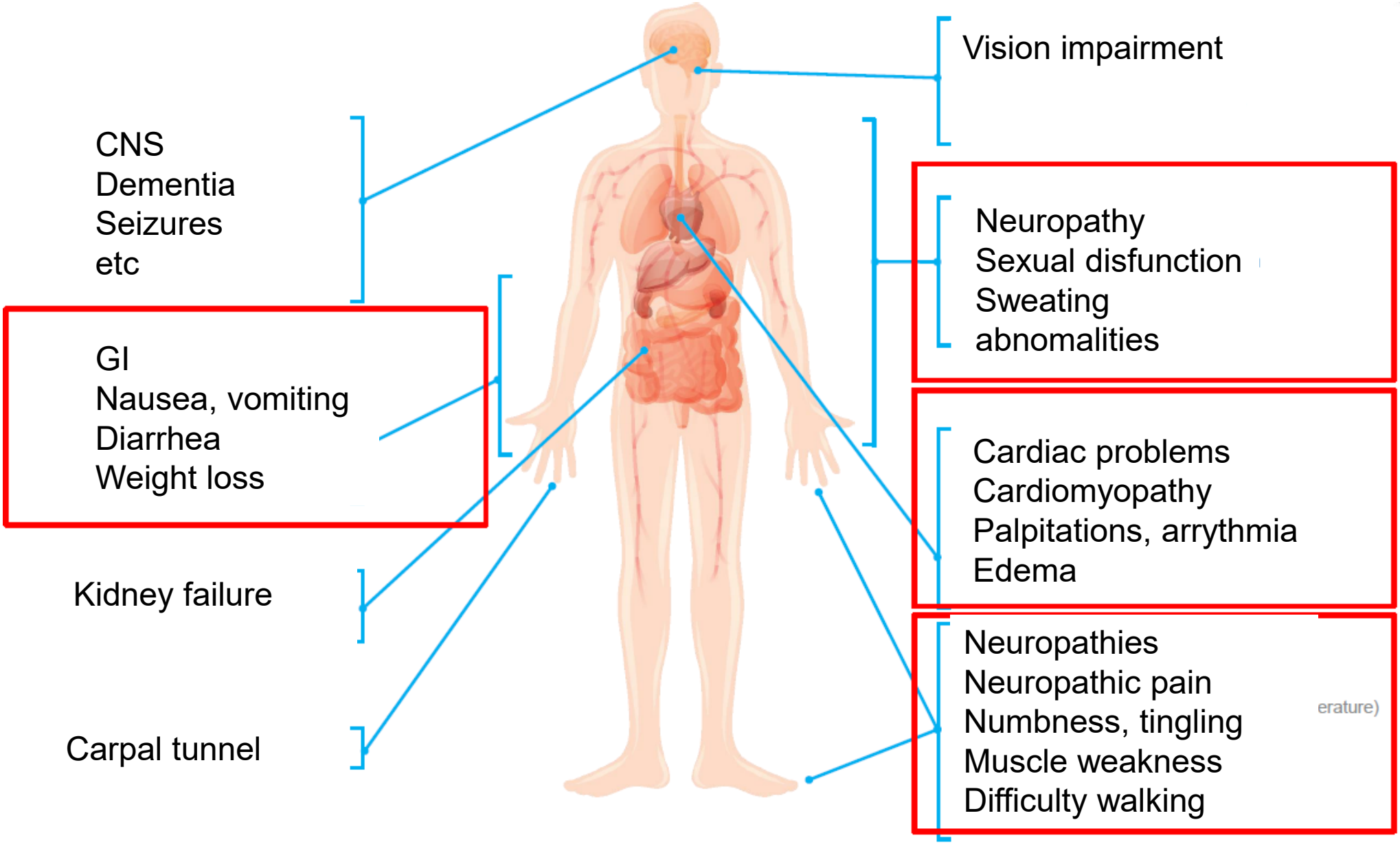
TTR is a tetrameric protein that is primarily expressed in the liver and transports serum retinol binding protein (RBP)



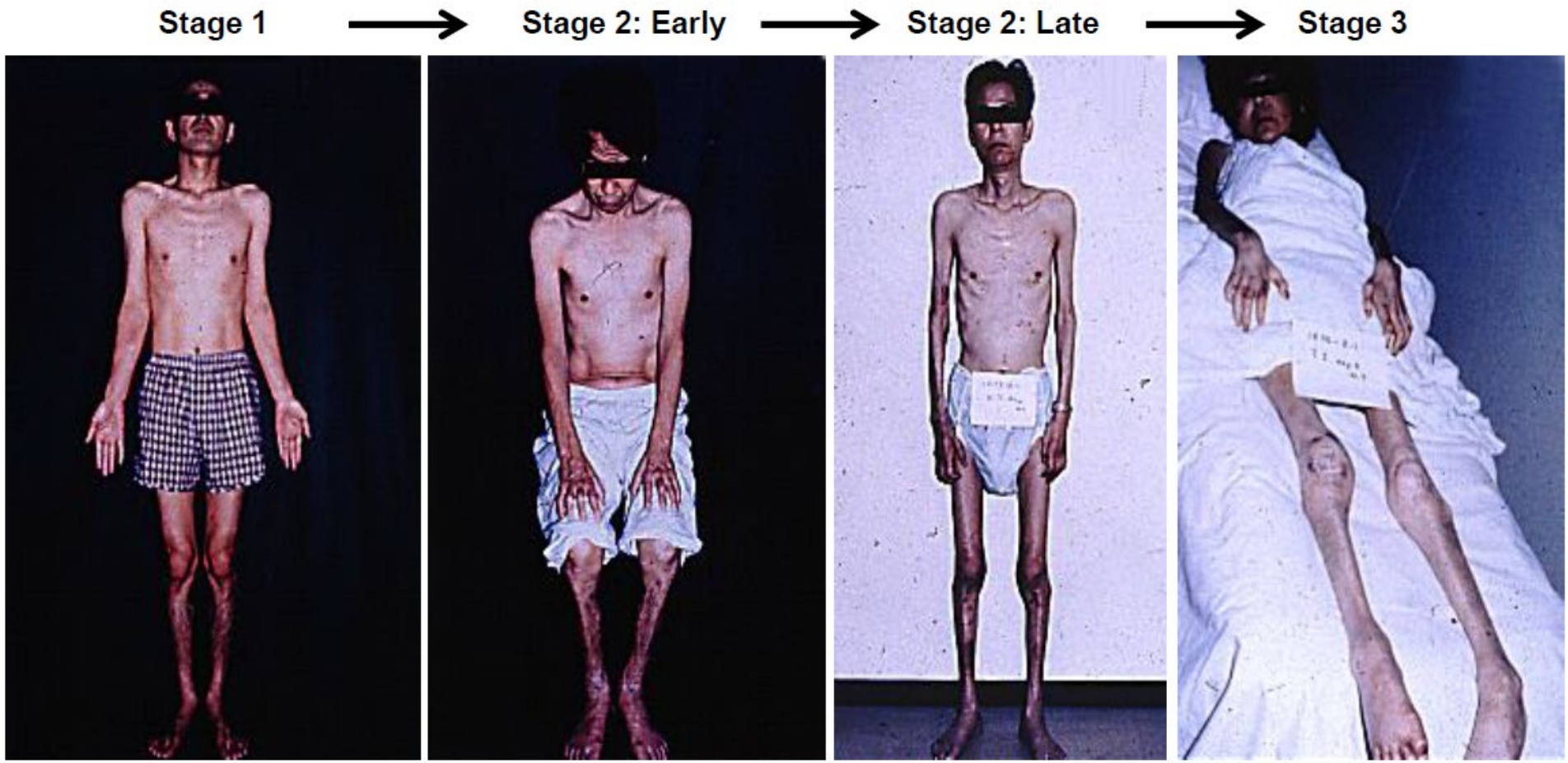
hATTR amyloidosis is a multisystem disease caused by extracellular deposits of TTR amyloid

- ~100 mutations in the TTR gene lead to amyloid deposition in:
  - Nerves : ~10,000 patients. extensive neuropathies
  - Heart: ~40,000 patients, cardiotoxicity leading to heart failure
- No effective therapy, usually fatal within five years of diagnosis

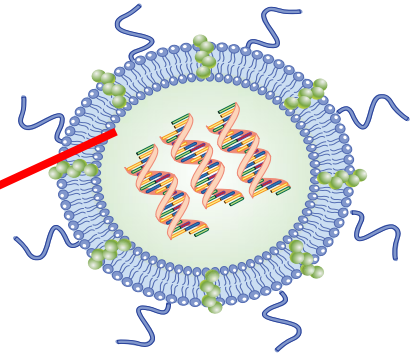
hATTR Amyloidosis: A Fatal Multi-Systemic Disease Causing Neuropathy and Cardiomyopathy Due to Liver-Derived TTR



hATTR Amyloidosis: A Rapidly Progressing Disease
Usually Fatal Within Five Years of Diagnosis



LNP TTR siRNA to Treat hATTR Amyloidosis: The Hypothesis



Use LNP TTR siRNA to silence mutant and wild type TTR production in the liver

Production of mutant and wild type TTR

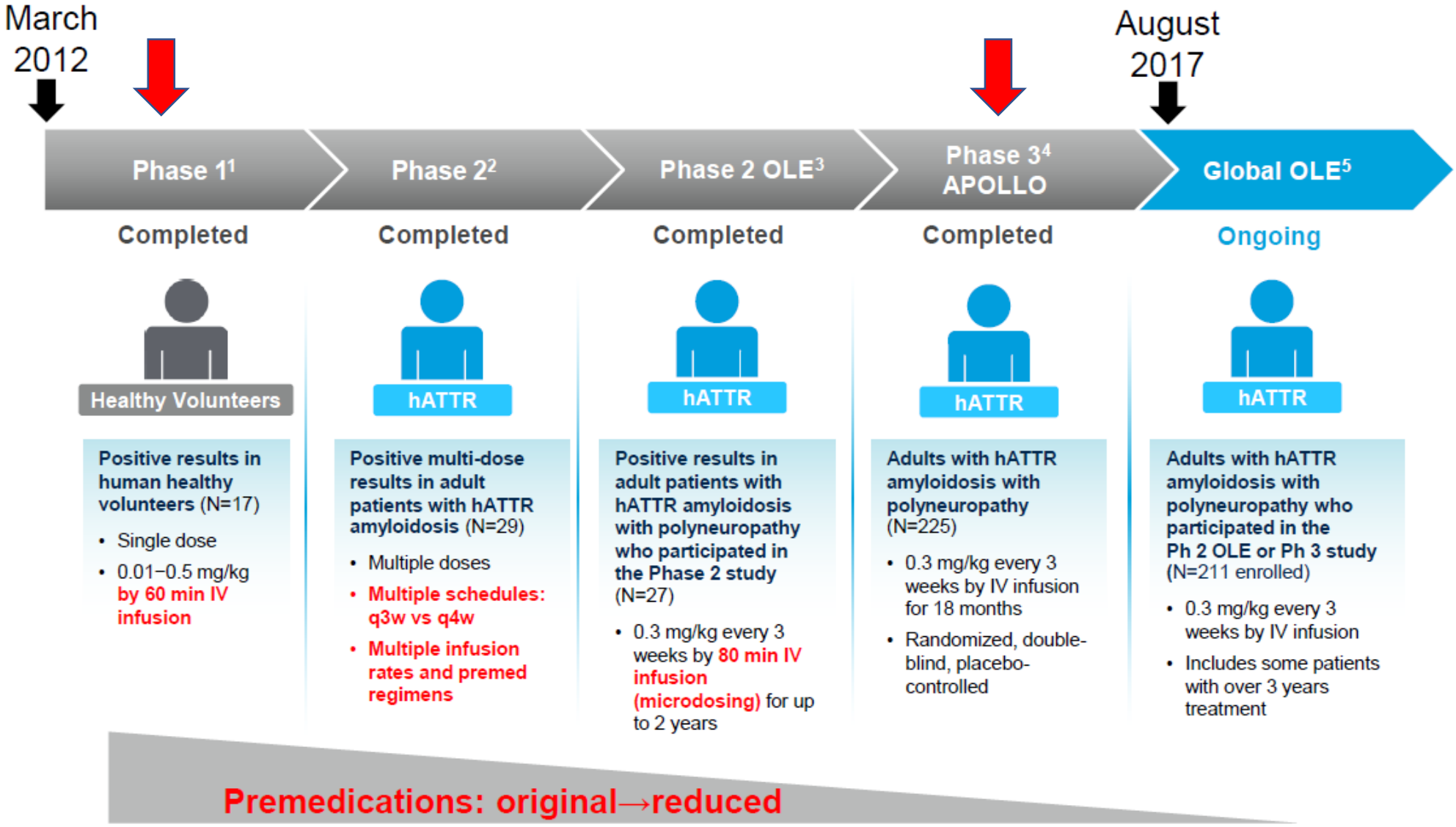
Unstable circulating TTR tetramers reduced

Organ deposition of amyloid fibrils prevented, clearance promoted

Stabilization and recovery from cardiomyopathy, neuropathy

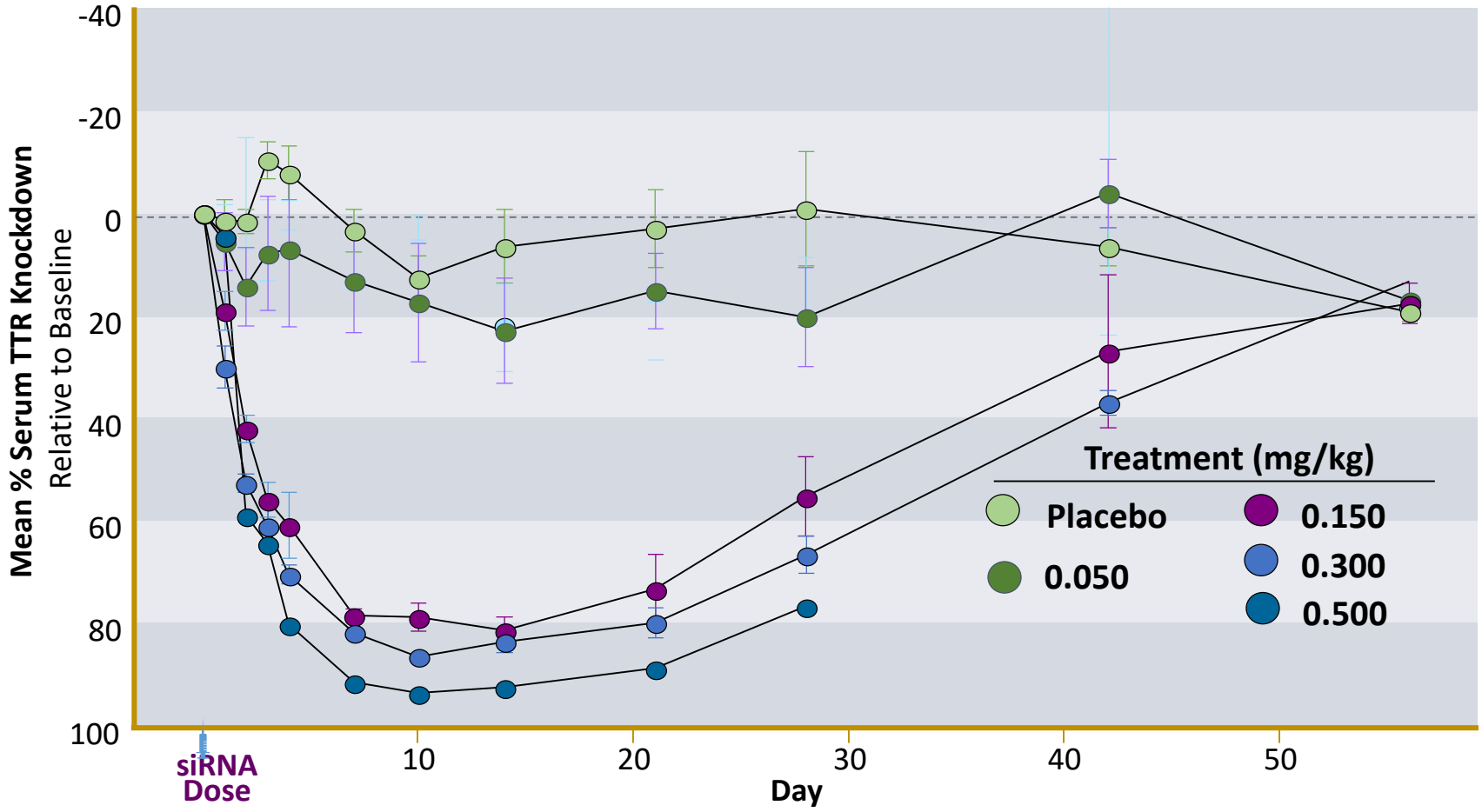
A potentially simple solution to a devastating disease

Clinical Development Program LNP TTR siRNA (Patisiran), A Potential RNAi Therapeutic for hATTR Amyloidosis



Phase I Study Results (Healthy Volunteers)

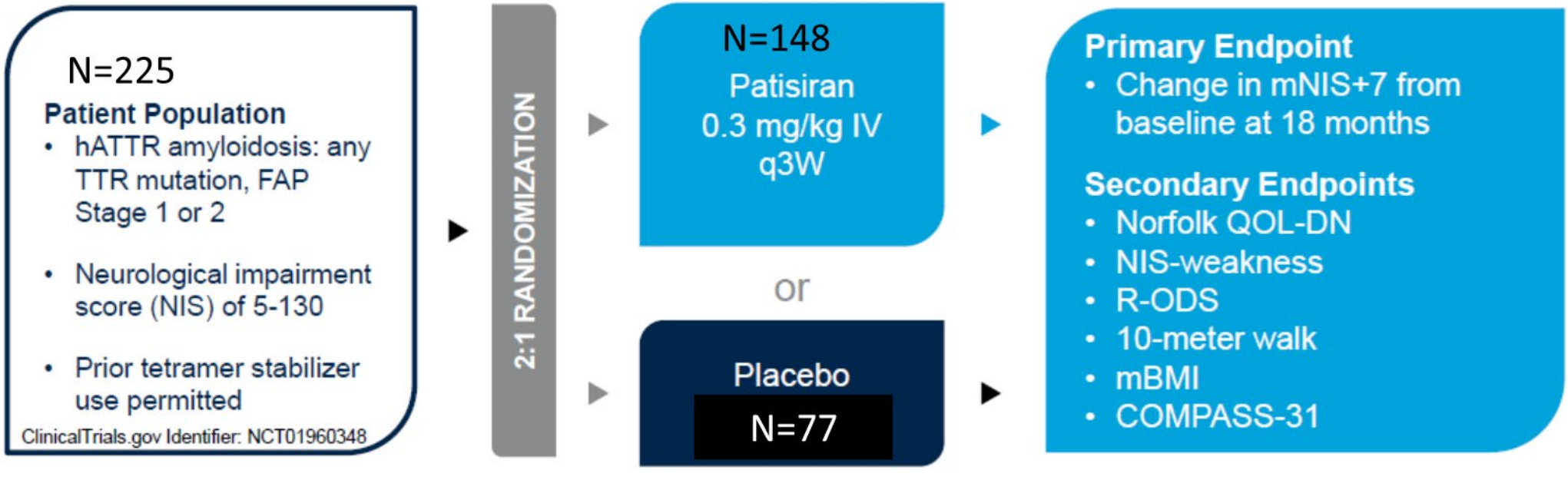
Effective TTR Gene Silencing at Dose Levels of 0.15 mg siRNA/kg Body Weight



Selected a dose of 0.3 mg siRNA/kg body weight every three weeks for subsequent trials

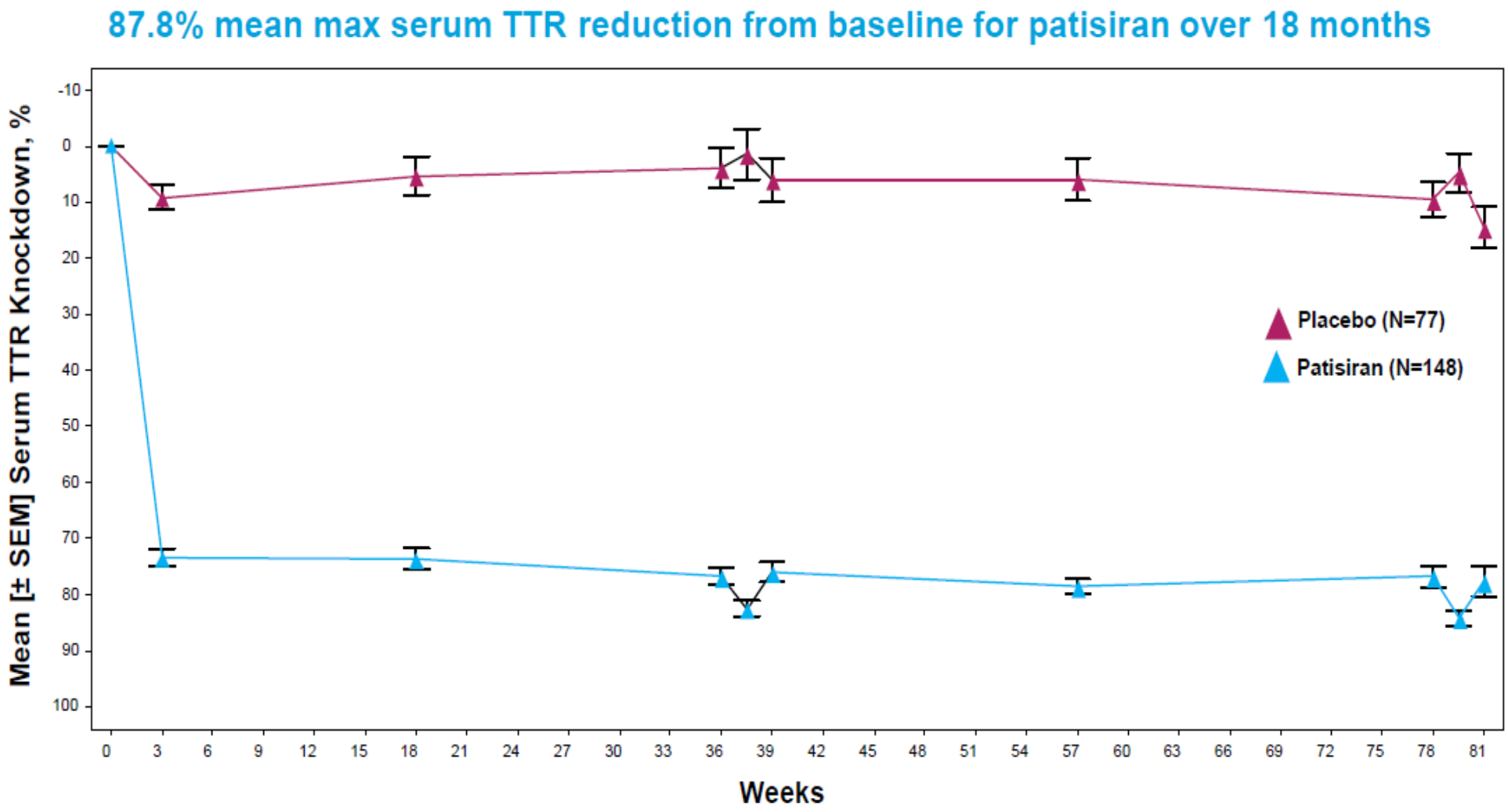
LNP siTTR (Patisiran) Phase 3 Study

Design

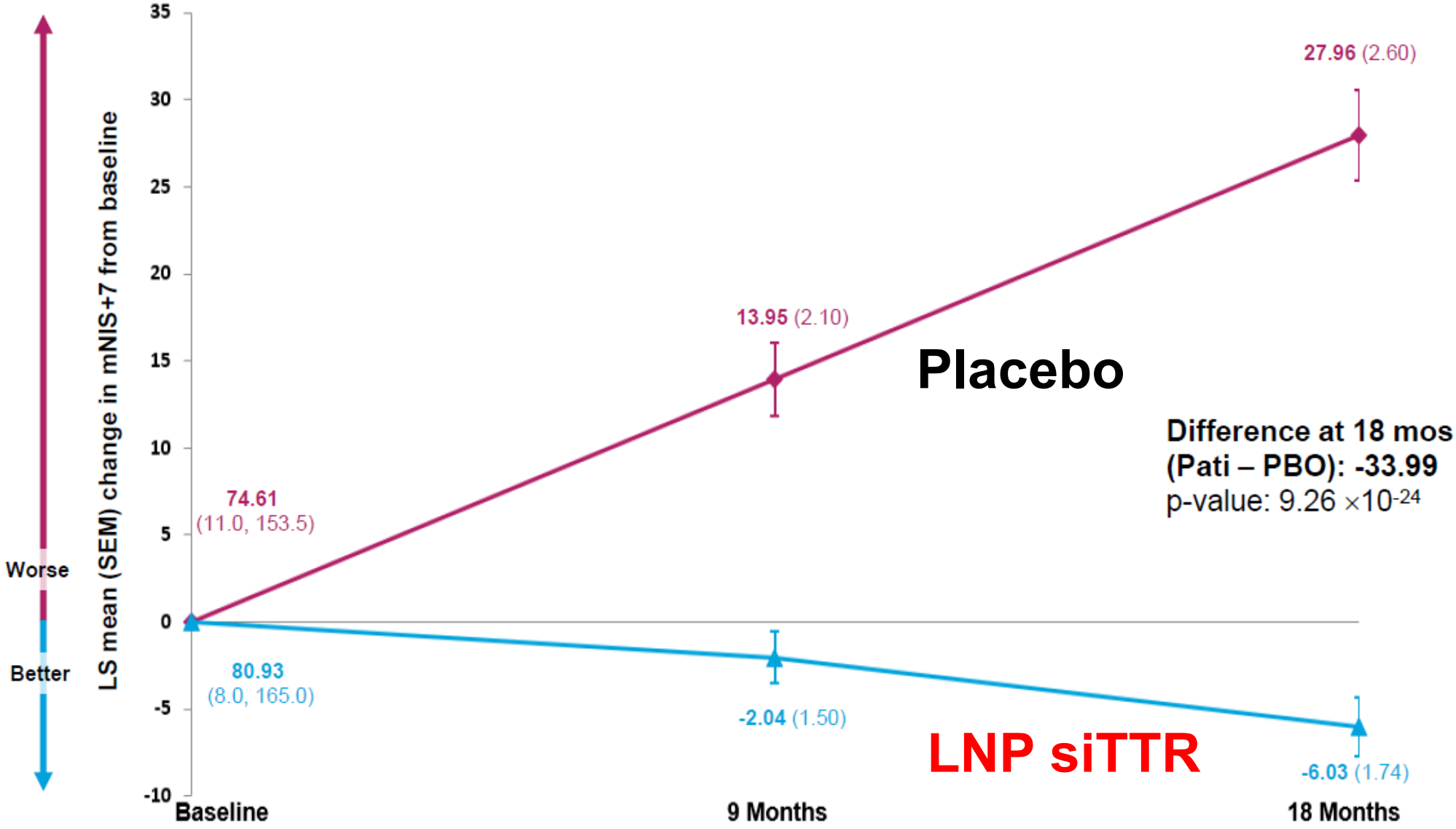


mNIS+7	**Modified neuropathy impairment score**
Norfolk QOL-DN	**Patients perception of neuropathy**
R-ODS	**Rasch-built Overall Disability Scale**
COMPASS-31	**Composite Autonomic Symptom Scale-31 (autonomic nervous system)**

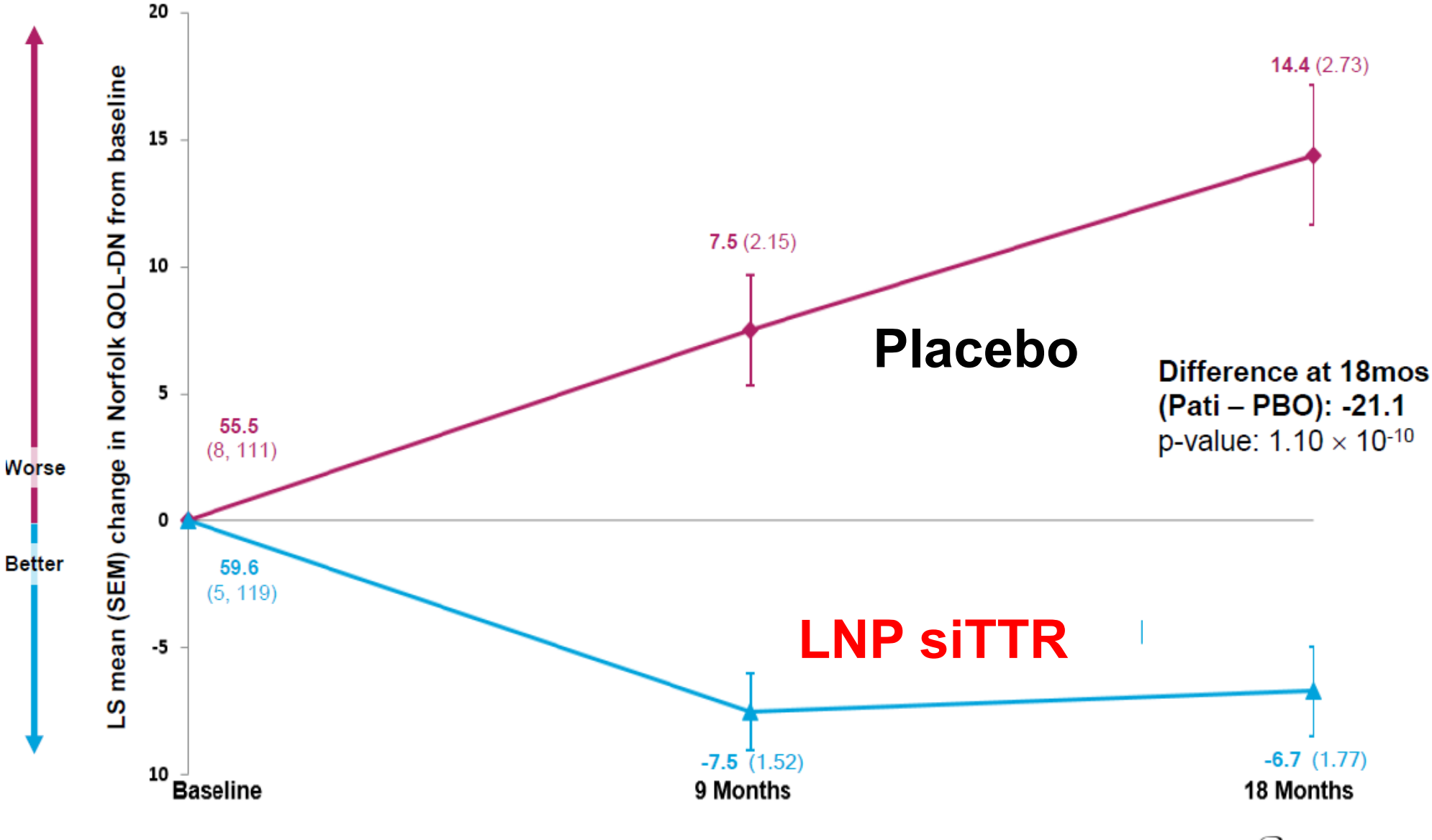
LNP siTTR (Patisiran) Phase 3 Study Results: Serum TTR Reduction



LNP siTTR (Patisiran) Phase 3 Study Results: LNP siTTR Results in Improvement in Neural Impairment Score (mNIS+7)



LNP siTTR (Patisiran) Phase 3 Study: LNP siTTR Results In Improvement in Quality of Life (Norfolk QOL-DN)



LNP siTTR (Patisiran) Phase 3 Trial Results Announced September 20, 2017: Hit Primary Endpoint and All Secondary Endpoints!

Primary Endpoint (18 mo.)		p-value
mNIS+7	Neuropathy improvement score better than placebo	9.26×10^{-24}

Secondary Endpoints (18 mo.)		p-value
Norfolk-QoL	Quality of life better than placebo	1.10×10^{-10}
NIS-W	Muscle strength better than placebo	1.40×10^{-13}
R-ODS	Overall disability scale better than placebo	4.07×10^{-16}
10MWT	Gait speed better than placebo	1.88×10^{-12}
mBMI	Nutritional status better than placebo	8.83×10^{-11}
COMPASS-31	Autonomic muscle function better than placebo	0.0008

Patisiran is a stabilizing, possibly curative therapy for a previously fatal disease

LNP siTTR (Patisiran; tradename Onpattro) approved by FDA Aug 10, 2018 for treatment of hATTR amyloidosis

First FDA approval of siRNA-based gene therapy drug

This is a big deal. Not only can we halt the progression of an hereditary disease, we can actually reverse the accumulated damage. Dramatically demonstrates the power of gene therapies.

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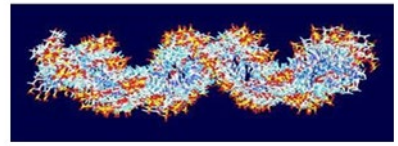
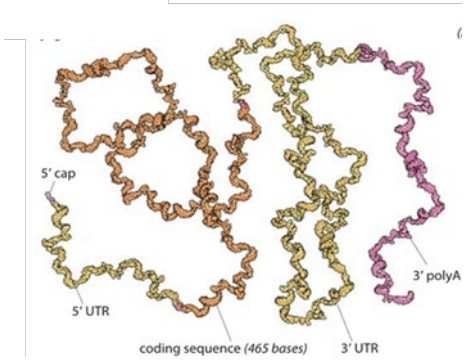
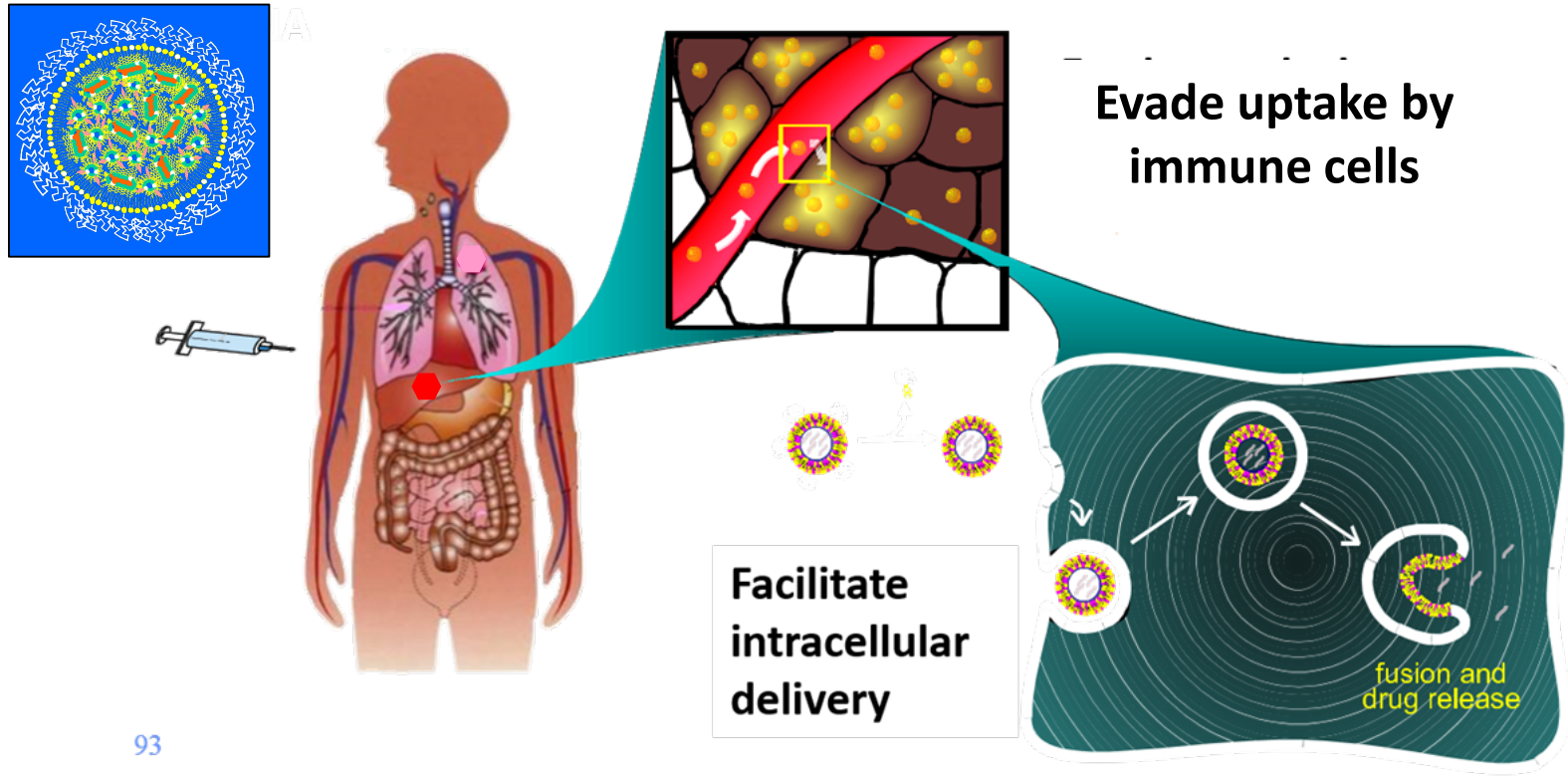




Does the LNP Technology Developed for siRNA Enable mRNA-Based Gene Therapies?

Package mRNA in LNP



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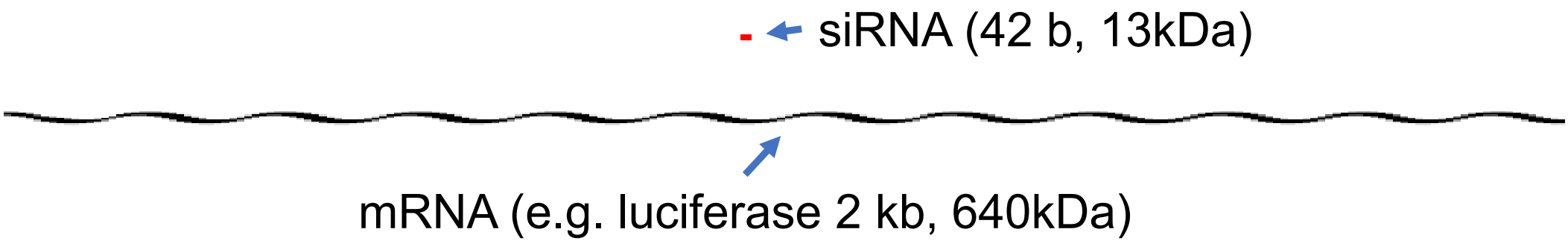
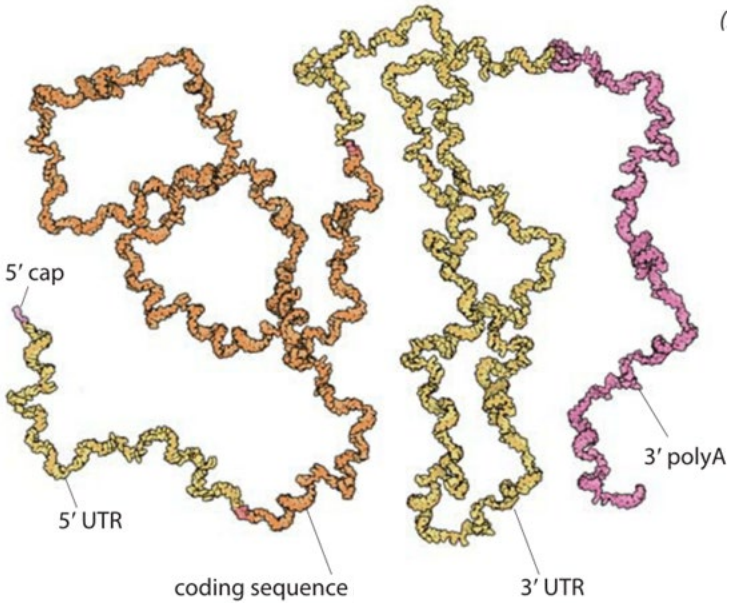


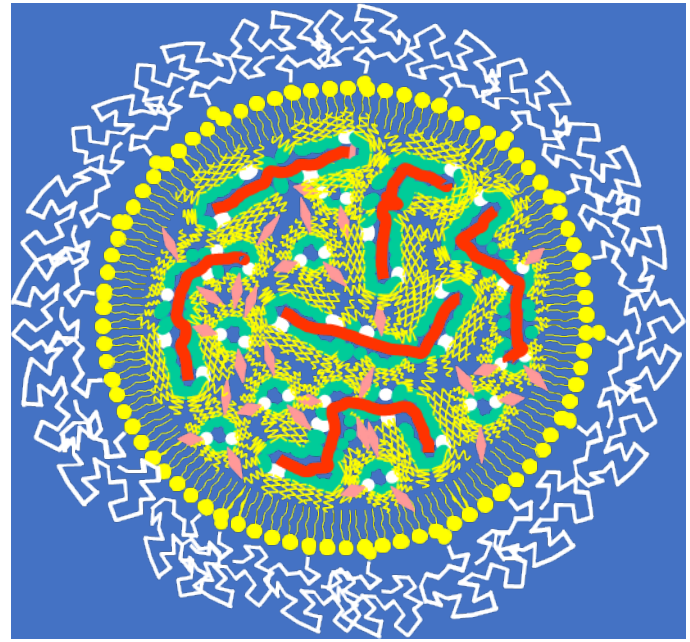
- Does ethanol dilution/rapid mixing allow efficient encapsulation of much larger mRNA in LNP?
- Are LNP containing ionizable cationic lipids such as MC3 effective for delivering mRNA to produce protein in hepatocytes?

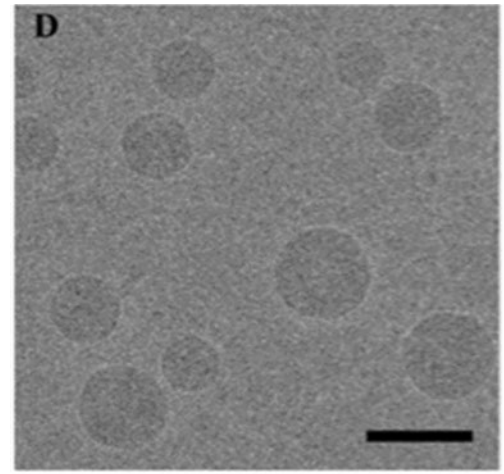
LNP Formulations of mRNA Can Be Generated Using Ionizable Cationic Lipids and Rapid Mixing-Ethanol Dilution Techniques

mRNA



+ **Lipid**

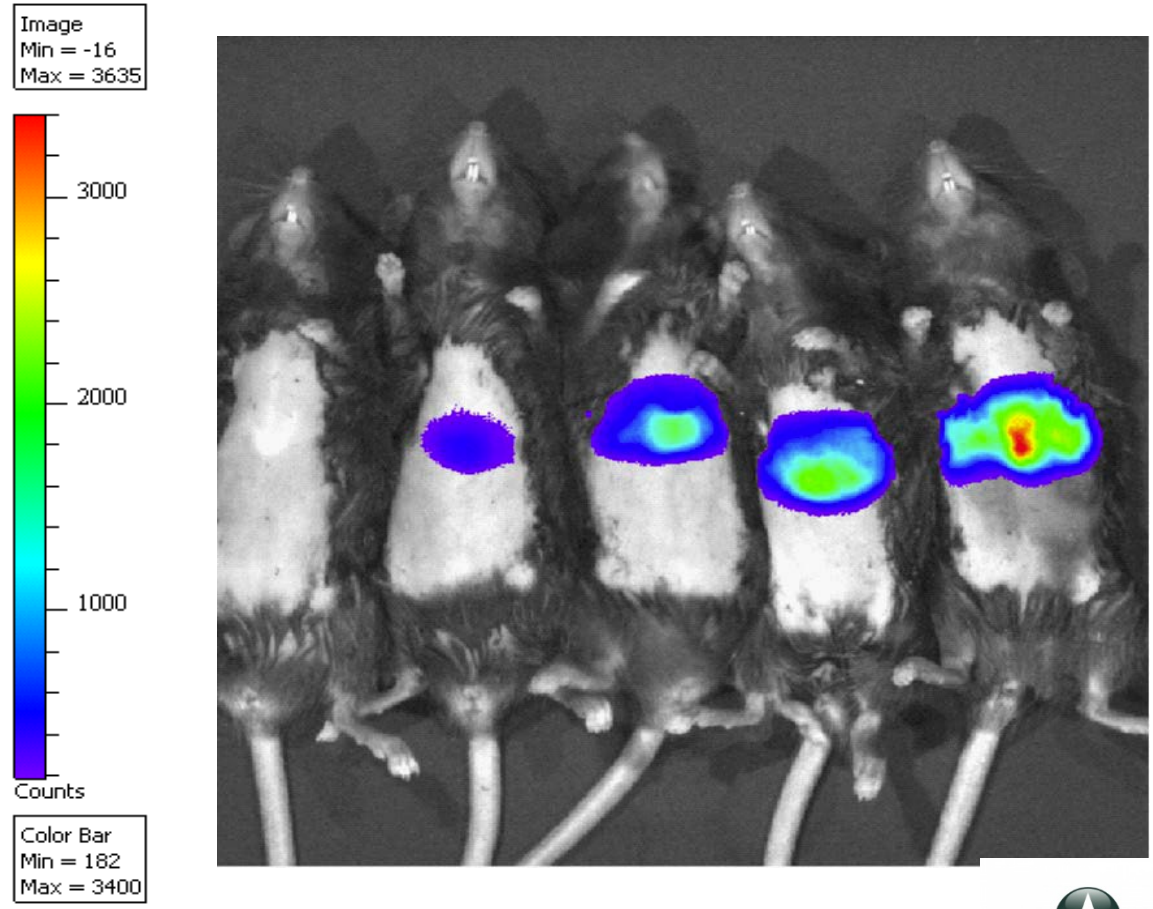




LNP Luc-mRNA Containing MC3 Result in Expression of Luciferase in Liver Following i.v. Administration

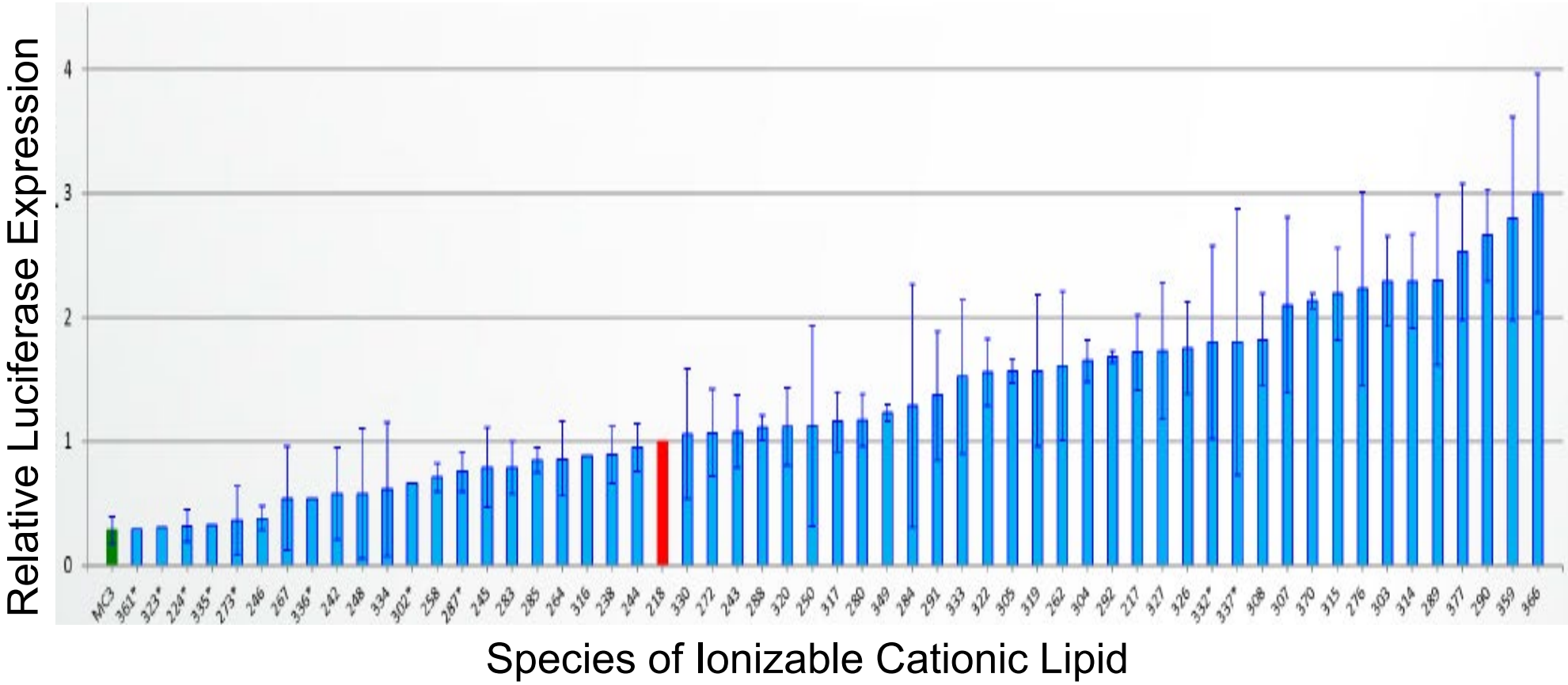
Modified (pseudouridine) mRNA required to see significant protein expression

- Live imaging at 4 h post-dosing (i.v)
- Luciferase expression primarily in liver
- Luciferase expression proportional to mRNA dose (0.1-3.0 mg/kg)



ACUITAS THERAPEUTICS

We (Acuitas) Have Screened Over 300 Ionizable Cationic Lipids Using the Luc Model to Develop 3rd Generation Lipids



3rd generation cationic lipids result in >20-fold improvement in gene expression levels in the liver for LNP mRNA systems

ACUITAS THERAPEUTICS

Serendipity: We (Acuitas) Were Approached by Drew Weissman (U Penn) Who Needed a Delivery System to Enable mRNA Vaccines

Drew Weissman

Drew had worked for many years with Katarin Kariko to explore the potential of mRNA therapeutics as vaccines, together they discovered that by modifying mRNA they could reduce immune activation and increase gene expression

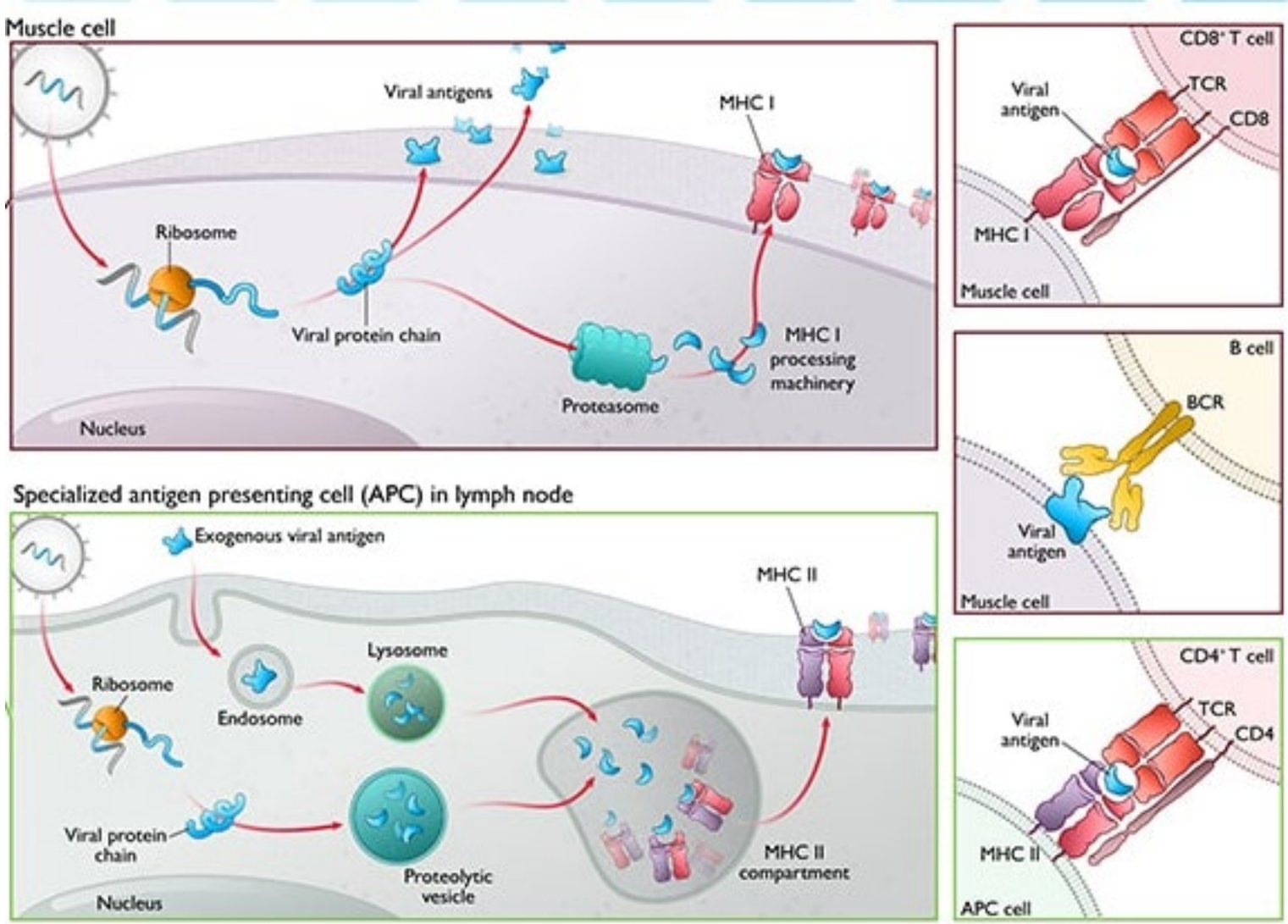
Katalin Kariko

Katalin Kariko had moved to BioNTech (Germany) in 2013 to further develop mRNA vaccines

"We have a delivery problem. How do we get mRNA coding for viral proteins into muscle and immune cells in vivo?"

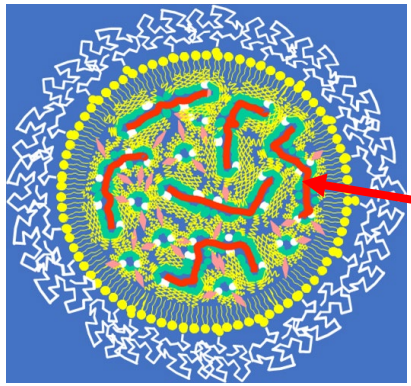
ACUITAS THERAPEUTICS

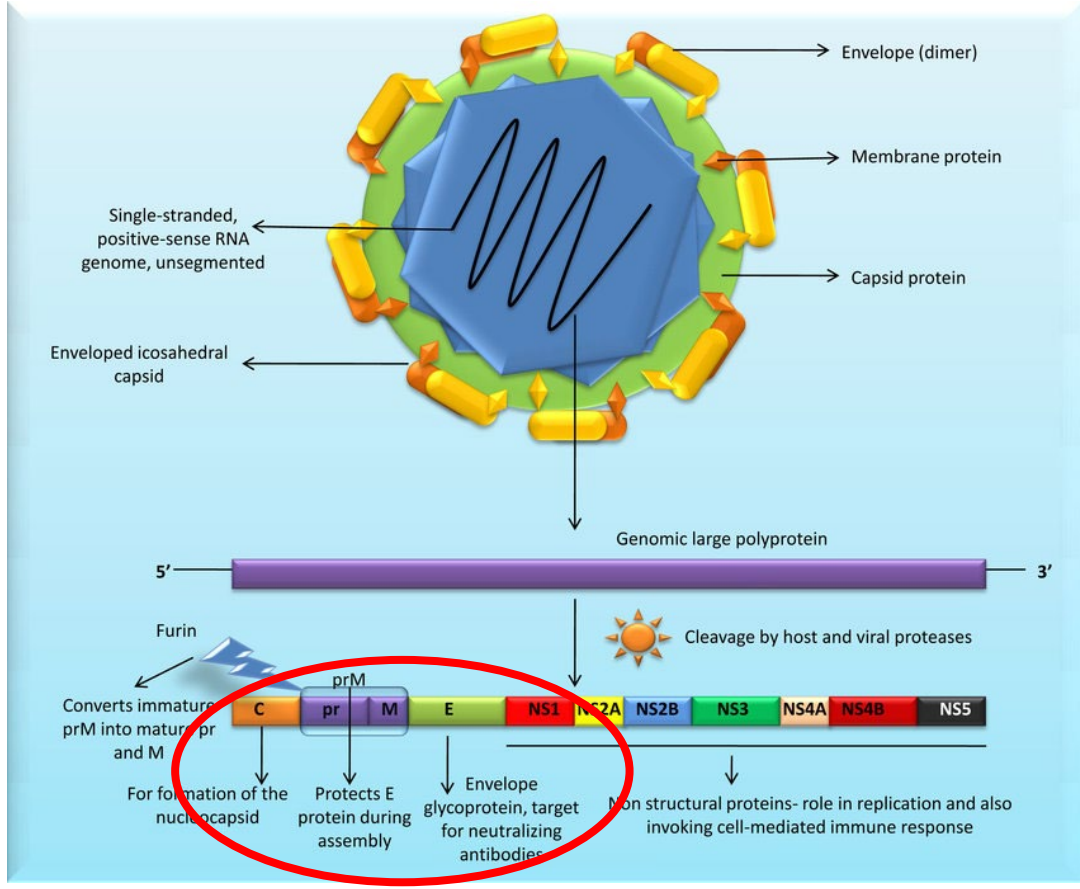
Drew Needed a Delivery System to Enable mRNA Vaccines



Zika Virus Vaccine

Microcephaly

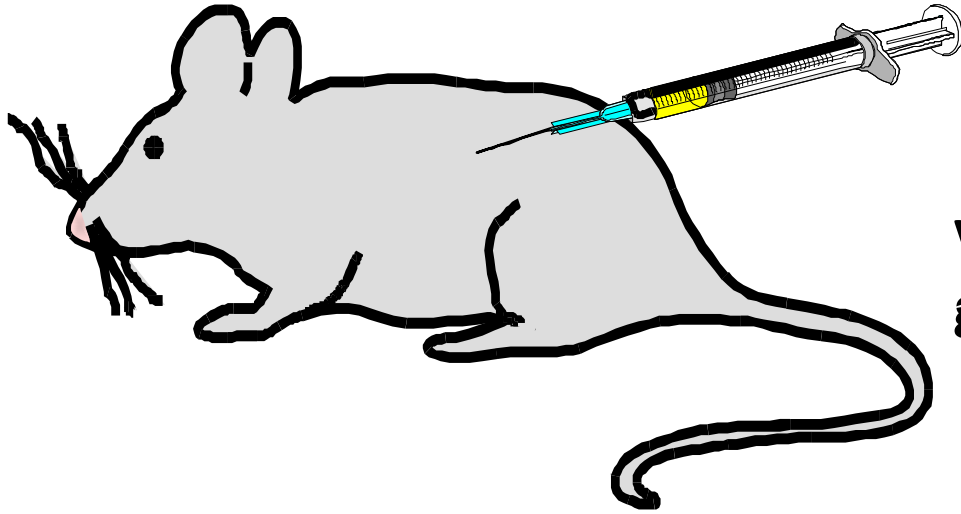




mRNA for ZIKV prM-E (Zika virus pre-membrane and envelope glycoprotein)

Pardi, Weissman et al. Nature 543, 248 (2017)

LNP mRNA Systems As Vaccines (I.D. Administration) Provide Total Protection Against Zika Virus

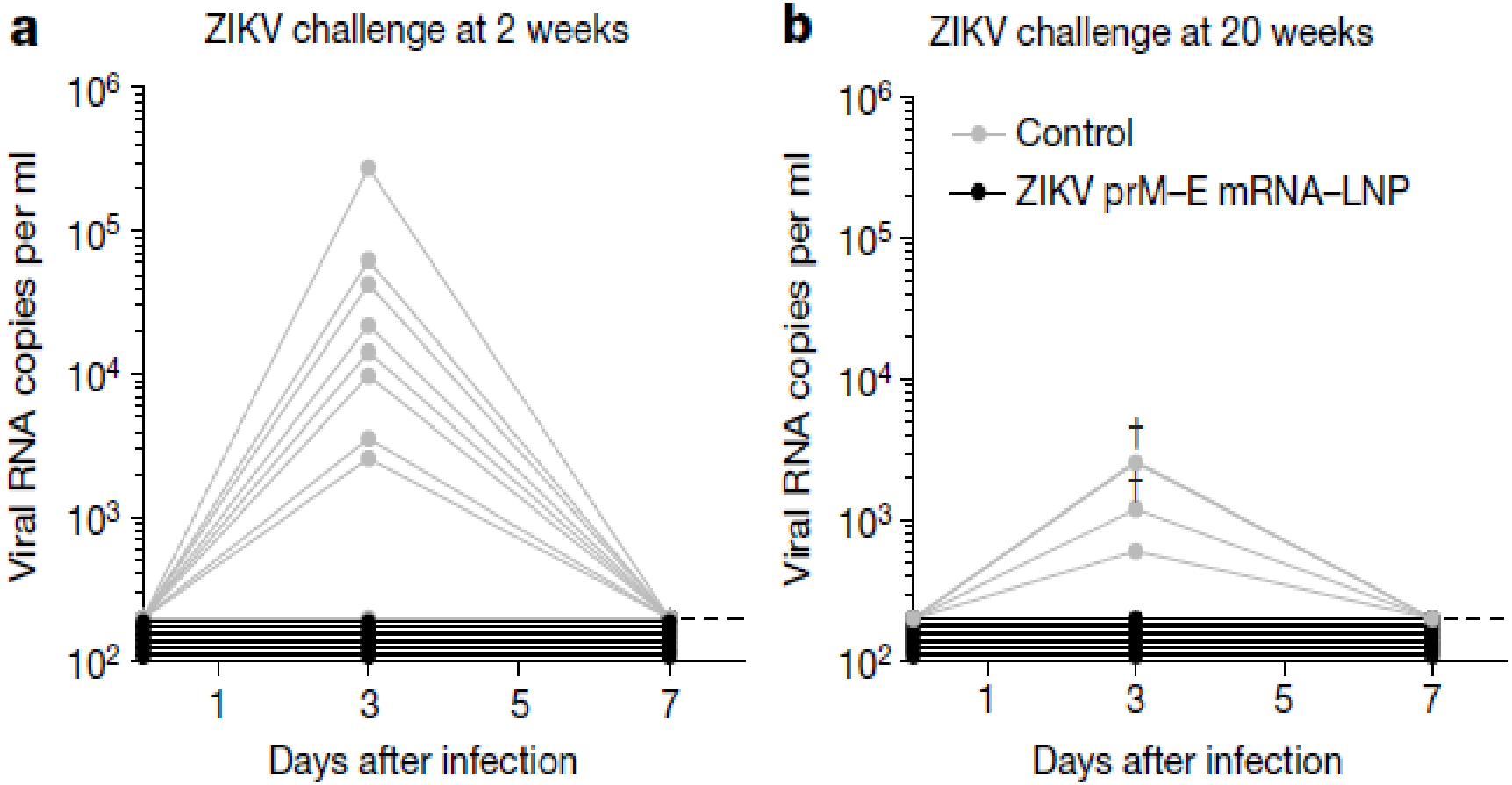


Inject LNP mRNA ZIKV prM-E (Zika virus pre-membrane and envelope glycoprotein)

Time 0 – inject i.d. 1.4 mg/kg LNP ZIKV prm-E mRNA
Challenge at 2 weeks or 20 weeks – inject i.v. 200 PFU ZIKV

Pardi, Weissman et al. Nature 543, 248 (2017)

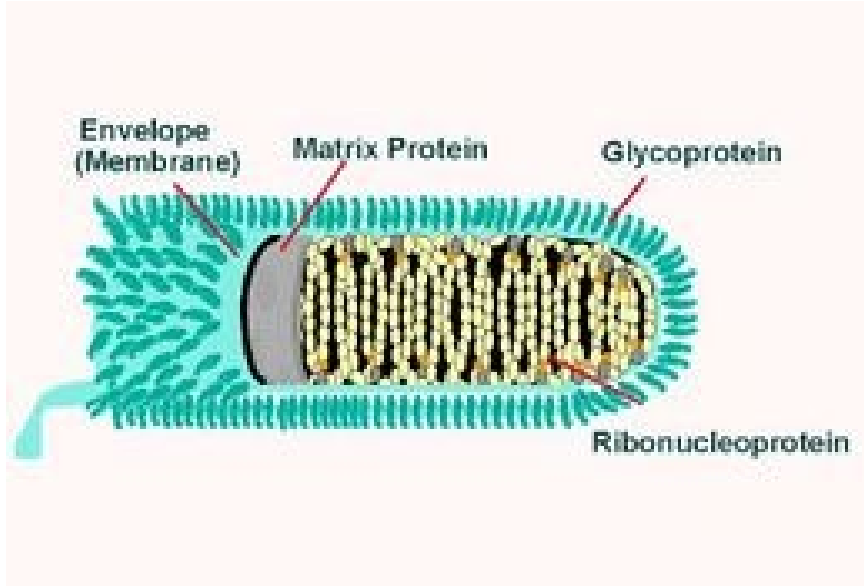
LNP mRNA anti-ZIKV Vaccine Provides Total Protection Against Zika Virus Infection in Mouse Model

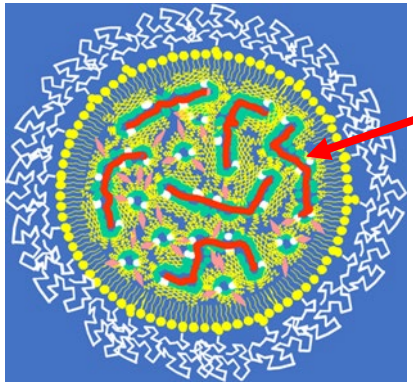


Pardi, Weissman et al. Nature 543, 248 (2017)

ACUITAS THERAPEUTICS

Jan 2020: Acuitas Partner CureVac Announces Effective LNP mRNA Rabies Vaccine





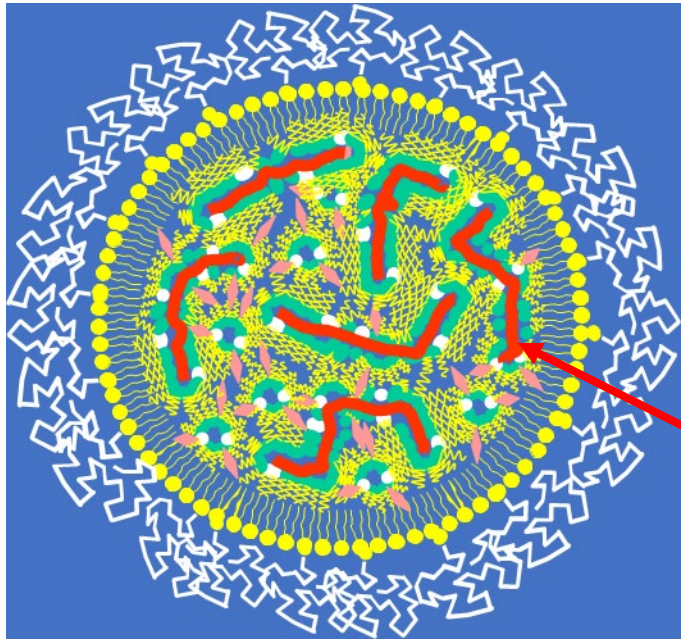
mRNA for rabies glycoprotein

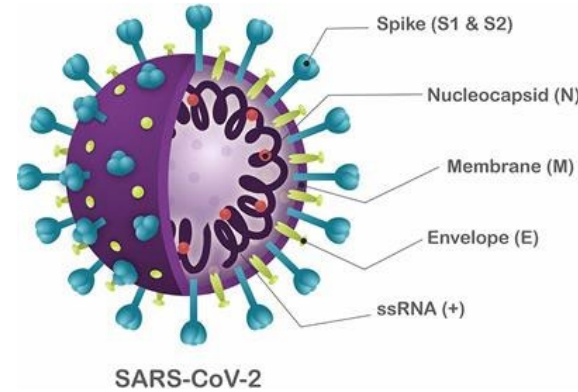
PRESS RELEASE

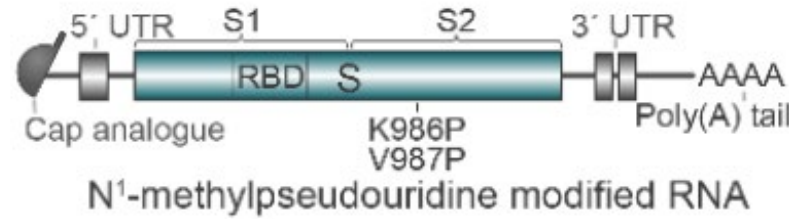
January 7, 2020: CureVac Announces Positive Results in Low Dose – 1 µg – Rabies Vaccine Clinical Phase 1 Study

ACUITAS THERAPEUTICS

Jan 2020: Acuitas Partner BioNTech Initiates LNP mRNA COVID-19 Vaccine Program With Pfizer







mRNA coding for the SARS-CoV-2 spike glycoprotein

Acuitas had begun working with BioNTech to develop influenza vaccines. BioNTech was also working with Pfizer. All efforts switched to a COVID-19 vaccine in January, 2020

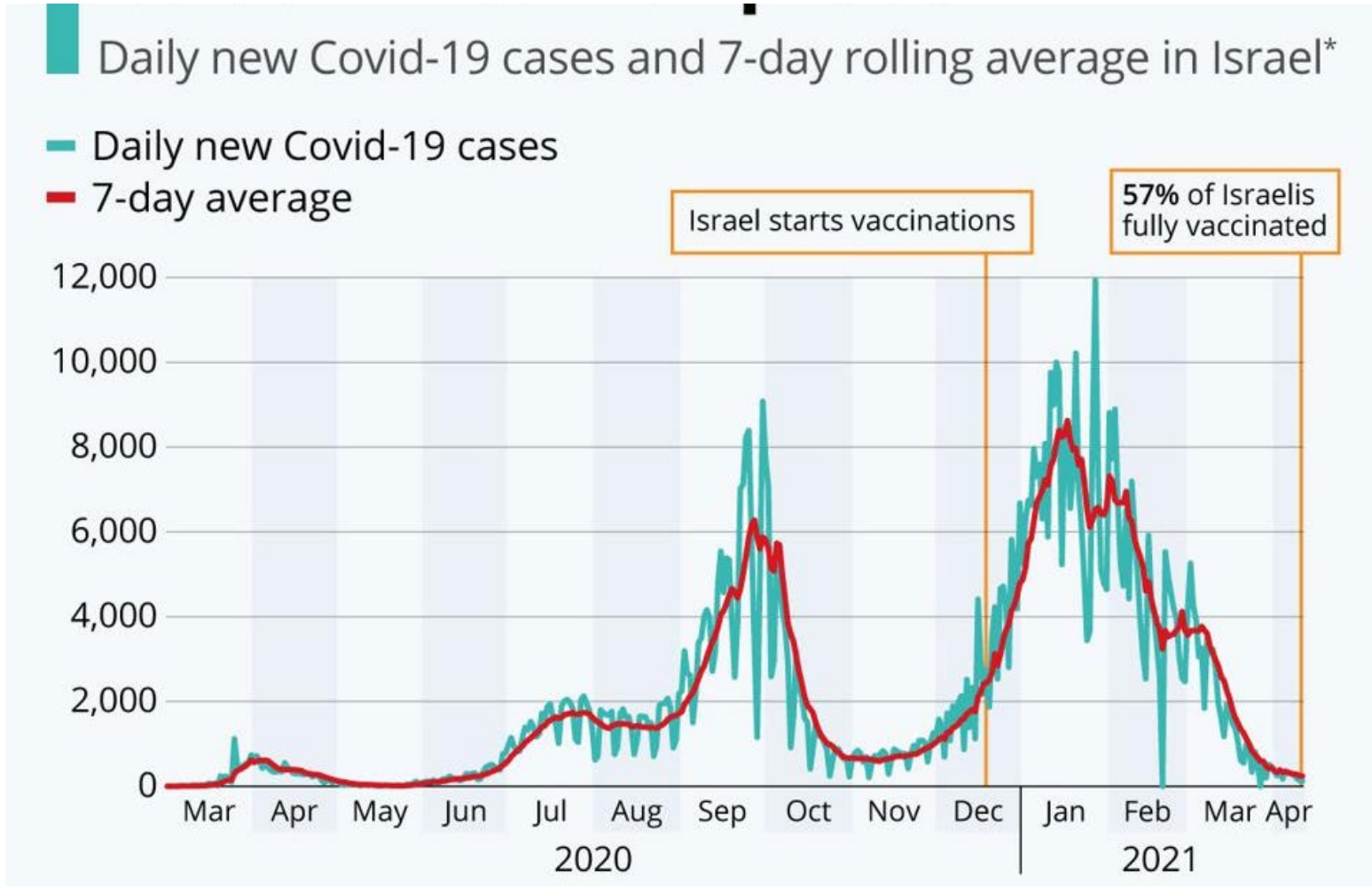
Pfizer And BioNTech Conclude Phase 3 Study Of Covid-19 Vaccine Candidate, Meeting All Primary Efficacy Endpoints

Press release Wednesday, November 18, 2020 - 06:59am

- Primary efficacy analysis demonstrates BNT162b2 to be 95% effective against COVID-19 beginning 28 days after the first dose;170 confirmed cases of COVID-19 were evaluated, with 162 observed in the placebo group versus 8 in the vaccine group
- Efficacy was consistent across age, gender, race and ethnicity demographics; observed efficacy in adults over 65 years of age was over 94%
- Safety data milestone required by U.S. Food and Drug Administration (FDA) for Emergency Use Authorization (EUA) has been achieved
- Data demonstrate vaccine was well tolerated across all populations with over 43,000 participants enrolled; no serious safety concerns observed; the only Grade 3 adverse event greater than 2% in frequency was fatigue at 3.8% and headache at 2.0%
- Companies plan to submit within days to the FDA for EUA and share data with other regulatory agencies around the globe
- The companies expect to produce globally up to 50 million vaccine doses in 2020 and up to 1.3 billion doses by the end of 2021

Approved by USA, UK, Canada, EU for emergency use December 2020
There is little doubt that LNP mRNA systems will play a major role in ending the COVID-19 pandemic!

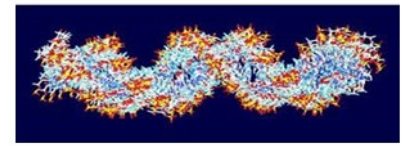
Real World Efficacy: Israel's Vaccine Rollout Curbs Covid-19 Spread



There is little doubt that LNP mRNA systems will play a major role in ending the COVID-19 pandemic!

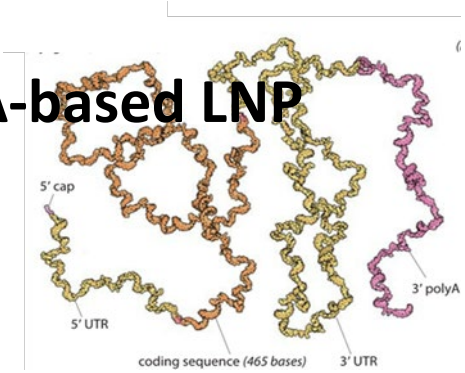
Lipid Nanoparticles That Enable Gene Therapies

- Design of LNP systems for delivery of nucleic acid polymers (1995-2020)
- The Patisiran (Onpattro) story (1995-2012): development of an siRNA-based LNP drug to treat hereditary amyloid transthyretin (hATTR) amyloidosis



  - ➢LNP siRNA program: gene silencing in the liver
  - ➢hATTR amyloidosis: the disease
  - ➢Clinical results: Patisiran

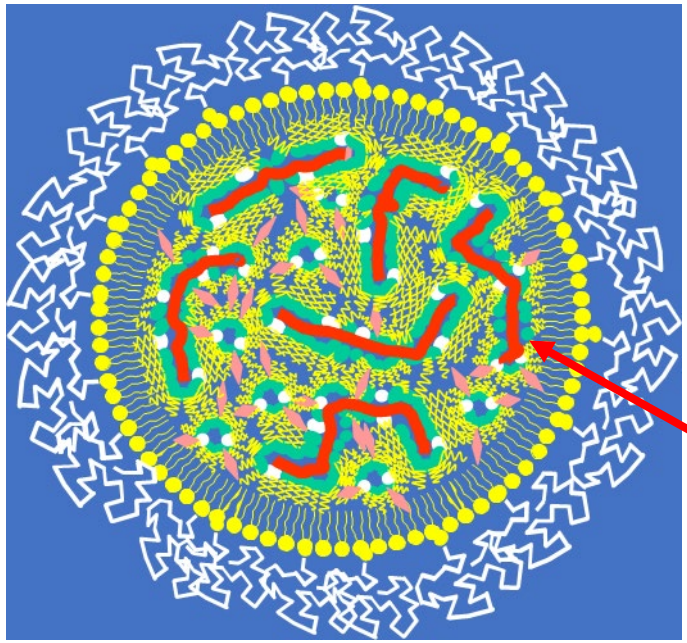
- The BNT162b2 story (2012-2020): development of an mRNA-based LNP drug as a COVID-19 vaccine

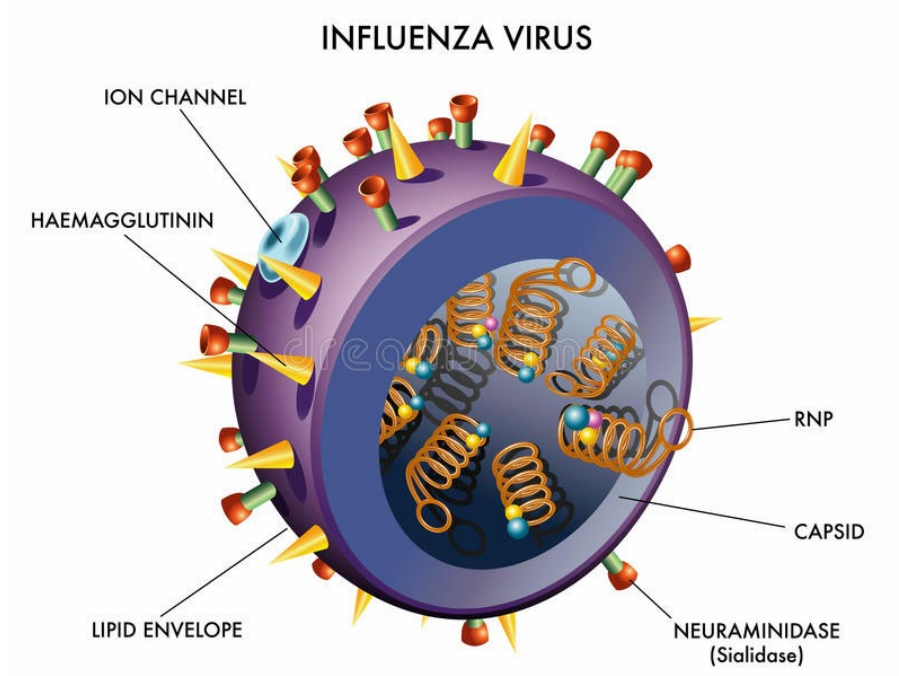


  - ➢LNP mRNA program: gene expression in the liver
  - ➢Clinical results: COVID-19 vaccine

It's been quite a journey!

Universal Flu Vaccine?





LNP mRNA encoding haemagglutinin

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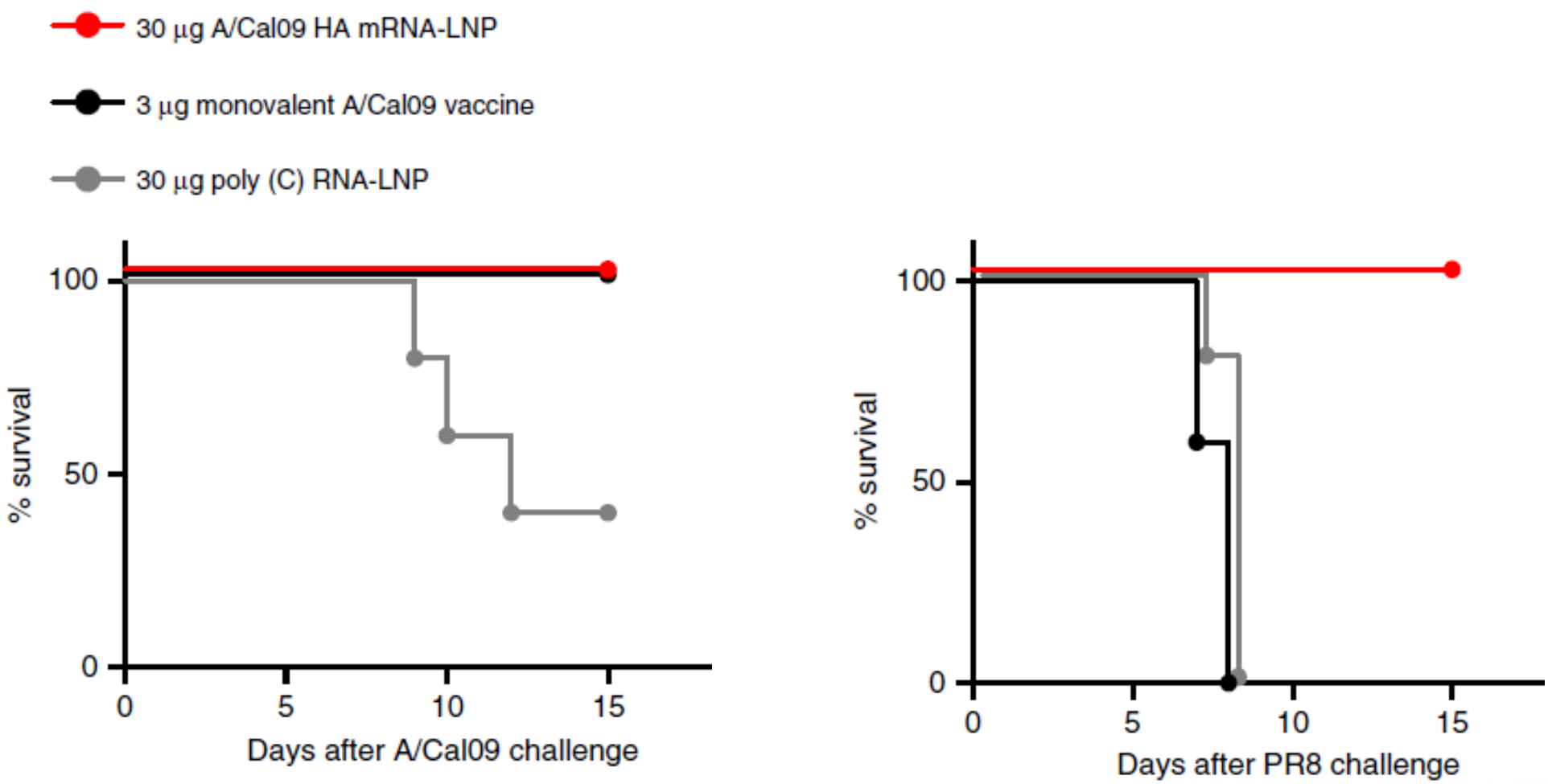
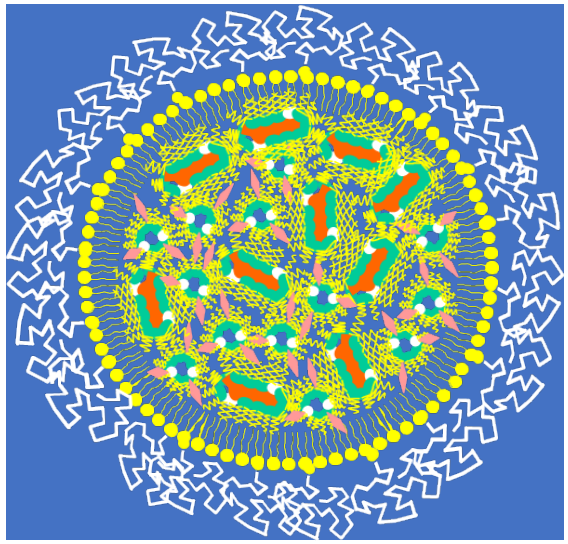
LNP mRNA Encoding HA From A/California /07/2009 Virus Protects Against A/Cal09 and A/Puerto Rico/B/1934 Influenza Virus



Pardi, Weissman et al. Nature Comm (2018

ACUITAS THERAPEUTICS

But Vaccine Applications are Just the Tip of the Iceberg, LNP mRNA Technology Will Enable a Multitude of Gene Therapies



Infectious Diseases (Vaccines):

- COVID-19
- Universal influenza vaccine
- HIV
- Zika
- Malaria, etc

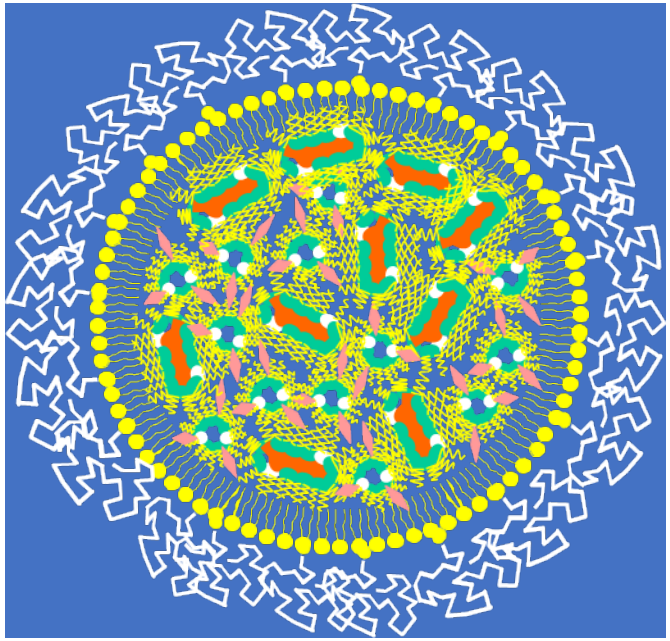
Chronic diseases

- Cancer
- Heart disease
- Alzheimer's, etc

Inherited diseases

- Sickle cell anemia
- Huntington's disease
- Cystic fibrosis, etc

The Future



Note extremely rapid and precise way in which LNP gene therapies can be devised and implemented:

- **Identify protein to be silenced/expressed/edited**
- **Produce siRNA/mRNA**
- **Encapsulate in LNP**
- **New medicine available in a time on the order of weeks**

ACKNOWLEDGEMENTS

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Carl Hansen

Arbutus

Ammen Sandhu

Ian MacLachlan

James Heyes

Alnylam

Mark Tracy

Akin Akinc

Martin Maier

Mano Manoharan

UBC Chemistry

Marco Ciufolini

UBC Brain Research

Brian MacVicar

Ravi Rungta

UBC Biochemistry

Chris Tam

Dominik Witzigmann

Genc Basha

Valentina Francia

Igor Zhigaltsev

Harrison Fan

Jay Kulkarni

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Drew Weissman