The Creation of RNA Targeting Technology

December 2019

Forward Looking Language Statement

This presentation includes forward-looking statements regarding our business, financial guidance and the therapeutic and commercial potential of SPINRAZA® (nusinersen), TEGSEDI® (inotersen), WAYLIVRA® (volanesorsen) and Ionis' technologies and products in development, including the business of Akcea Therapeutics, Inc., Ionis' majority owned affiliate. Any statement describing Ionis' goals, expectations, financial or other projections, intentions or beliefs is a forward-looking statement and should be considered an at-risk statement. Such statements are subject to certain risks and uncertainties, particularly those inherent in the process of discovering, developing and commercializing medicines that are safe and effective for use as human therapeutics, and in the endeavor of building a business around such medicines. Ionis' forward-looking statements also involve assumptions that, if they never materialize or prove correct, could cause its results to differ materially from those expressed or implied by such forward-looking statements. Although Ionis' forward-looking statements reflect the good faith judgment of its management, these statements are based only on facts and factors currently known by Ionis. As a result, you are cautioned not to rely on these forward-looking statements. These and other risks concerning Ionis' programs are described in additional detail in Ionis' annual report on Form 10-K for the year ended December 31, 2018 and our most recent Form 10-Q quarterly filing, which are on file with the SEC. Copies of these and other documents are available at www.ionispharma.com.

In this presentation, unless the context requires otherwise, "Ionis," "Company," "we," "our," and "us" refers to Ionis Pharmaceuticals and its subsidiaries.

IONIS

Presenters

Stanley T. Crooke, M.D., Ph.D.
Chief Executive Officer
and Chairman

Brett P. Monia, Ph.D.
Chief Operating Officer

C. Frank Bennett, Ph.D.
Senior Vice President
Research & Neurology

Eric Swayze, Ph.D.
Vice President
Chemistry

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Introduction

Stanley Crooke, M.D., Ph.D.

Chief Executive Officer and Chairman

Agenda

• Introduction	*Stan*
• Optimization of Antisense	*Eric*
• Enhancing Productive Delivery *in vivo* (LICA)	*Frank*
• New Routes of Local Delivery	*Frank*
• Commercially Attractive Oral Delivery	*Brett*
• Bridging the Gap Between the Gene and the Patient	*Brett*
• New Advances in Core Antisense Research	*Stan*
• Conclusions	*Stan*
• *Q&A*	*All*

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Ionis: The Leader in RNA-Targeted Drug Discovery Technology

Key Messages

- Advances in the technology and incorporated into our pipeline enhance the value of every medicine
- Advances in our technology are incorporated more rapidly today
- Given the breadth of advances today, we believe we can realize the dream of “designer medicines”

Technology

We Set Out to Create and Advance a New Platform for Drug Discovery

Antisense

- Invest broadly and aggressively in every element necessary to create a new platform
- Identify and solve potential problems
- Persevere in investing in understanding and advancing the technology for the long-term
- Prolong control of the technology by consistently enhancing the performance of drugs based on the technology and effective patenting

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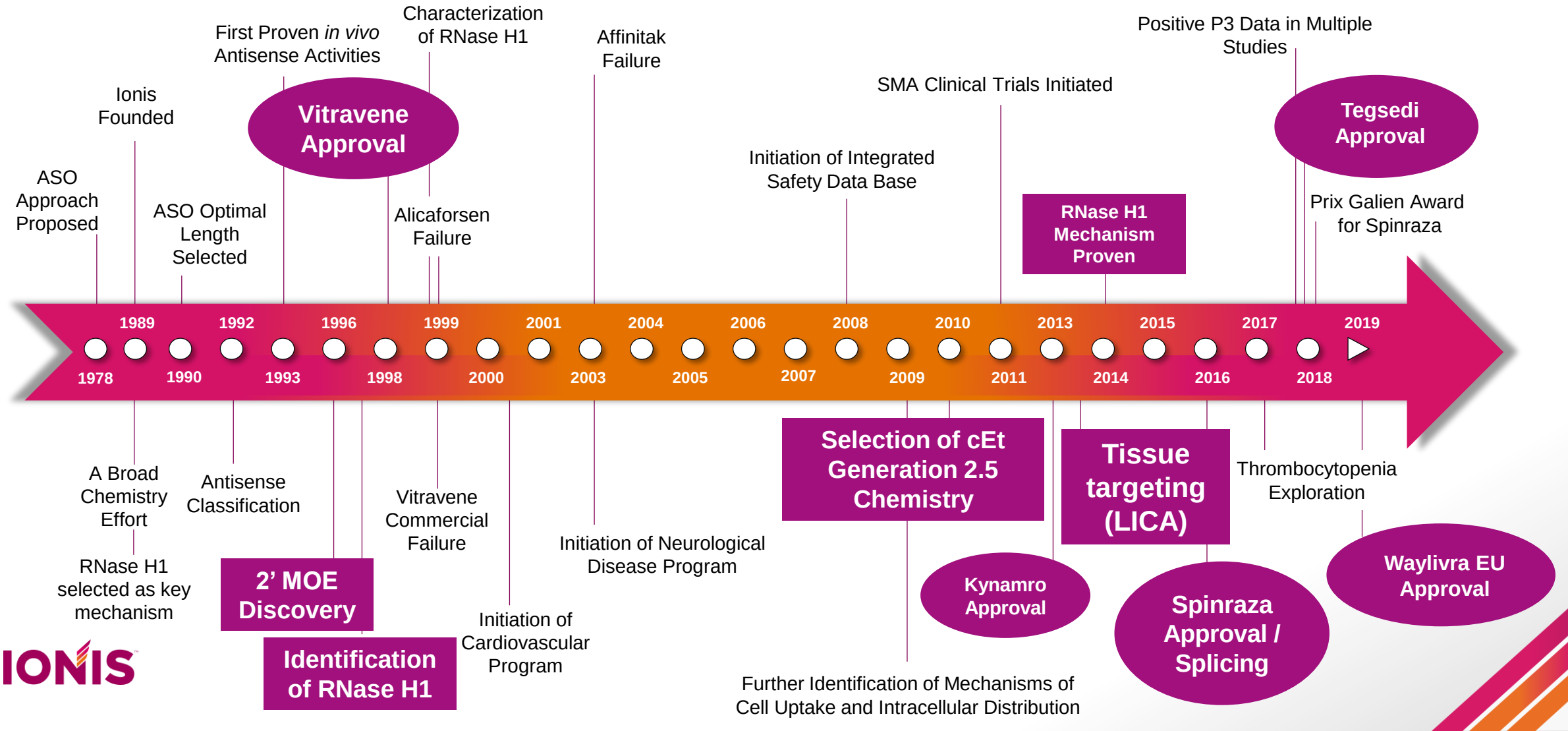
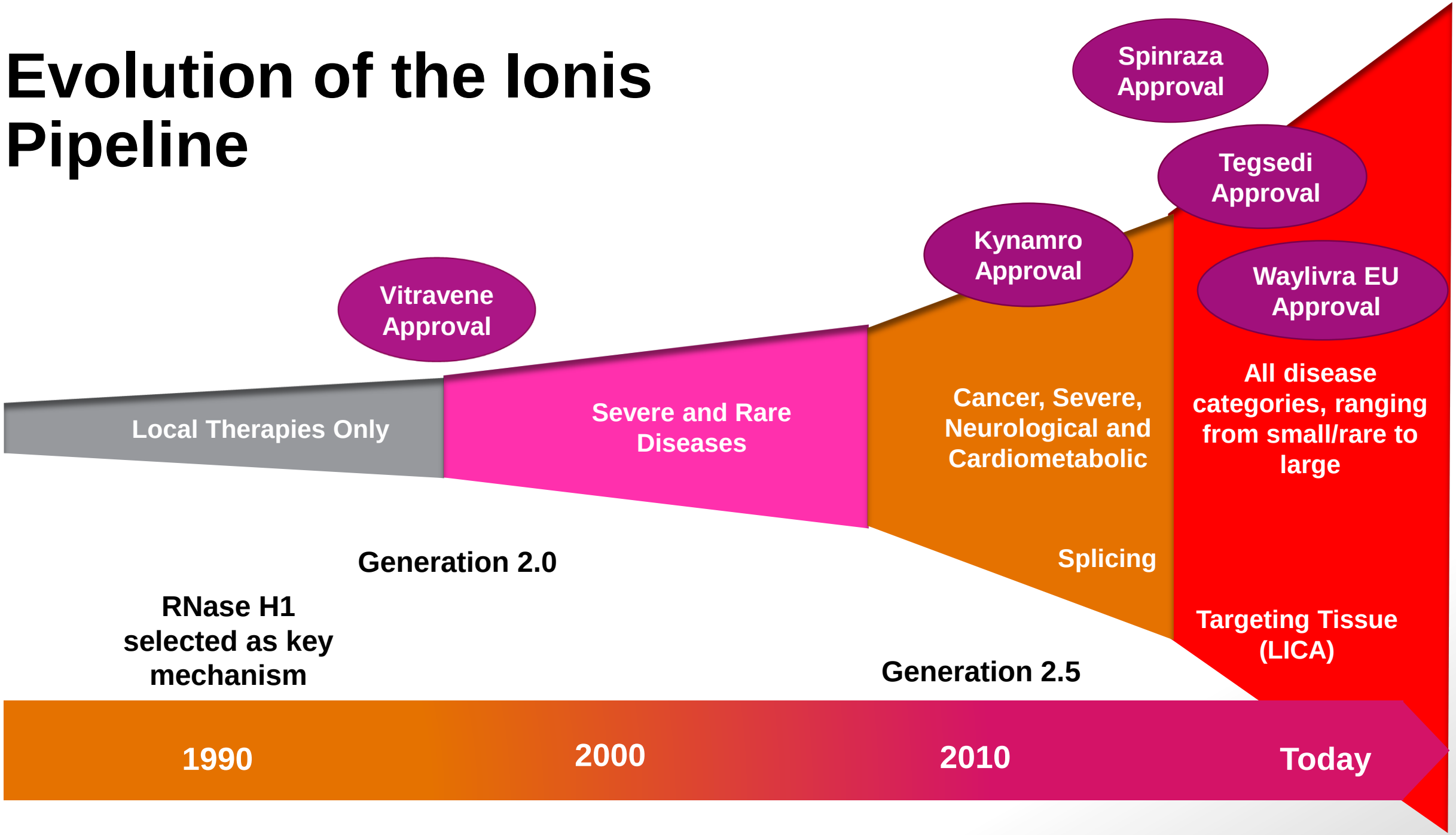
Technology

Timeline: 30 Years of Innovation



Evolution of the Ionis Pipeline



Advancing and Growing Pipeline of Over 40 Medicines

CLINICAL PIPELINE: IONIS-OWNED & PARTNERED MEDICINES

	MEDICINES	INDICATION	PARTNER	PHASE 1	PHASE 2	PHASE 3
NEUROLOGICAL	IONIS-HTT$_{Rx}$ (RG6042)	Huntington's Disease	Roche			
	Tofersen (IONIS-SOD1$_{Rx}$)	ALS	Biogen			
	IONIS-MAPT$_{Rx}$	Alzheimer's Disease	Biogen			
	IONIS-C9$_{Rx}$	ALS	Biogen			
	ION859 (LRRK2)	Parkinson's Disease	Biogen			
	IONIS-DNM2-2.5$_{Rx}$	Centronuclear Myopathy	Dynacure			
RARE	WAYLIVRA® (volanesorsen)	FPL	Akcea			
	AKCEA-TTR-L$_{Rx}$	hATTR Polyneuropathy	Akcea			
	IONIS-GHR-L$_{Rx}$	Acromegaly	Ionis			
	IONIS-PKK-L$_{Rx}$	Hereditary Angioedema	Ionis			
	IONIS-TMPRSS6-L$_{Rx}$	β-Thalassemia	Ionis			
	IONIS-ENAC-2.5$_{Rx}$	Cystic Fibrosis	Ionis			
	ION357	Retinitis Pigmentosa	ProQR			
CARDIOMETABOLIC & RENAL	AKCEA-APO(a)-L$_{Rx}$	CVD	Akcea / Novartis			
	AKCEA–TTR-L$_{Rx}$	ATTR Cardiomyopathy	Akcea			
	AKCEA–ANGPTL3-L$_{Rx}$	NAFLD/Met. Comp.	Akcea / Pfizer			
	AKCEA-APOCIII-L$_{Rx}$	CVD	Akcea / Novartis			
	IONIS-GCGR$_{Rx}$	Diabetes	Ionis / Suzhou-Ribo*			
	IONIS-FXI$_{Rx}$	Clotting Disorders	Bayer			
	IONIS-AGT-L$_{Rx}$	Hypertension	Ionis			
	IONIS-AZ4-2.5-L$_{Rx}$	CVD	AstraZeneca			
	IONIS-FXI-L$_{Rx}$	Clotting Disorders	Bayer			
	ION839	NASH	AstraZeneca			
CANCER	IONIS-AR-2.5$_{Rx}$	Prostate Cancer	Ionis / Suzhou-Ribo*			
	Danvatirsen	Cancer	AstraZeneca			
OTHER	IONIS-HBV$_{Rx}$ /HBV-L$_{Rx}$	Hepatitis B Virus Infection	GSK			
	IONIS-FB-L$_{Rx}$	Complement Mediated Diseases	Roche			

*China rights only

MEDICINES EXPECTED TO ENTER THE CLINIC WITHIN THE NEXT 18 MONTHS

	MEDICINES	INDICATION	PARTNER
NEUROLOGICAL	ION581	Neurodegenerative Disease	Biogen
	ION260	Neurodegenerative Disease	Biogen
	ION283	Lafora Disease	Ionis
	ION373	Neurodegenerative Disease	Ionis
	ION464	Neurodegenerative Disease	Biogen
	ION541	Neurodegenerative Disease	Biogen
RARE	ION663	Severe Disease	Ionis
CARDIOMETABOLIC & RENAL	ION547	Cardiometabolic Disease	Ionis
	ION904	Cardiometabolic Disease	Ionis
	ION224	NASH	Ionis
	ION532	Kidney Disease	AstraZeneca

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	MEDICINES	INDICATION	PARTNER
CANCER	ION929	Cancer	Ionis / Suzhou-Ribo*
	ION537	Cancer	MD Anderson
	ION251	Cancer	Ionis
	ION674	Cancer	Suzhou-Ribo*
	ION736	Cancer	AstraZeneca
OTHER	ION781	Inflammatory Disease	Ionis
	ION253	GI Autoimmune Disease	Janssen

- Ionis consistently adds 3 to 5 new medicines per year
- Preclinical medicines are evaluated in IND-enabling studies for ~12 to 18 months before entering clinical trials

Optimization of Antisense

Eric Swayze, Ph.D.

Vice President, Chemistry and Neuro Drug Development

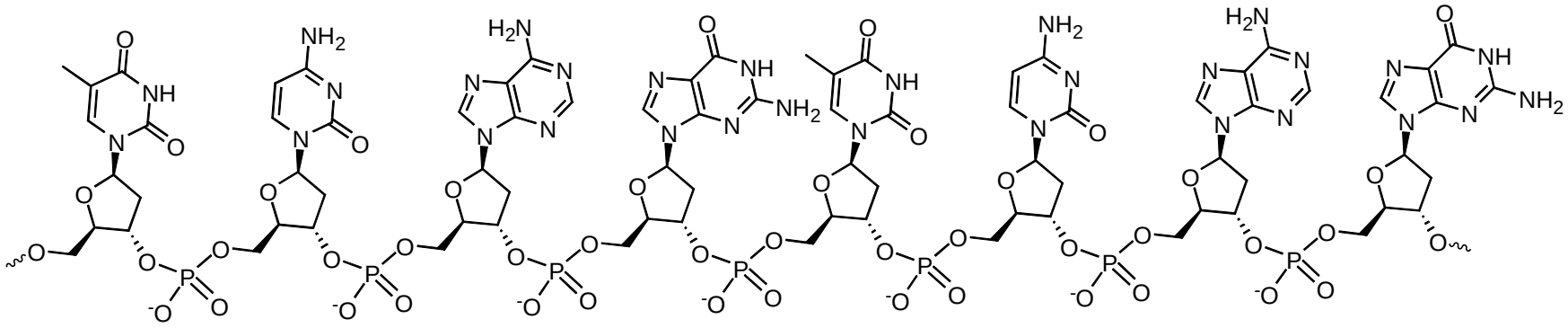
Optimization of Antisense

Leading Chemistry Based Drug Discovery Platform

- Antisense has more in common with small molecule drugs than, for example, gene therapy

- When we started in 1989, there was no oligonucleotide medicinal chemistry

Deoxyribonucleic acid (DNA)



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Optimization of Antisense

Goals of Ionis' Medicinal Chemistry Program

Create a broad program to investigate every modification to

- Improve potency dramatically
- Increase stability to extend dosing interval
- Maximize distribution to intended tissues
- Decrease proinflammatory effects to allow many routes of dosing
- Decrease class toxicities
- Increase scale and quality of ASO manufacturing while reducing cost

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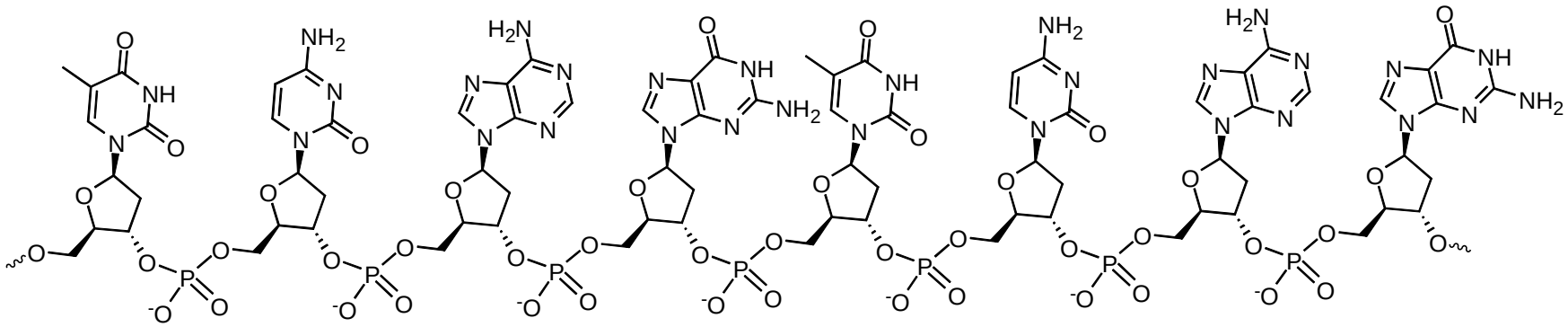
Optimization of Antisense

Initial Focus

- Enhance affinity for RNA targets to increase potency
- Enhance nuclease stability to support less frequent dosing
- Reduce proinflammatory effects
  - Enable dosing via all routes of administration



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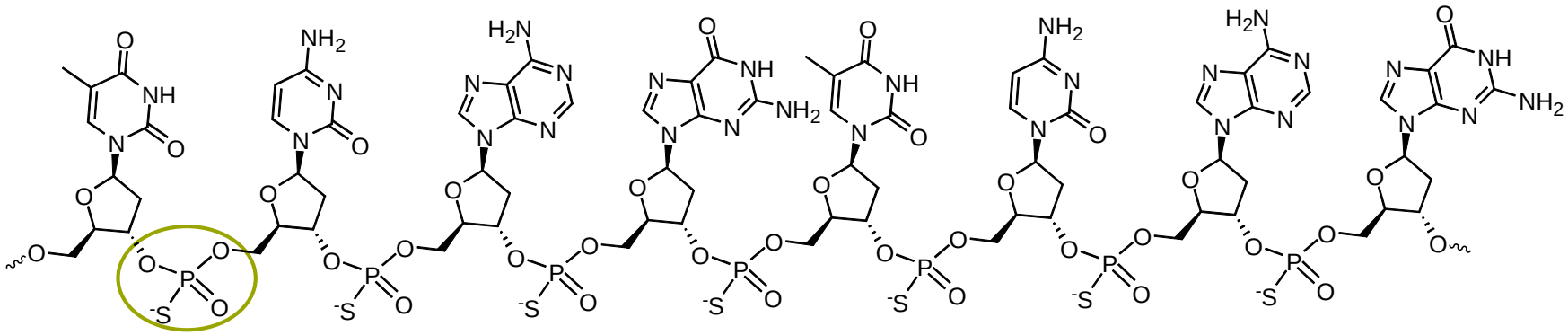
Optimization of Antisense

Why Phosphorothioates

- To increase nuclease resistance – adds stability in cells and animals
- To increase plasma protein binding – facilitates distribution to tissues
  - ASOs are approximately 7,000 Dalton water soluble molecules
  - Without plasma protein binding, ASOs are rapidly cleared by renal filtration
  - Phosphorothioates define the general distribution to organs



Phosphorothioate

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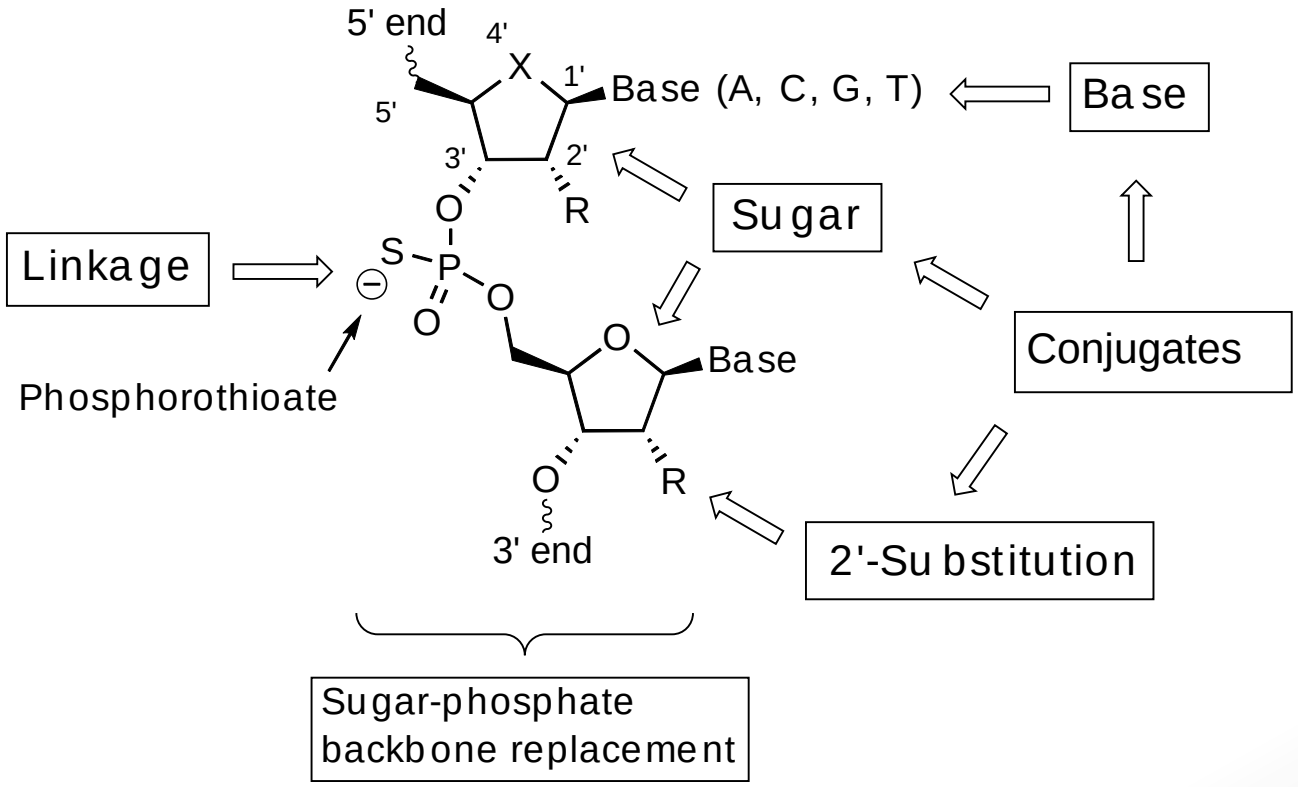
Eckstein, F.; Gindl, H. *FEBS Lett.* **1969**, *2*, 262-264.
Eckstein, F. *Antisense Nucleic Acid Drug Dev.* **2000**, *10*, 117-121.

Optimization of Antisense

Ionis' Medicinal Chemistry Program

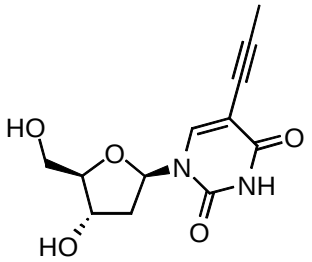
- Antisense is a chemically based drug discovery platform
- We created RNA-targeted medicinal chemistry from scratch



Optimization of Antisense

Modifications We Chose Not to Develop

- Unnatural heterocyclic modifications
  - Known toxicity of metabolites



Shen, et al. *Antisense Nucleic Acid Drug Dev.* **2003**, *13*, 129-142.

Optimization of Antisense

Modifications We Chose Not to Develop

- Unnatural heterocyclic modifications
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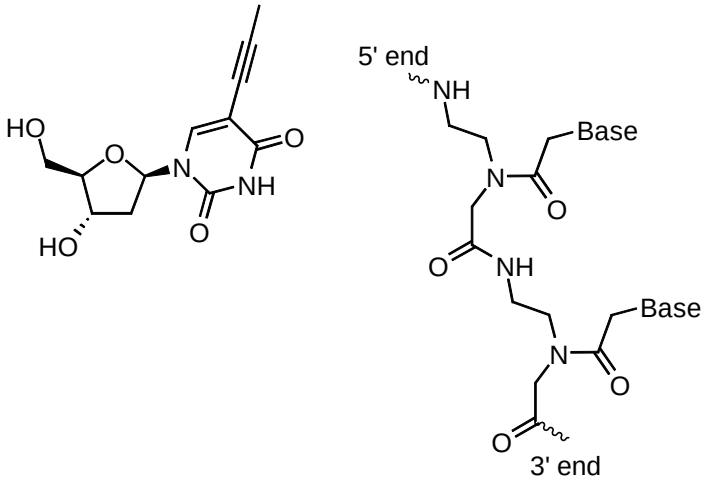
- Neutral backbones
  - Poor solubility and distribution to tissues



Shen, et al. *Antisense Nucleic Acid Drug Dev.* **2003**, *13*, 129-142.
Wancewicz, et al. *J. Med. Chem.* **2010**, *53*, 3919-3926.

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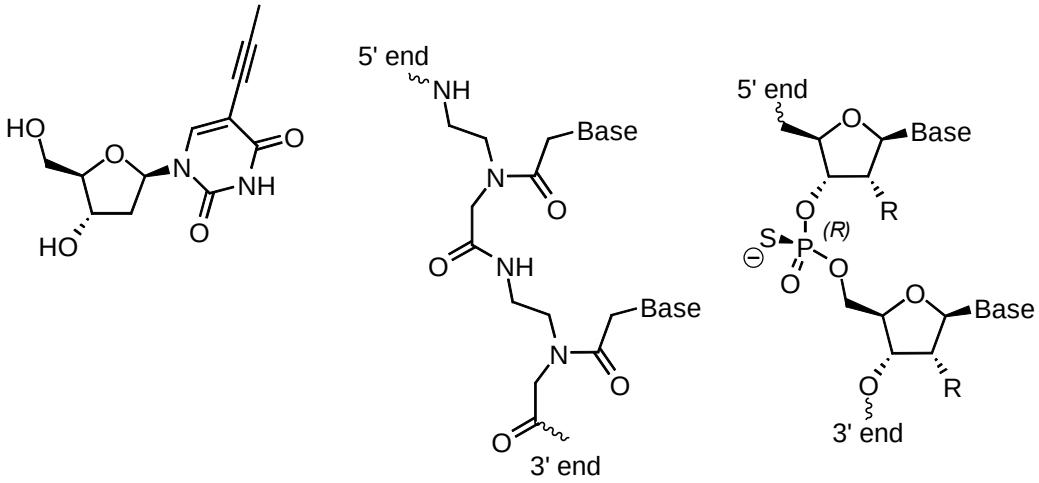
Optimization of Antisense

Modifications We Chose Not to Develop

- Unnatural heterocyclic modifications
  - Known toxicity of metabolites
- Neutral backbones
  - Poor solubility and distribution to tissues
- Chiral phosphorothioate backbones
  - Our extensive investigations have found no added value
  - Greatly increases cost and complexity of manufacture



Shen, et al. *Antisense Nucleic Acid Drug Dev.* **2003**, *13*, 129-142.
Wancewicz, et al. *J. Med. Chem.* **2010**, *53*, 3919-3926.
Wan, et al. *Nucleic Acids Res.* **2014**, *42*, 13456-13468.
Li, et al. *Chem Commun (Camb)* **2017**, *53*, 541-544.

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Optimization of Antisense

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  - Known toxicity of metabolites
- Neutral backbones
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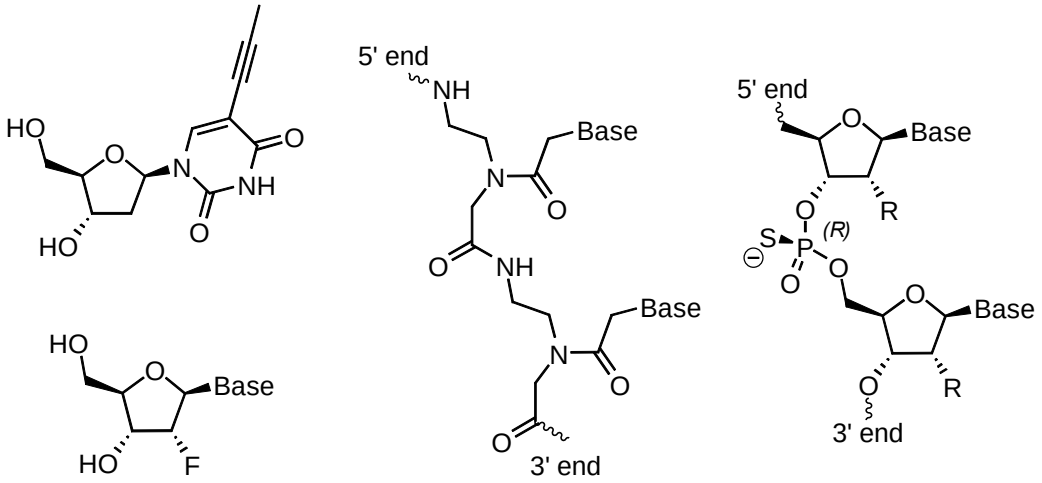
- Chiral phosphorothioate backbones
  - Our extensive investigations have found no added value
  - Greatly increases cost and complexity of manufacture
- 2’-Fluoro nucleosides
  - Poor nuclease stability
  - Known toxicity and incorporation of metabolites into DNA and RNA



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Li, et al. *Chem Commun (Camb)* **2017**, *53*, 541-544.
Shen, et al. *Nucleic Acids Res.* **2015**, *43*, 4569-4578.
Richardson, et al. *Chem. Res. Toxicol.* **2002**, *15*, 922-926.

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Optimization of Antisense

Our Strategy Focused Primarily on the 2'-Position

Known to have the potential to improve affinity for RNA

- Expected to improve potency

Known to reduce susceptibility to nucleases

- Expected to increase distribution and duration of effect

As a bonus, we also observed a decrease in proinflammatory effects

This strategy led to two key chemistries that are used in our drugs today

- Generation 2 Chemistry (MOE)
- Generation 2.5 Chemistry (cEt)

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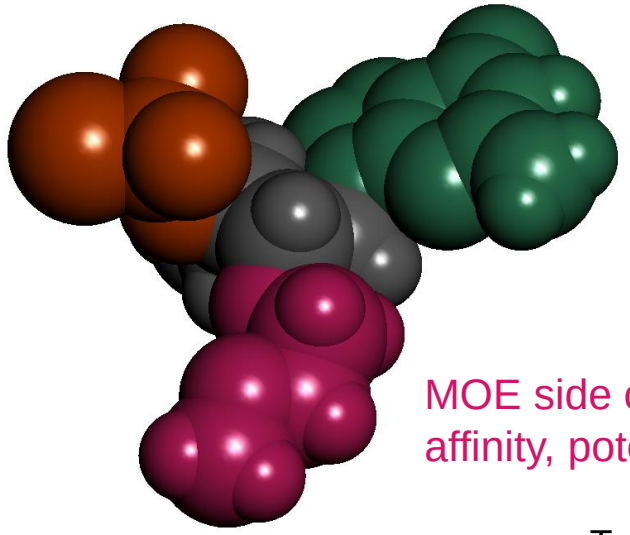
Optimization of Antisense

Benefits of Generation 2 Chemistry (MOE)

Stability of MOE allows use of ***either*** phosphorothioate ***or*** phosphodiester backbone



Heterocyclic Base
Recognizes target RNA

MOE side chain improves binding affinity, potency and safety

Teplova, et al. *Nat. Struct. Biol.* 1999, *6*, 535-539.

- Extends dosing interval by increasing stability
- Increases potency due to improved binding affinity and stability
- Increases safety by decreasing proinflammatory effects and class toxicities

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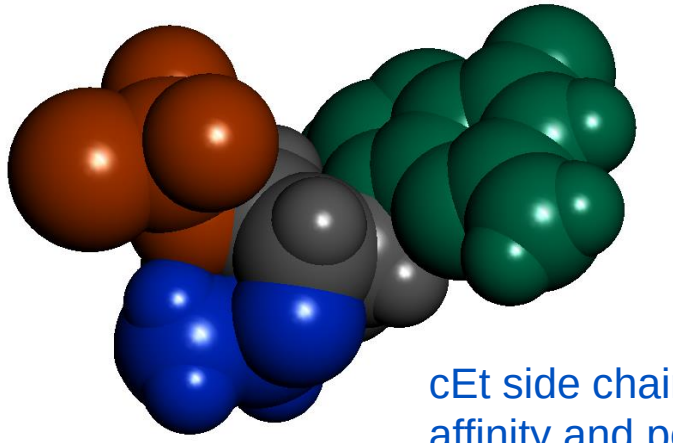
Optimization of Antisense

Benefits of Generation 2.5 Chemistry (cEt)

Stability of cEt allows use of ***either*** phosphorothioate ***or*** phosphodiester backbone



Heterocyclic Base
Recognizes target RNA

cEt side chain improves binding affinity and potency

Pallan, et al. *Chem Commun (Camb)* **2012**, *48*, 8195-8197.

- Further increases potency by greatly increasing affinity
- Maintains extended dosing interval and safety
- This improves therapeutic index, and facilitates activity in tissues with lower accumulation (e.g. tumors)

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Optimization of Antisense

More Recent Focus

Realizing most of the administered dose is wasted – we have **enhanced** the productive **delivery** of **ASOs** to desired **organs** and **cells**

This led to the creation of our **LICA** strategy

Liver **LICA** (GalNAc) is delivering **value** in patients today

- 30x increase in potency
- 16 LICA drugs in our pipeline

Optimizing ASO distribution sets the stage for future gains in performance

- Targeting new tissues with other LICAs
- Optimizing intracellular distribution

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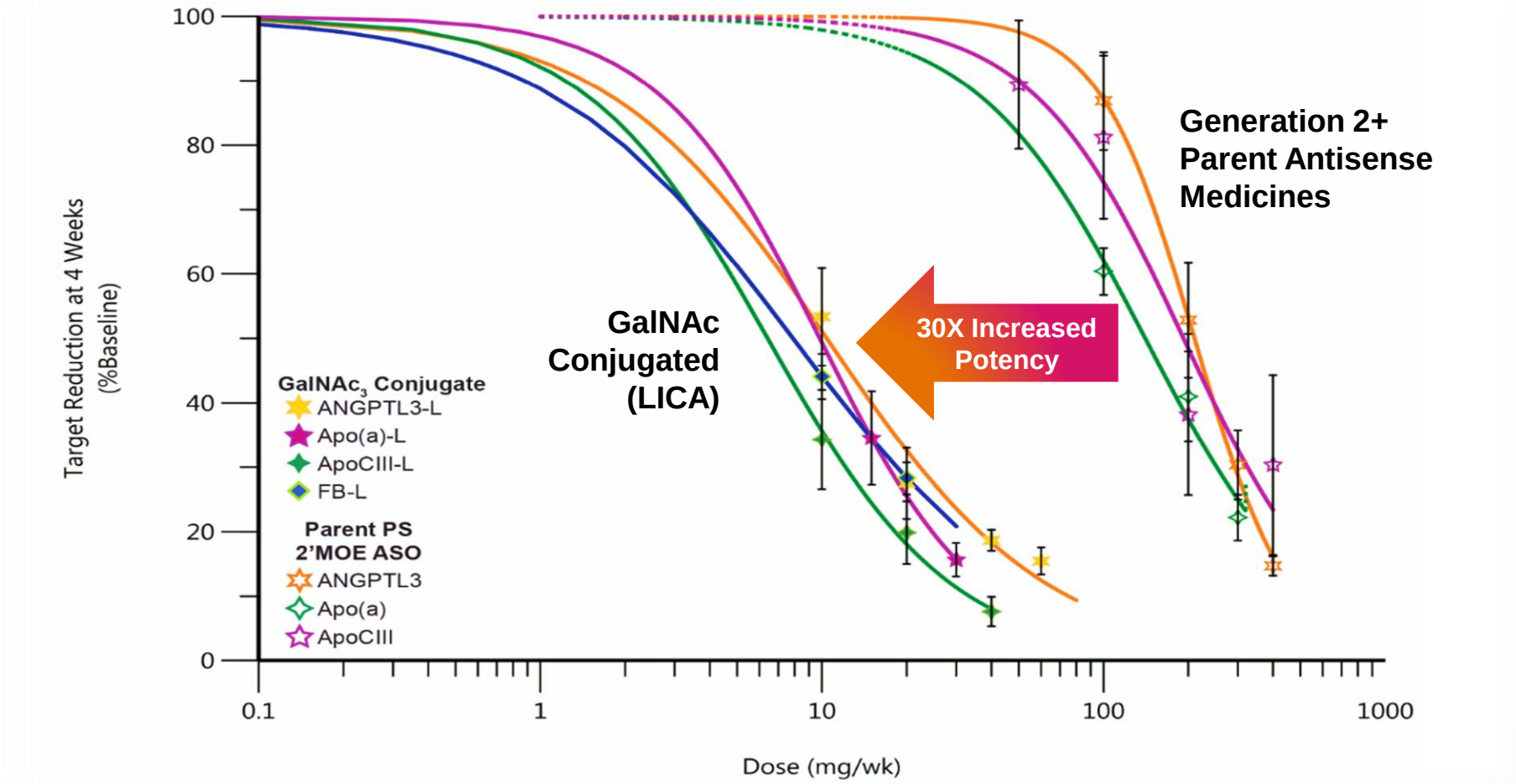
Optimization of Antisense

Liver LICA (GalNAc) Increases Potency of ASOs in Humans by ~30-Fold

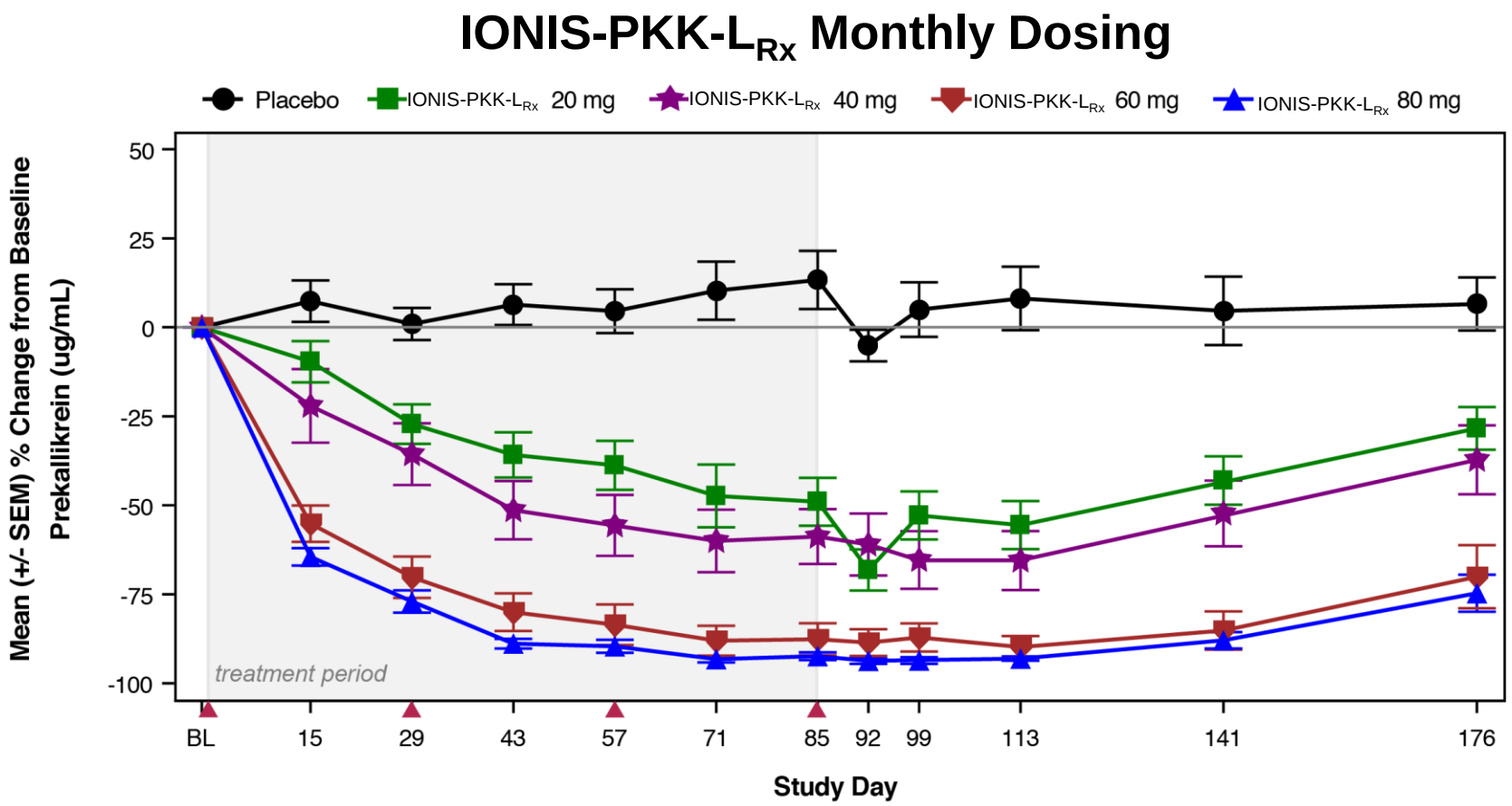


Greater than 30-fold increase in potency of LICAs targeting the liver

Crooke, S., et al. (2018), Nucleic Acid Therapeutics. http://doi.org/10.1089/nat.2018.0753

Optimization of Antisense

Liver LICA (GalNAc) Extends Dosing Intervals and Increases Therapeutic Margins



Long duration of effect supports monthly to quarterly dosing interval

Low dose reduces side effects and improves therapeutic margins

Optimization of Antisense

Growing Liver LICA Safety Database Demonstrates Increased Therapeutic Margins

- **16** in development and we have clinical data available for **> 10** GalNAc ASOs
- **> 1,000** subjects treated in Ionis integrated safety database
  - Treatment duration: 6 to 52 weeks
  - Dose range: 10 mg to 120 mg monthly (ED_{50} for target reduction less than ~5 mg)
- **No effects observed** on kidney, liver, hematological parameters
- **No clinically meaningful changes** in platelet count; no abnormally low values
- **No discontinuations** for AEs
- **No flu** like symptoms
- **Very low incidence** of mild injection site observations

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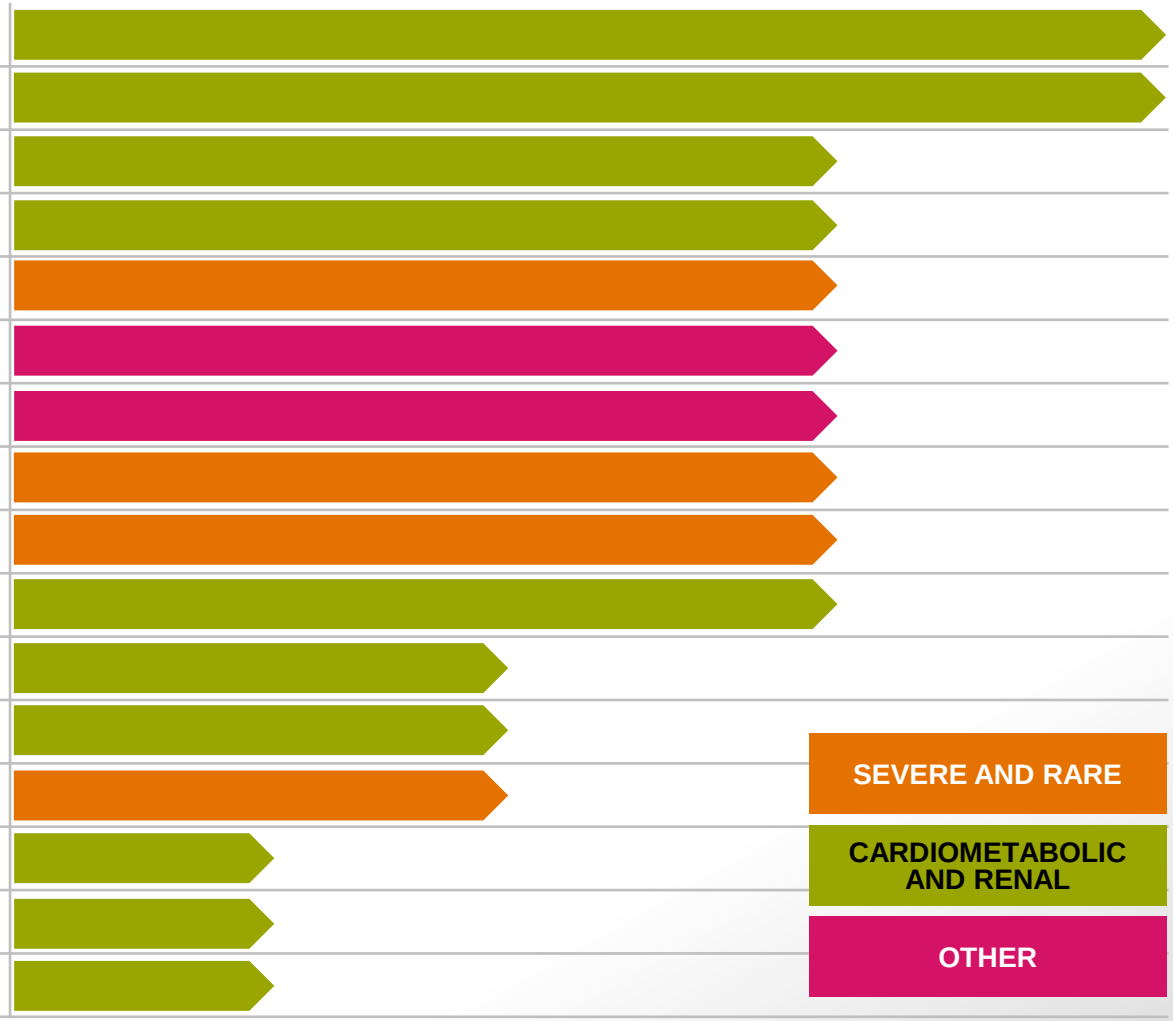
Crooke, ST., et al., (2018) NAT 28(1):10-22

Technology Advances Enhancing the Value of Our Pipeline

16 LIVER-TARGETED LICA MEDICINES IN PIPELINE

MEDICINE	INDICATION
AKCEA-APO(a)-L_{Rx}	Cardiovascular disease
AKCEA-TTR-L_{Rx}	Transthyretin amyloidosis
AKCEA-ANGPTL3-L_{Rx}	Cardiometabolic disorders
AKCEA-APOCIII-L_{Rx}	Cardiovascular disease
IONIS-GHR-L_{Rx}	Acromegaly
IONIS-HBV-L_{Rx}	Hepatitis B virus infection
IONIS-FB-L_{Rx}	Complement-mediated diseases
IONIS-PKK-L_{Rx}	Hereditary Angioedema
IONIS-TMPRSS6-L_{Rx}	β-Thalassemia
IONIS-AGT-L_{Rx}	Treatment-resistant hypertension
IONIS-FXI-L_{Rx}	Clotting disorders
IONIS-AZ4-2.5-L_{Rx}	Cardiovascular disease
ION839	Nonalcoholic steatohepatitis
ION547	Cardiometabolic disease
ION904	Cardiometabolic disease
ION224	Nonalcoholic steatohepatitis



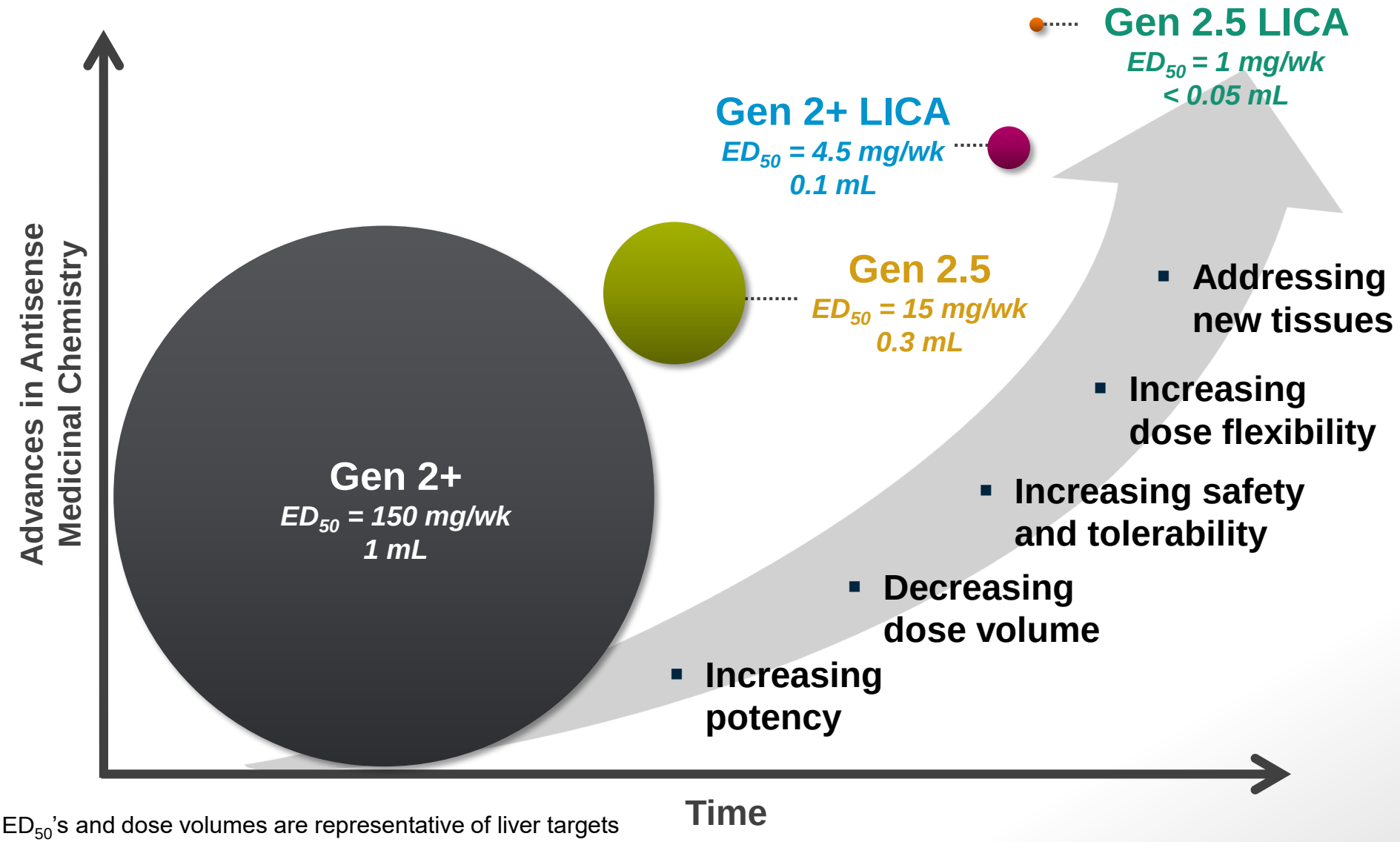
Technology Advances Expand Potential to Address Broad Patient Populations

MEDICINE	INDICATION	PHASE I	PHASE II	PHASE III
AKCEA-APO(a)-L_{Rx}	Cardiovascular disease			
AKCEA-TTR-L_{Rx}	Transthyretin amyloidosis			
AKCEA-ANGPTL3-L_{Rx}	Cardiometabolic disorders			
AKCEA-APOCIII-L_{Rx}	Cardiovascular disease			
IONIS-GHR-L_{Rx}	Acromegaly			
IONIS-HBV-L_{Rx}	Hepatitis B virus infection			
IONIS-FB-L_{Rx}	Complement-mediated diseases			
IONIS-PKK-L_{Rx}	Hereditary Angioedema			
IONIS-TMPRSS6-L_{Rx}	β-Thalassemia			
IONIS-AGT-L_{Rx}	Treatment-resistant hypertension			
IONIS-FXI-L_{Rx}	Clotting disorders			
IONIS-AZ4-2.5-L_{Rx}	Cardiovascular disease			
ION839	Nonalcoholic steatohepatitis			
ION547	Cardiometabolic disease			
ION904	Cardiometabolic disease			
ION224	Nonalcoholic steatohepatitis			

MEDICINES ADDRESSING LARGE PATIENT POPULATIONS

Optimization of Antisense

Advances in Our Technology Substantially Improve the Utility of Our Medicines



ED_{50}'s and dose volumes are representative of liver targets

Optimization of Antisense

Lessons Learned and Importance

- Focused **medicinal chemistry** has greatly impacted antisense technology **performance**
  - Significant increases in potency
  - Extended duration of effect
  - Selective targeting to the desired tissue and cell type
  - Decreased side effects and improved therapeutic margins
- Translated to a **robust RNA targeting** platform technology
- **Advances** support efficiently creating a large pipeline of transformative medicines
  - Targeting multiple tissues
  - Across a range of disease areas
  - Large, medium, and rare commercial opportunities
- Provides a knowledge base to **improve the platform even more**
- And this is just **the beginning**

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Ionis Created, Validated, and Continues to Advance an Efficient RNA-Targeting Platform

DELIVERING GREAT VALUE TODAY AND BEYOND

Most Direct Route from Gene to Medicine	**Uniquely specific and broadly applicable**
Efficient Discovery & Early Development	**Dramatically reduced cost and increased success through clinical proof of concept**
Consistent Performance Within Chemical Classes	**Higher success rate in discovery and development**
Advances Rapidly Incorporated Across the Entire Pipeline	**Chemistry, manufacturing, formulation, analytical methods**
Consistent Pipeline Growth	**Robust, mature, diversified pipeline, adding 3-5 new medicines per year**

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Enhancing Productive Distribution to Tissues In Vivo

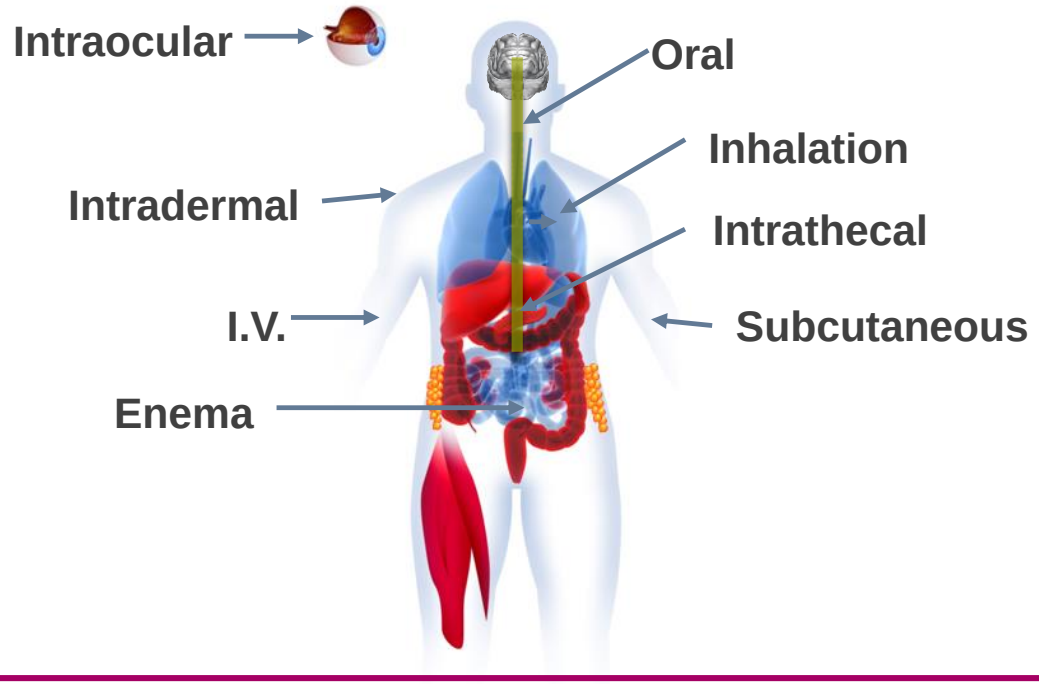
C. Frank Bennett, Ph.D.

Senior Vice President of Research

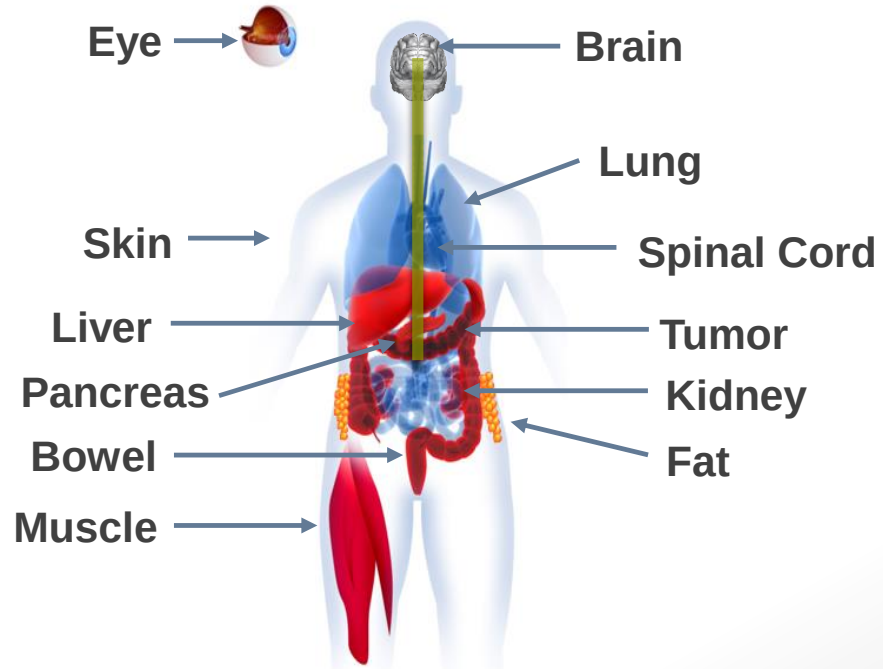
Enhancing Productive Distribution

Multiple Delivery Routes and Target Tissues Enable Our Broad Pipeline

ADMINISTERED THROUGH MULTIPLE ROUTES OF DELIVERY



BROAD CLINICAL ACTIVITY IN MULTIPLE TISSUES



Multiple routes of delivery, multiple target tissues

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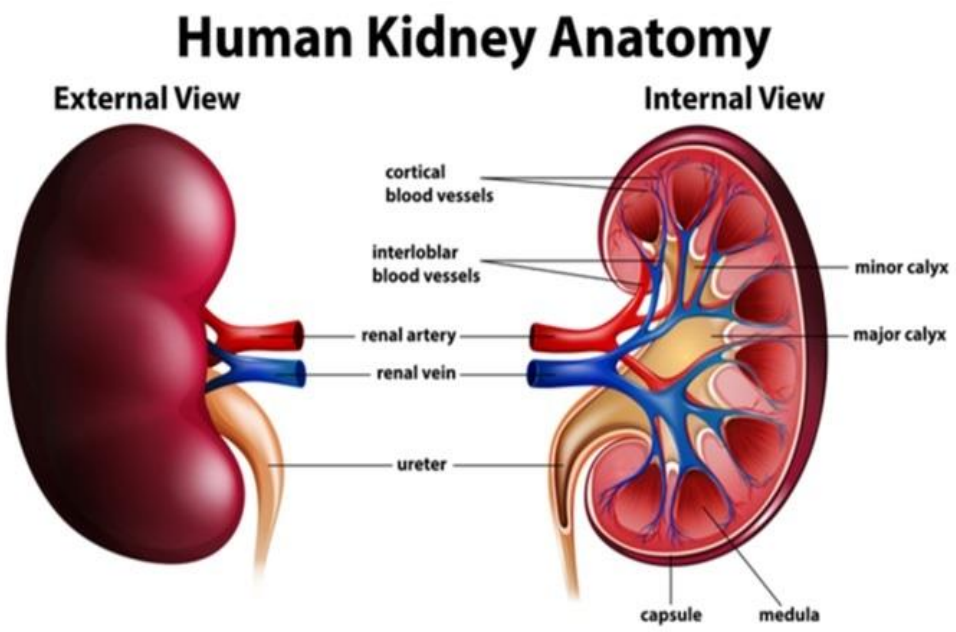
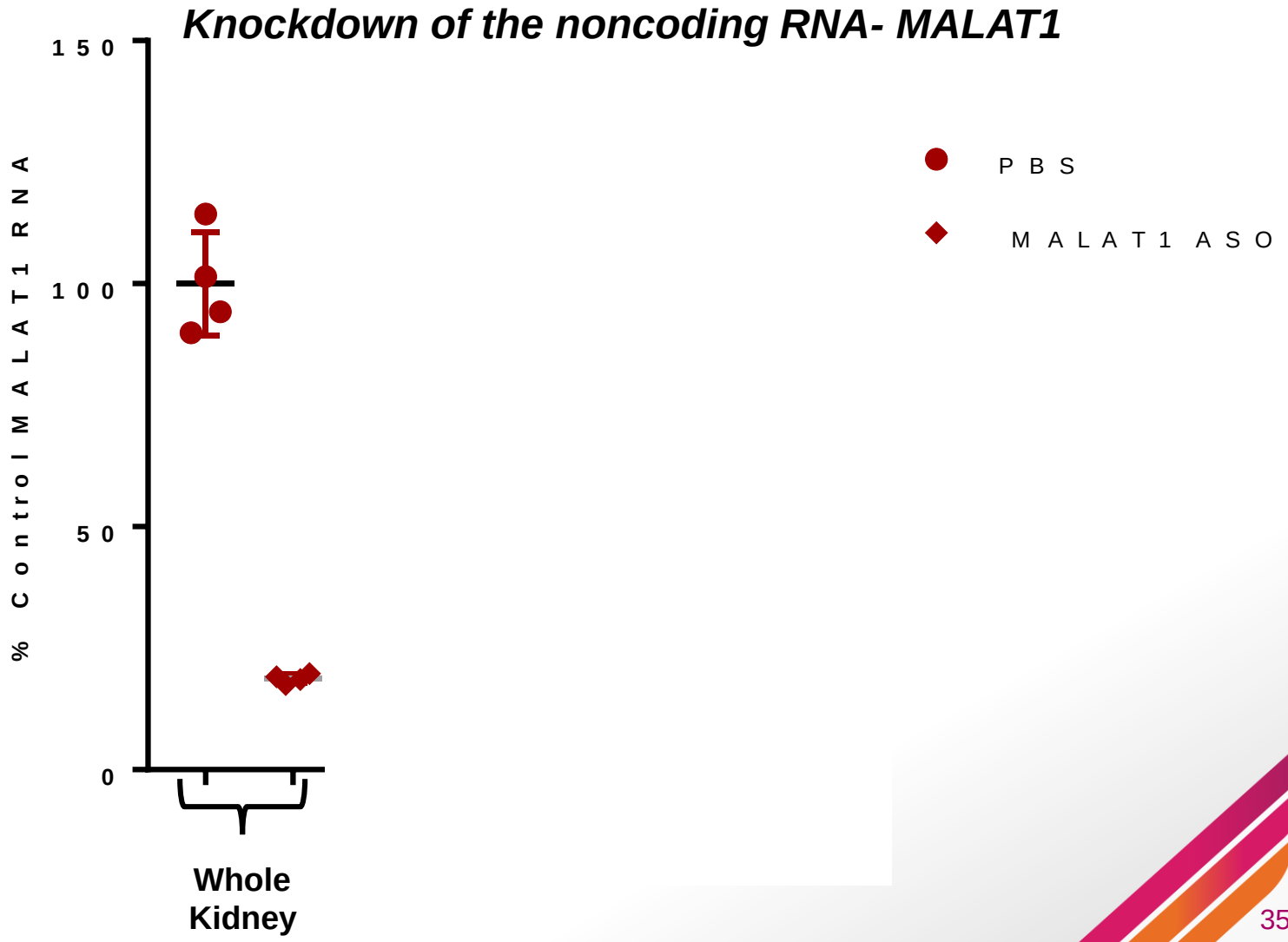
Enhancing Productive Distribution

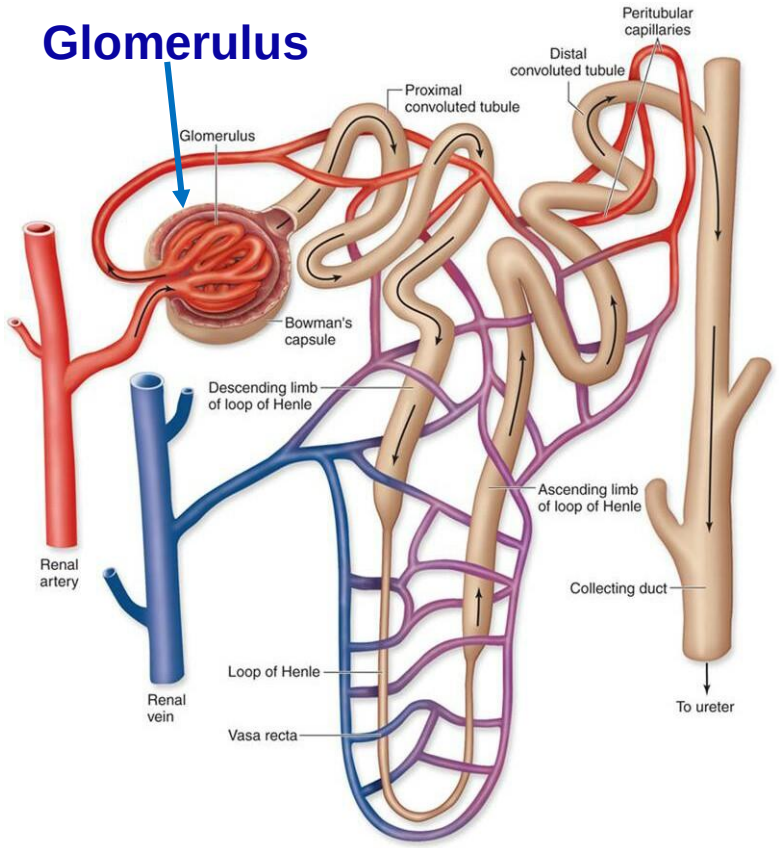
Robust MALAT1 Reduction in All Kidney Regions Enabled by Generation 2.5 Chemistry

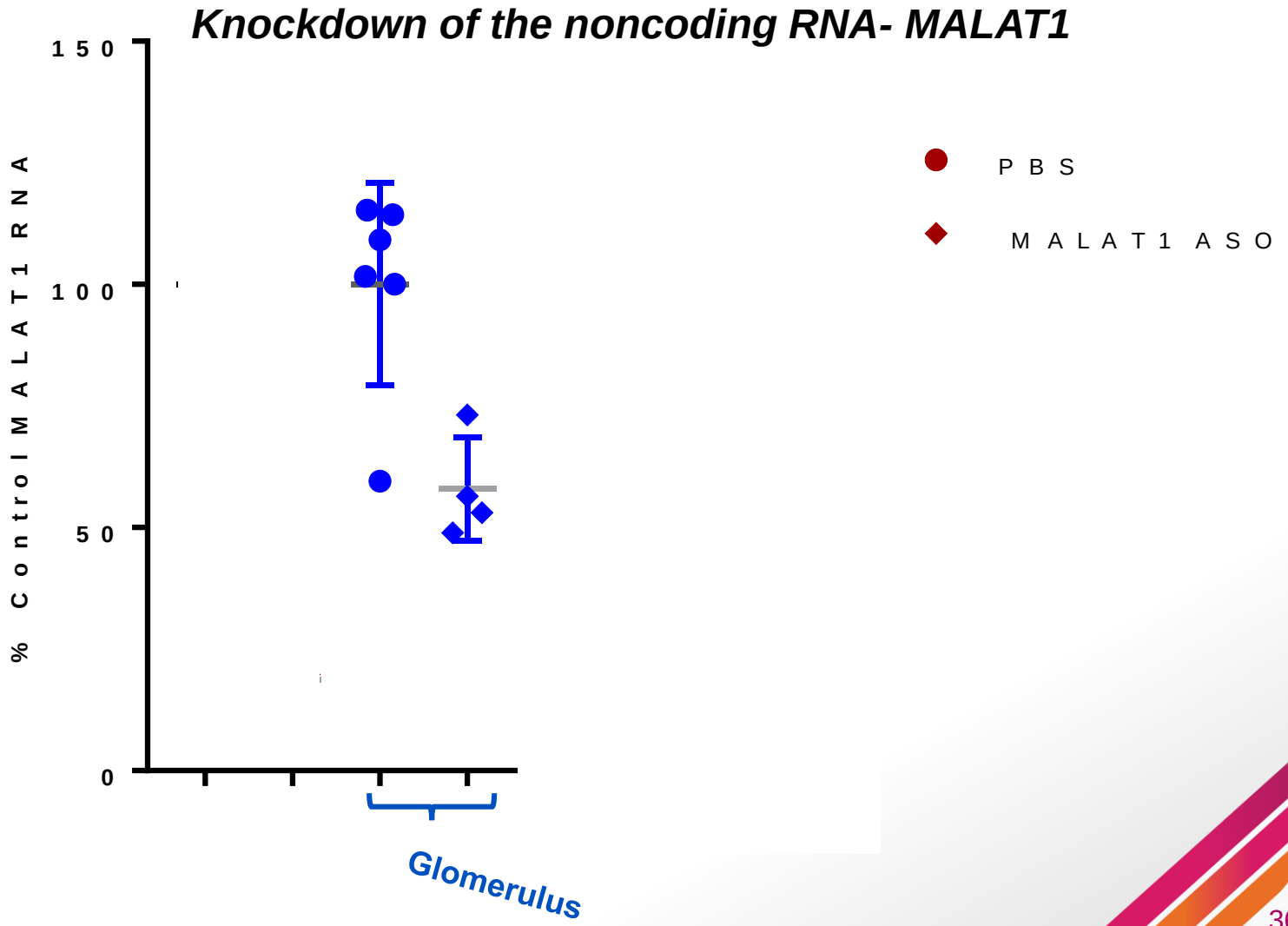




Enhancing Productive Distribution

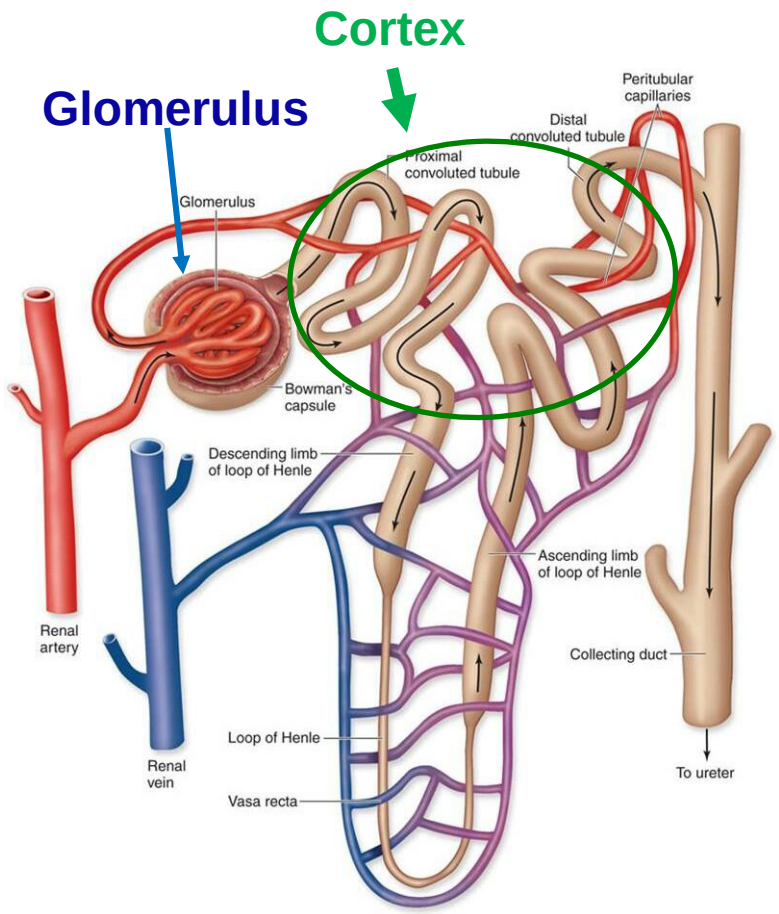
Robust MALAT1 Reduction in All Kidney Regions Enabled by Generation 2.5 Chemistry

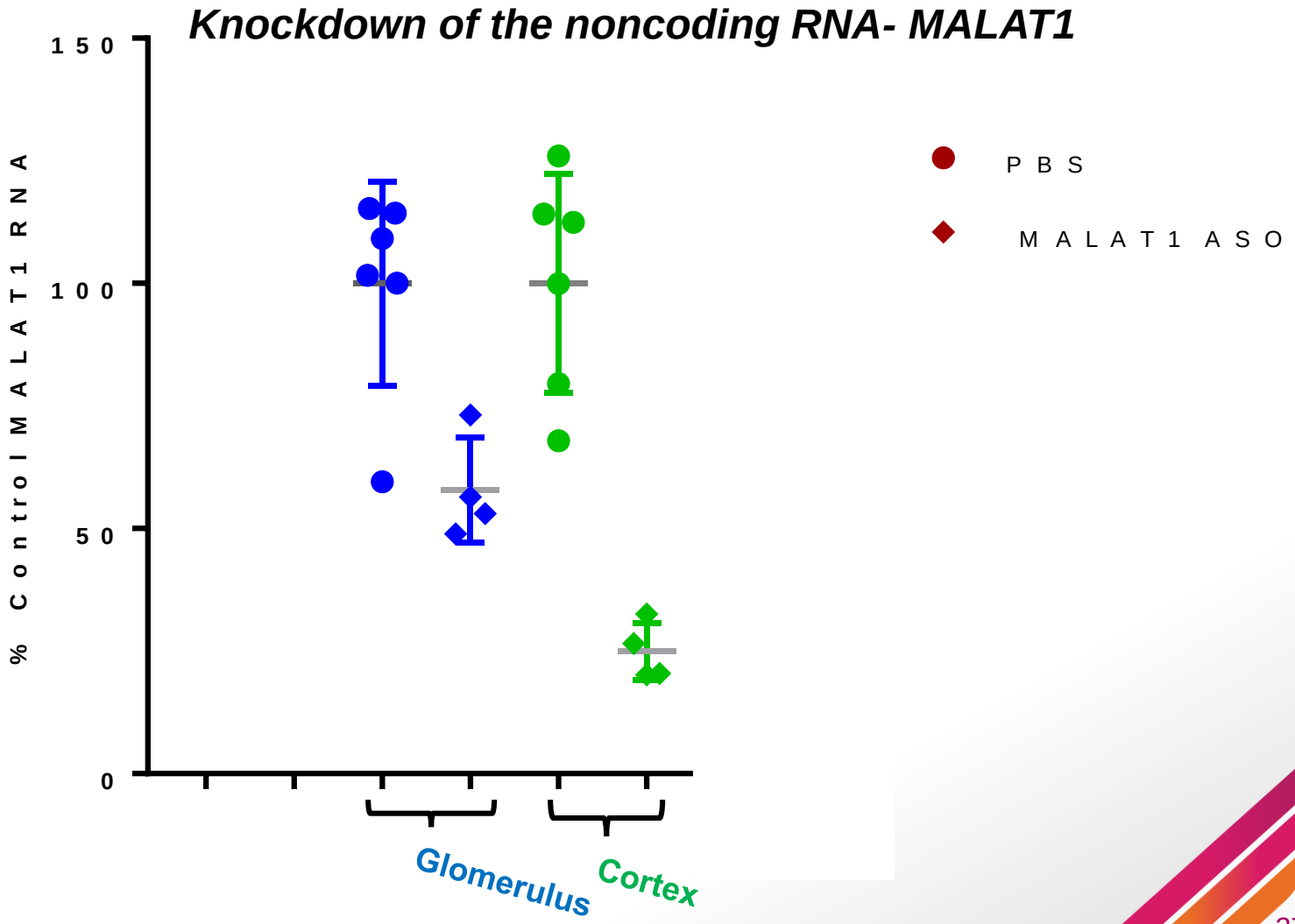




Enhancing Productive Distribution

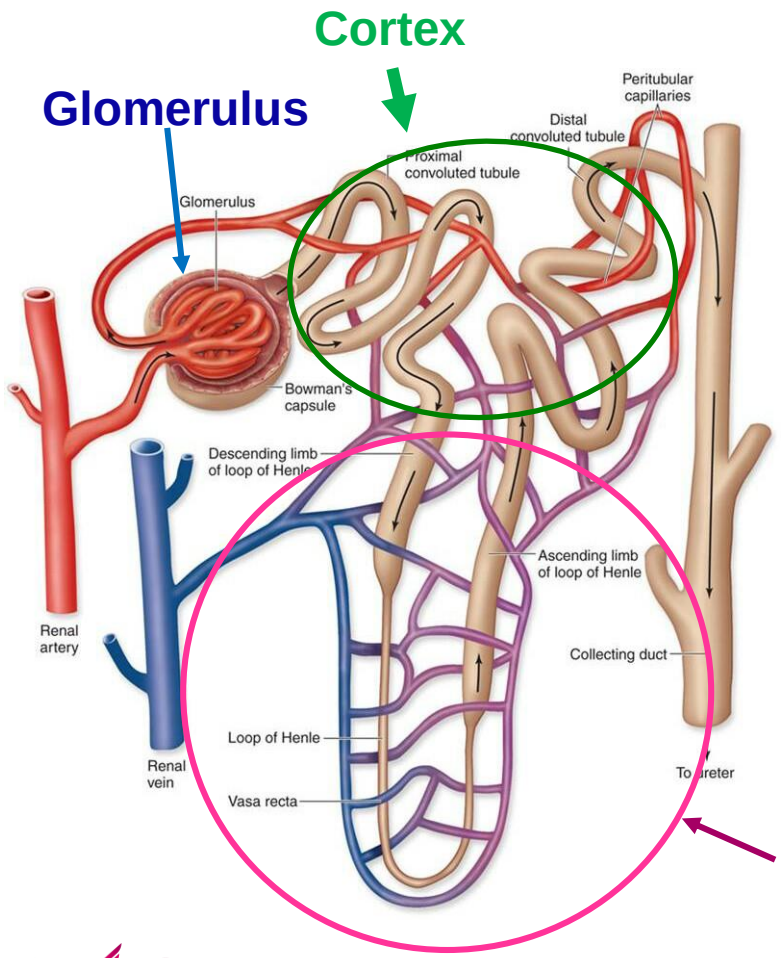
Robust MALAT1 Reduction in All Kidney Regions Enabled by Generation 2.5 Chemistry

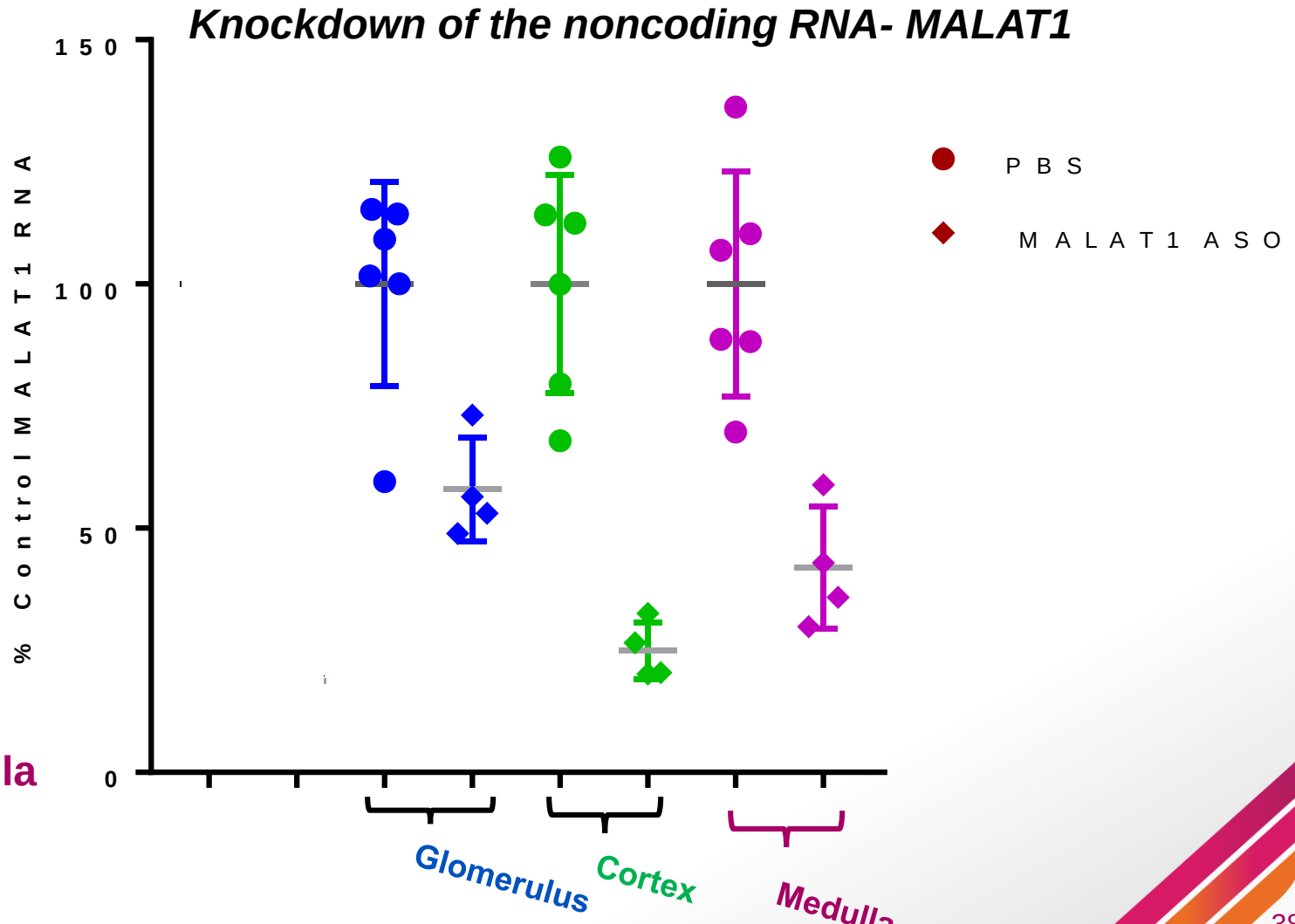




Enhancing Productive Distribution

Robust MALAT1 Reduction in All Kidney Regions Enabled by Generation 2.5 Chemistry





Enhancing Productive Distribution

Importance

- Understanding sub-organ pharmacokinetics supports effective drug discovery and development
- Diseases are typically not organ wide but involve sub-structures of the organ
  - This knowledge focuses our programs on diseases for which our drugs should work
- Sub-organ pharmacokinetics understood
  - Liver
  - Kidney
  - CNS
  - Lung
  - Heart
- Performance of Ionis medicines in the clinic demonstrate the value of this approach

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Enhancing Productive Delivery of ASOs to Tissues: LICA Technology

LICA Technology
Introduction

In contrast to double strand ASOs (siRNA), single strand ASOs distribute broadly to multiple tissues and are active in multiple tissues

Nevertheless, a significant fraction of ASO in every organ is wasted in non-productive sites

Therefore, if productive distribution to specific organs can be achieved, potency should be dramatically enhanced

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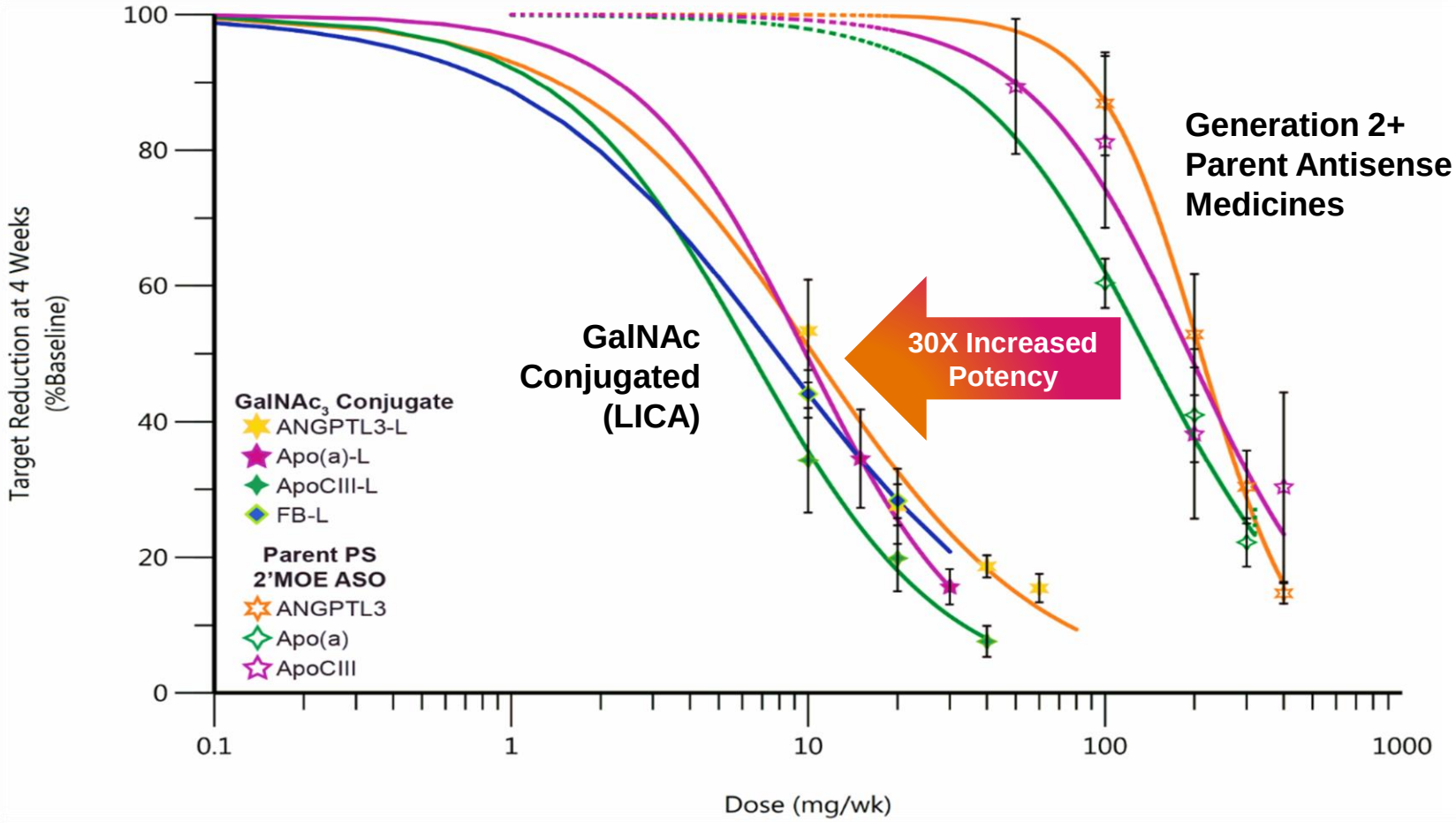
LICA Technology

Liver LICA (GalNAc) Increases Potency of ASOs in Humans by ~30-Fold



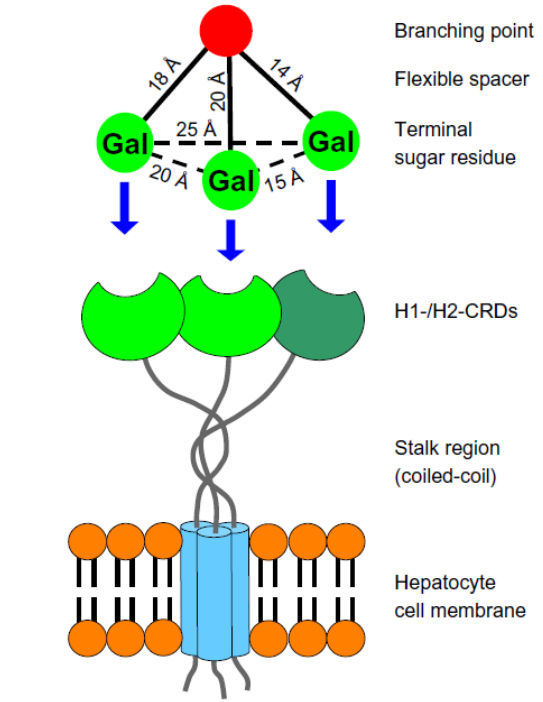


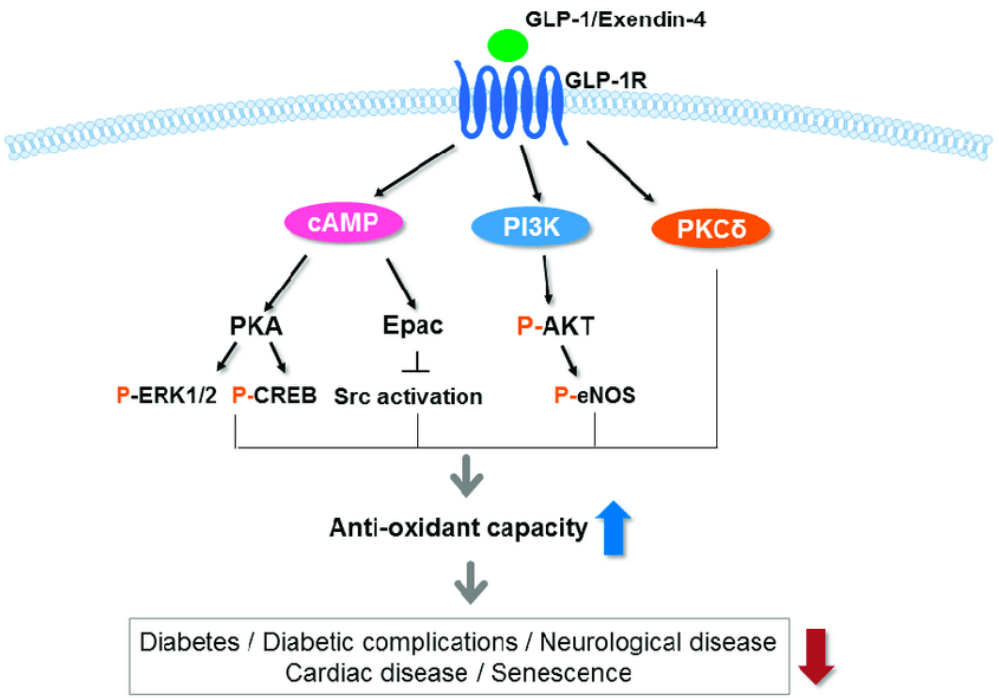
Figure 1. Binding model for ASGP-R ligands in an optimal conformation to the heterooligomeric receptor consisting of H1 and H2 subunits. Dashed line indicates the distance between the C-4 of each Gal moieties; filled line represents approximate distance between branching point and C-6 of Gal (14–20 Å). Adapted from Lee et al.[16]

Greater than 30-fold increase in potency of LICAs targeting the liver

Crooke, S., et al. (2018), Nucleic Acid Therapeutics. http://doi.org/10.1089/nat.2018.0753

LICA Technology

Pancreatic Beta-cell LICA (GLP1) Substantially Enhances Potency

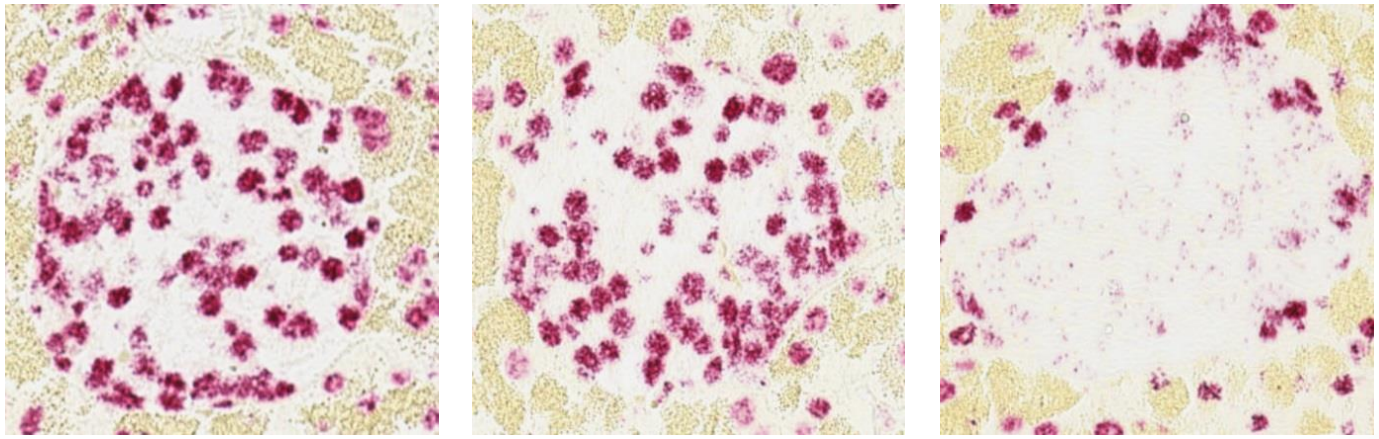


Oh and Jun (2017) Int. J. Molec. Sci. **19:***26*

> 50-fold Increase in Potency in Mouse Pancreas

Gen 2+ and Gen 2.5

- Improves uptake in pancreas beta cells



Saline | **Gen 2.5 ASO** | **Gen 2.5 + LICA ASO**

Pink dots represent MALAT1 RNA in the nucleus

*Ammala et al, Sci Adv.,*v.4(10); 17 Oct 2018

LICA Technology

Strategy to Identify Additional LICAs

Determine the cell types to be targeted

Identify best acceptors (receptors) on target cells

Identify ligands that interact with target acceptors

Optimize ASO-ligand structure

Additional target **tissues** actively being investigated

- Heart
- Skeletal muscle
- Immune cells and others

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LICA Technology

LICA Could Broaden Therapeutic Potential of ASOs in Tissues

Example: The Heart

- Enormous opportunity to use the specificity, versatility and breadth of ASOs to treat cardiac diseases
- Cardiac arrythmias by altering the specific molecular causes
  - Na channels
  - K channels
  - Ca channels
  - Cl channels
- Positive inotropic agents for congestive heart failure
- The management of reactive oxygen species and tissue damage
- Enhance mitochondrial function

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LICA Technology

Lessons Learned and Importance

- Understanding the **sub-organ distribution** of ASO has been of tremendous benefit in **guiding** our drug discovery programs
- **Targeted** delivery of **ASOs** to specific **organs** and **cells** is feasible
  - Liver, pancreas
- **Enhance** productive **delivery** leads to very substantial **enhancements** in potency, safety, tolerability, ease of administration and infrequent dosing schedules
- **New approaches** to identifying potential ligands to **enhance** productive **delivery increase** likelihood of **success**

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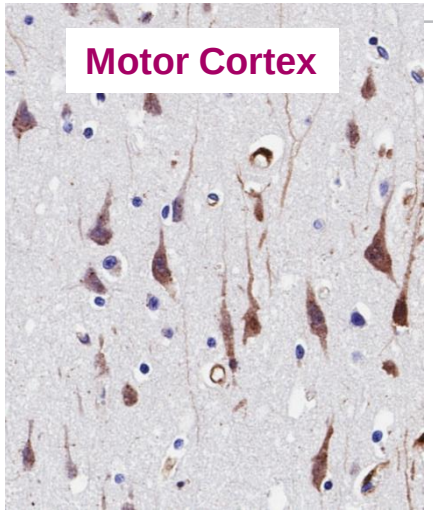
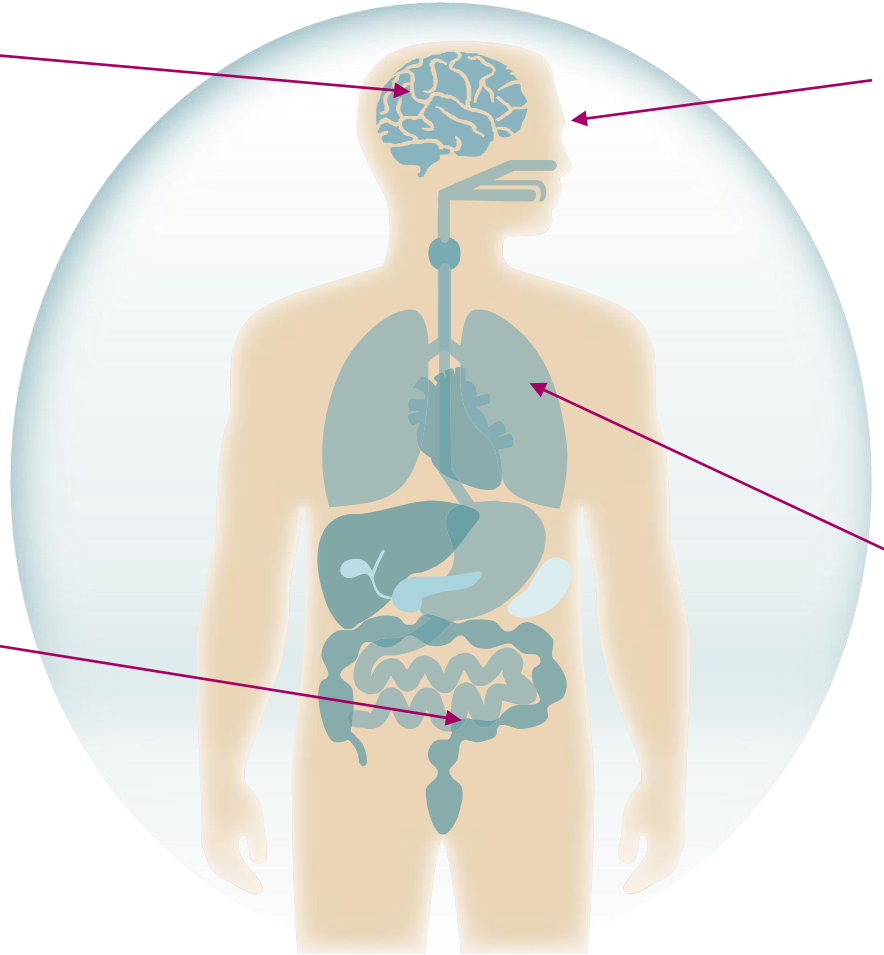
Local Delivery of ASOs to the CNS and Lung

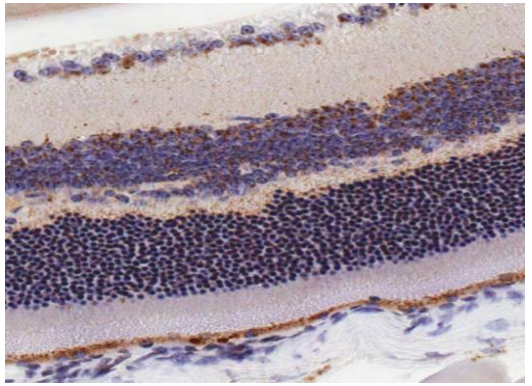
Local Delivery of Antisense Drugs Further Broadens the Tissues We Can Practice

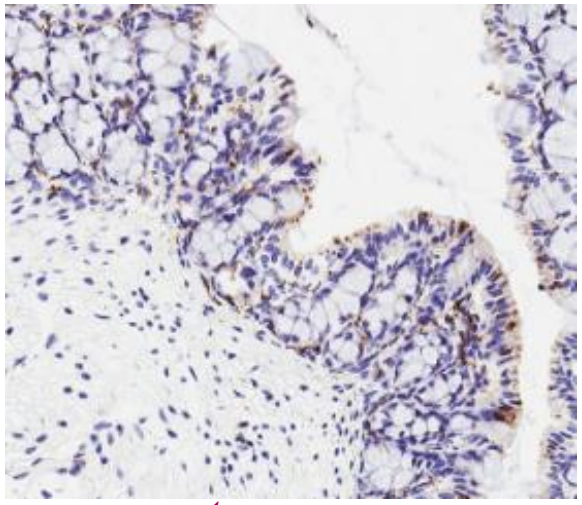


Brain



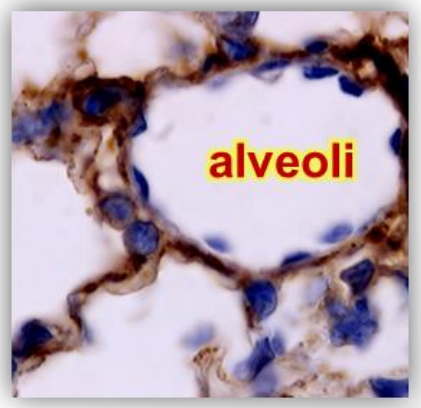
Eye





Intestine

Lung



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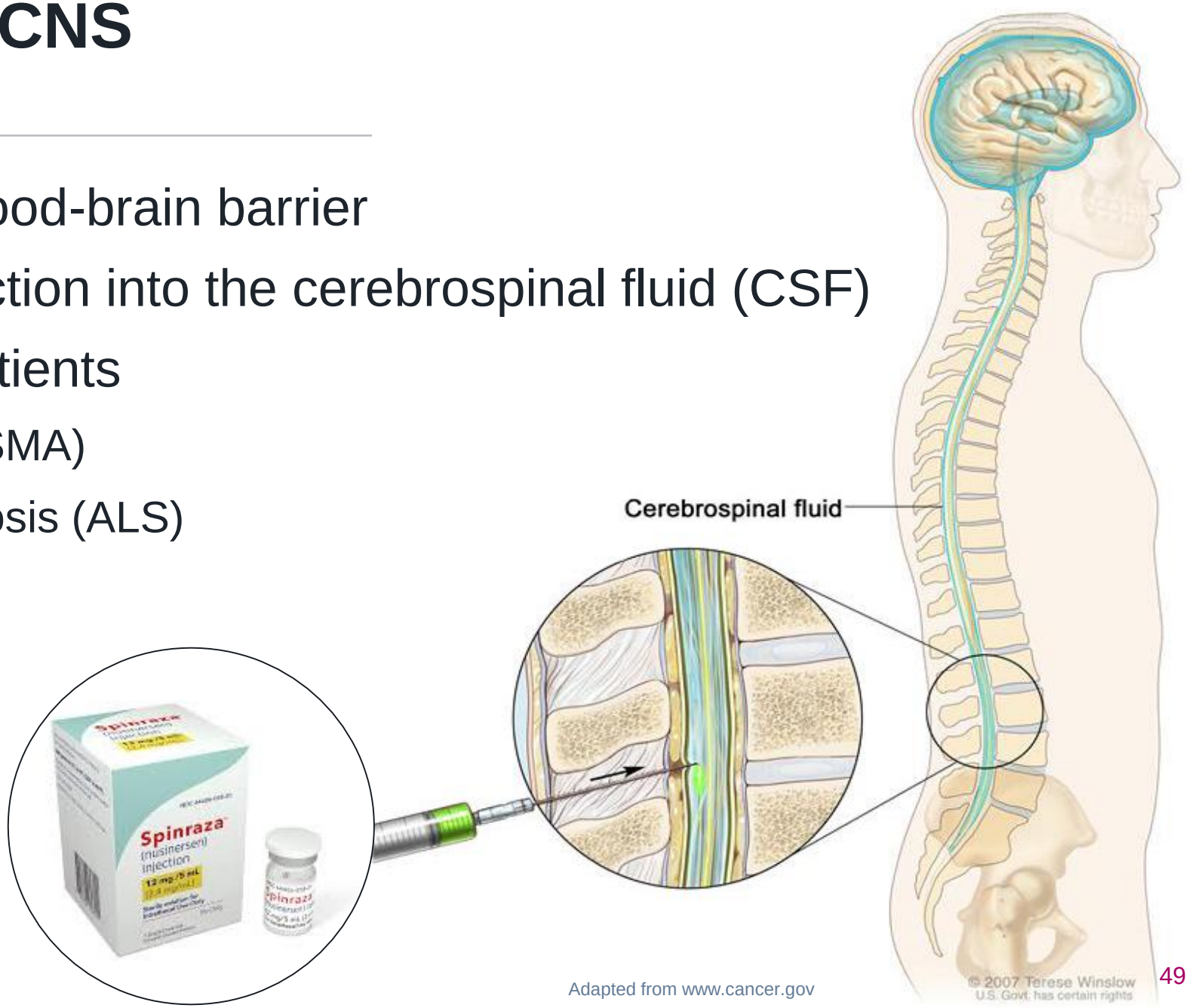
Local Delivery to the CNS

Targets in the Brain

- ASOs do not cross the blood-brain barrier
- Bolus intrathecal (IT) injection into the cerebrospinal fluid (CSF)
- IT experience: > 9,300 patients
  - Spinal muscular atrophy (SMA)
  - Amyotrophic Lateral Sclerosis (ALS)
  - Huntington's disease
  - Alzheimer's disease
  - Parkinson's disease



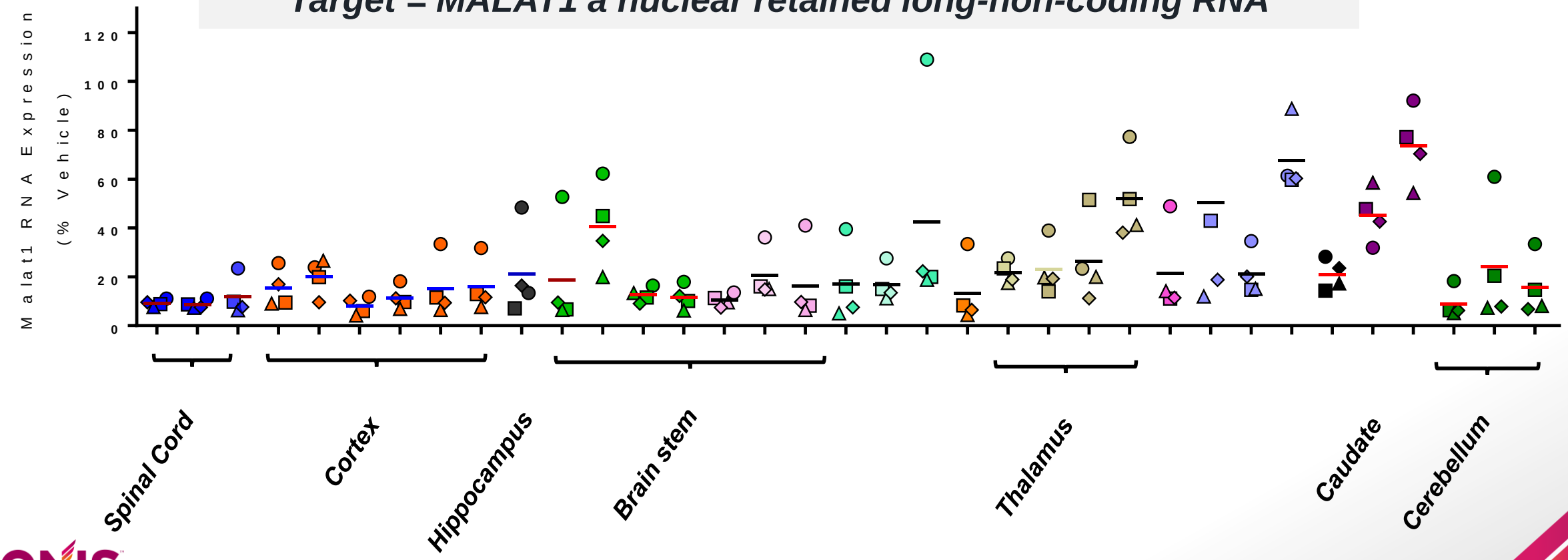
Adapted from www.cancer.gov

Local Delivery to the CNS

Broad Activity Throughout CNS Demonstrated for ASOs

Non-Human Primate

Target = MALAT1 a nuclear retained long-non-coding RNA

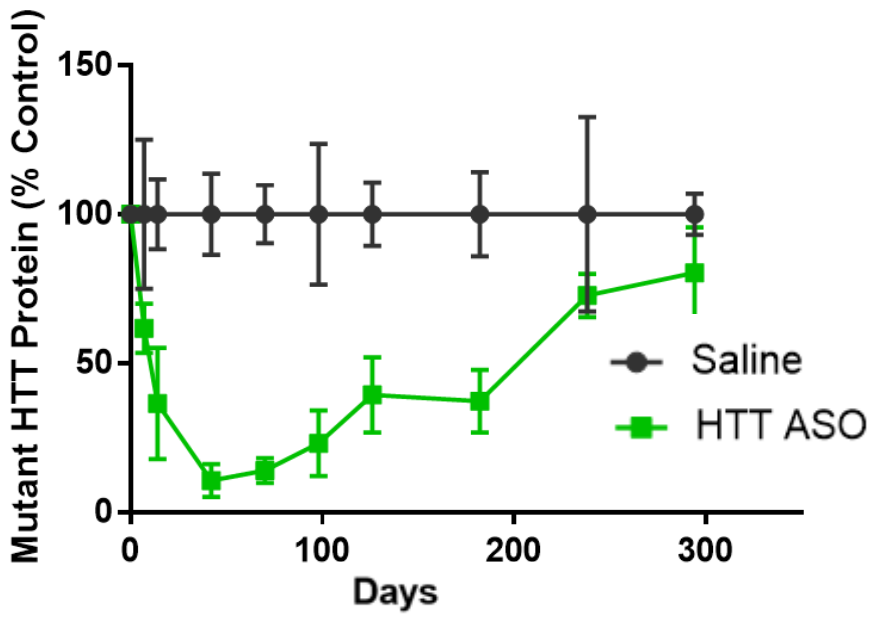


Jafar-nejad et al. (in preparation)

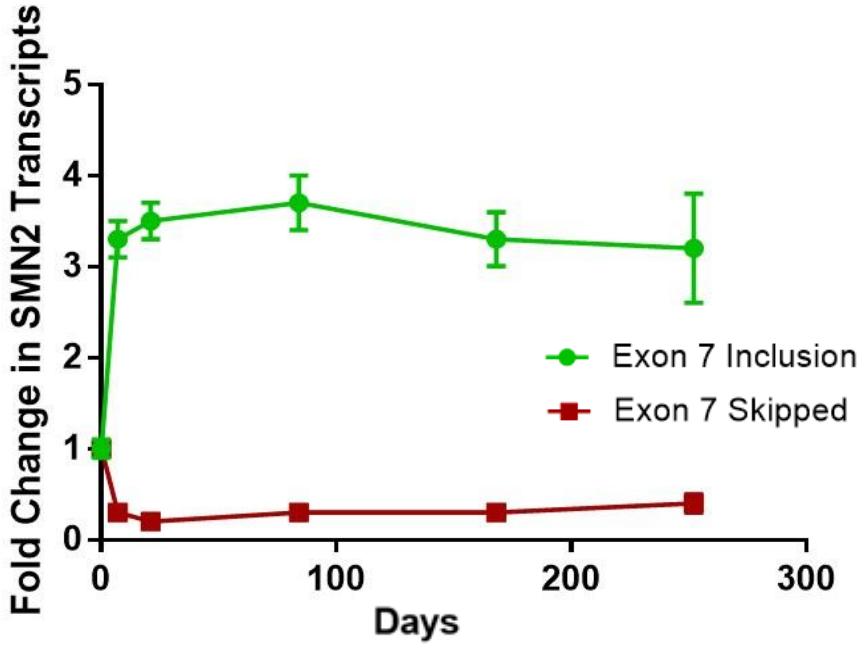
Local Delivery to the CNS

Long Duration of Effect Extends Dosing Intervals of CNS-Administered Medicines

Reduction of HTT Protein in Mouse Brain by an RNase H1 ASO



SPINRAZA



Long duration of effect supports quarterly or longer dosing interval

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Southwell et al., JCI 2018; Rigo et al, JPET. 2014

Local Delivery to the CNS

Lessons Learned

- **ASOs** may be **safely** administered intrathecally
- Nearly **all brain** regions are **accessible** to ASOs
- Every **4** to **12** month dosing is feasible
- Newer **chemistries** may **enhance** potency and **extend dosing** intervals even further

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Local Delivery to the CNS

Importance

An extremely large and important therapeutic opportunity is now accessible to the technology

- Genetic diseases
- Neurodegenerative diseases
- Motor disorders
- Cognitive disorders

The technology is advancing and these advances will result in even greater opportunities

- Once a year dosing
- Chronic pain
- Severe affective disorders

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Local Delivery to the CNS

Ionis Neurological Pipeline

SPINRAZA
(Spinal Muscular Atrophy)

TEGSEDI *(hATTR)*

ATTR Amyloidosis
AKCEA-TTR-L_{Rx}

Huntington's Disease
IONIS-HTT-$_{Rx}$ (RG6042)

Dementia (Alzheimer and FTD)
IONIS-$MAPT_{Rx}$ (BIIB080)

Amyotrophic Lateral Sclerosis
Tofersen (IONIS-$SOD1_{Rx}$)
IONIS-$C9_{Rx}$ (BIIB078)

Centronuclear Myopathy
IONIS-DNM2-2.5$_{Rx}$

Neurodegenerative Diseases
Multiple Programs

Alexander Disease
IONIS-$GFAP_{Rx}$

Prion Disease

Lafora Disease

Charcot-Marie-Tooth

Parkinson's Disease

Myotonic Dystrophy

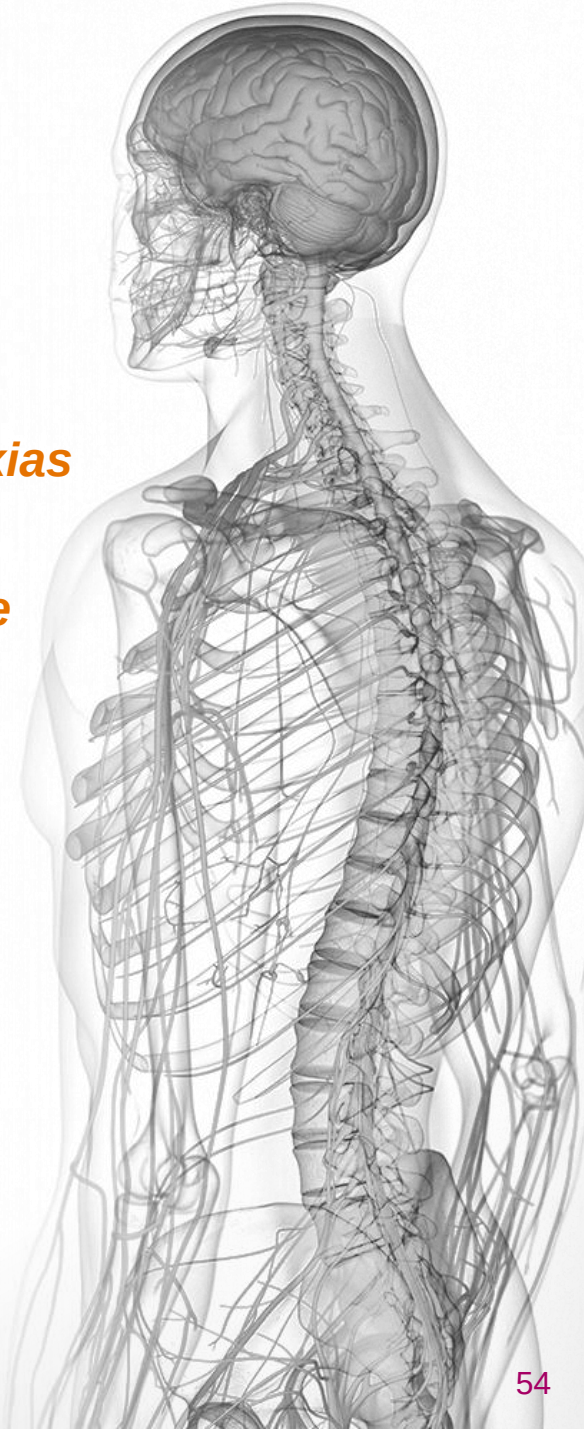
Spinocerebellar Ataxias

Angelman Syndrome

Multiple Sclerosis

Pain

And many more in research stage



Aerosol Delivery for Lung Diseases

Aerosol Delivery

Opportunities and Early Challenges

Pulmonary disease is a common and significant cause of illness and death around the world, responsible for most of hospital stays among children and ICU utilization in the U.S.

Potential challenges

- Tolerability of antisense oligonucleotides (ASOs) in the lung?
- Access to lung cell types of interest with sufficient potency?
- Levels of systemic exposure from locally delivered ASO?
- Translatability from rodent models to human diseases?

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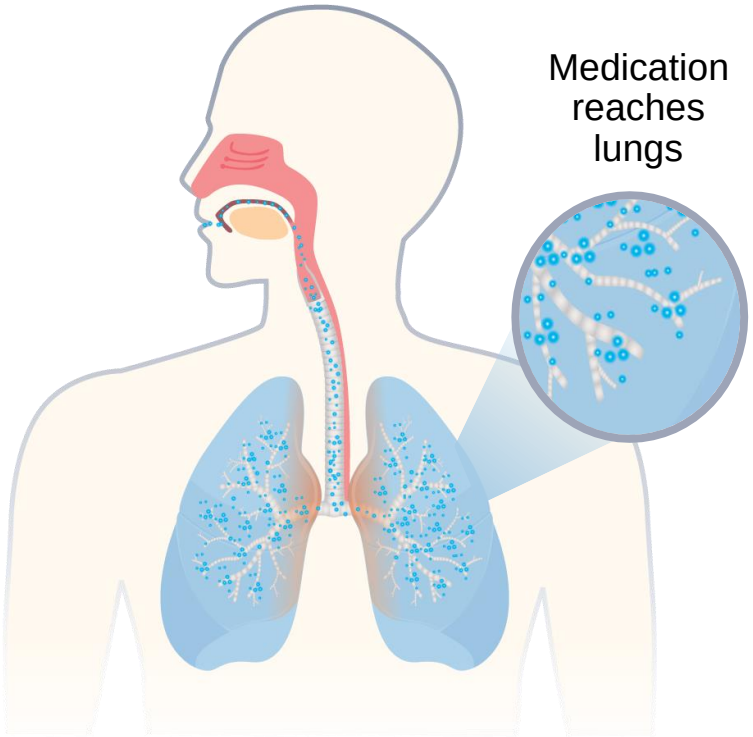
Aerosol Delivery

Advancements in ASO Technology Enable Pulmonary ASO Delivery



http://www.asthma.ca/napa/juillet2011enewsletter.php

- **Antisense drugs are compatible with inhalation devices, such as nebulizers**
- **IL-4Rα ASO, AIR645 (ISIS 369645), was evaluated in a clinical PoC trial for asthma treatment**
  - Gen 2 chemistry
  - Demonstrated pulmonary safety of ASOs in patients
  - Evidence of target inhibition
- **Positive attributes demonstrated for an aerosolized ASO therapy**
  - Excellent tolerability profile
  - Broad distribution in lung
  - Minimal systemic exposure
  - Long tissue half-life (~14 days)
- **Medicinal chemistry advances and extensive screening process significantly improves ASO potency in lung**
  - Gen 2.5 chemistry expands range of targetable cell types

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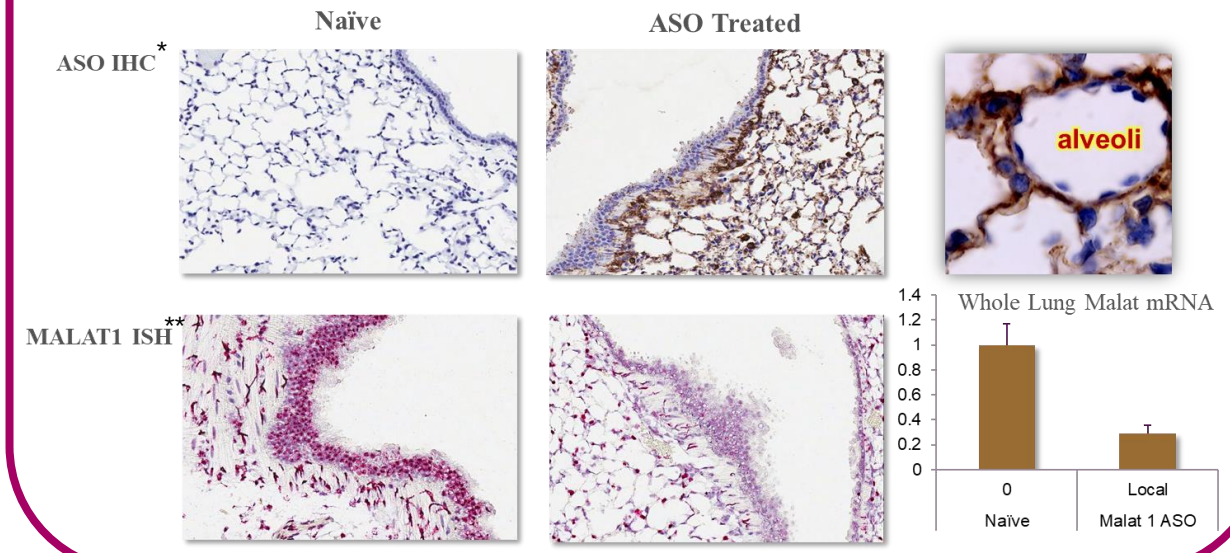
Aerosol Delivery

Improved Cellular Activity and Broad Distribution with Gen 2.5 Chemistry

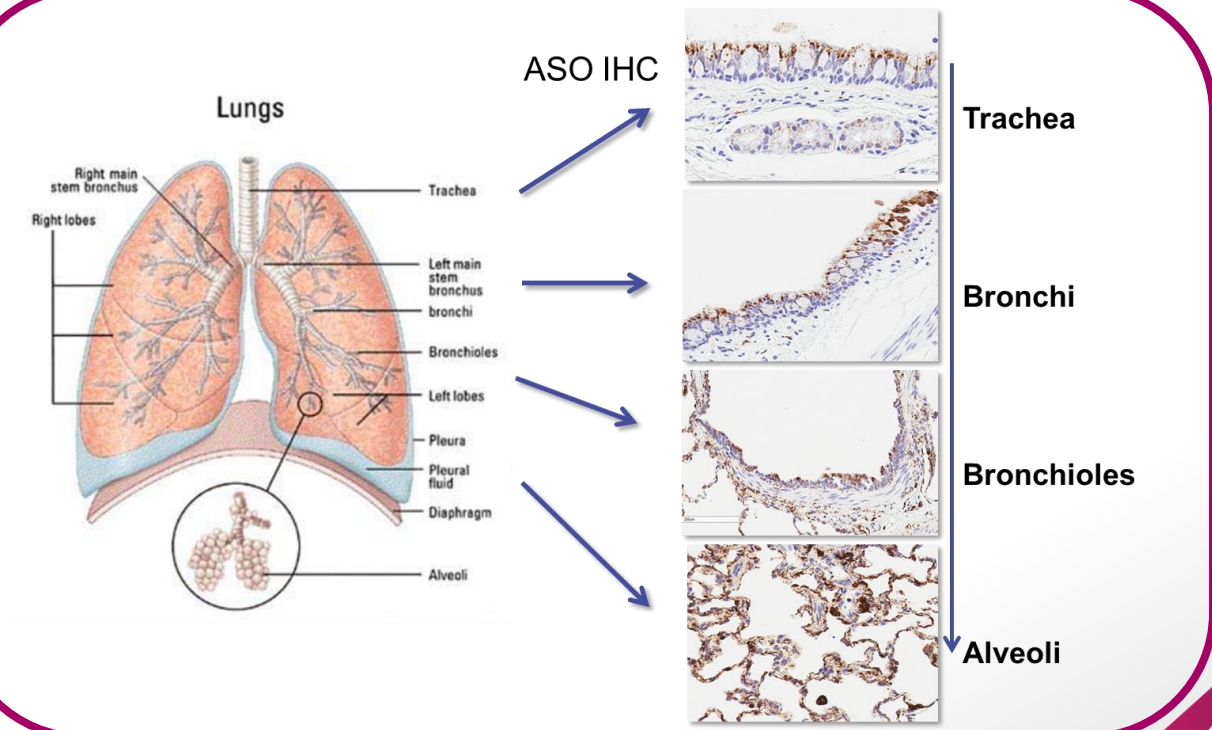
Aerosolized ASO Distributes Well in Mouse Lung

Aerosol delivery of ASO targeting MALAT1, an abundant nuclear ubiquitously expressed noncoding RNA



*IHC: Immunohistochemistry (Brown Staining = ASO)
**ISH: In Situ Hybridization

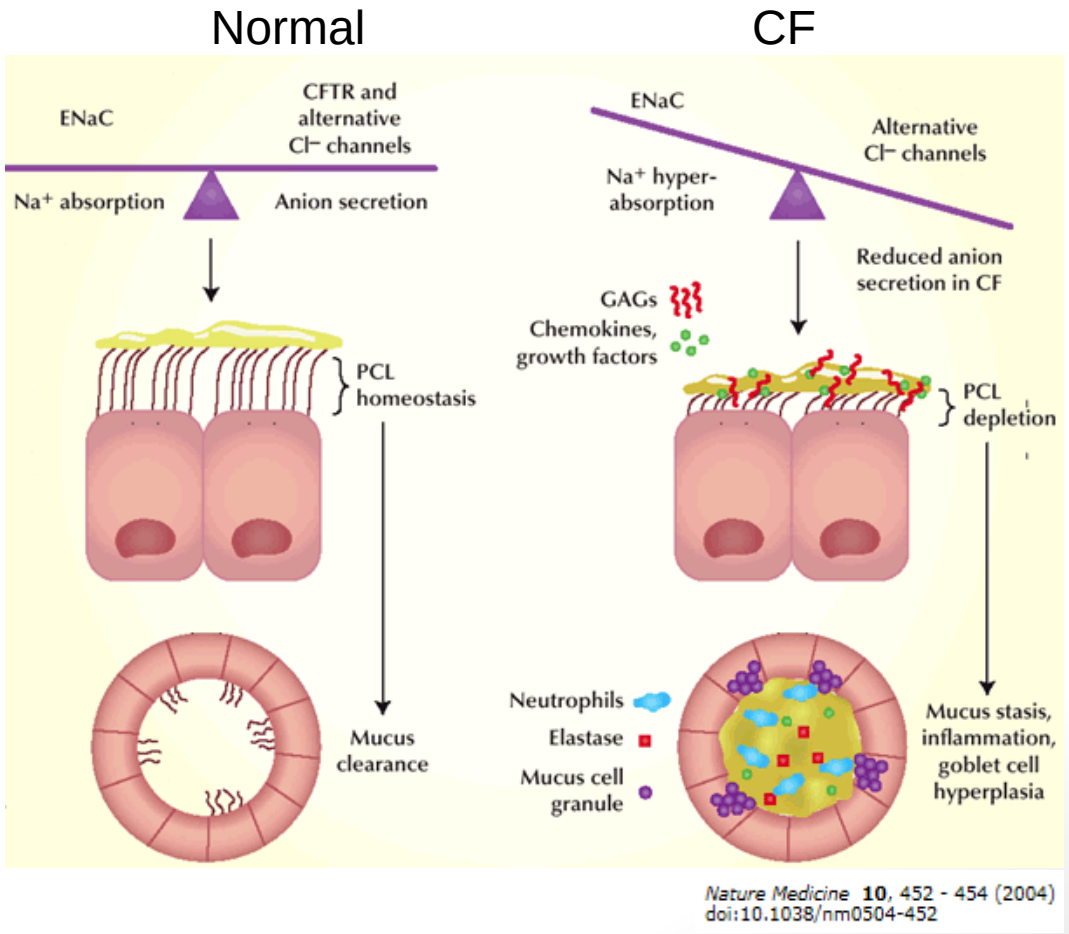
Aerosolized ASO Distributes Well in Monkey Lung



Aerosol Delivery

Targeting ENaC for the Treatment of Cystic Fibrosis

- Cystic Fibrosis (CF) is caused by mutations in the CFTR gene
  - Over 2,000 CFTR mutations identified
- The epithelial sodium channel (ENaC) in the apical membrane sodium channel expressed in lung, kidney and intestine
- In CF airways, hyperactive ENaC leads to increased Na+ and H2O absorption, reduced airway surface liquid hydration, reduced mucus clearance and persistent infection and inflammation
- ENaC small molecules greatly limited due to unwanted activity in kidney (hyperkalemia)

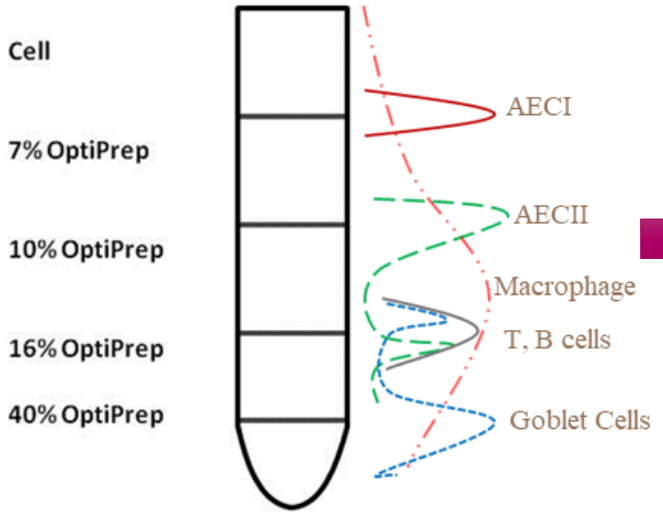


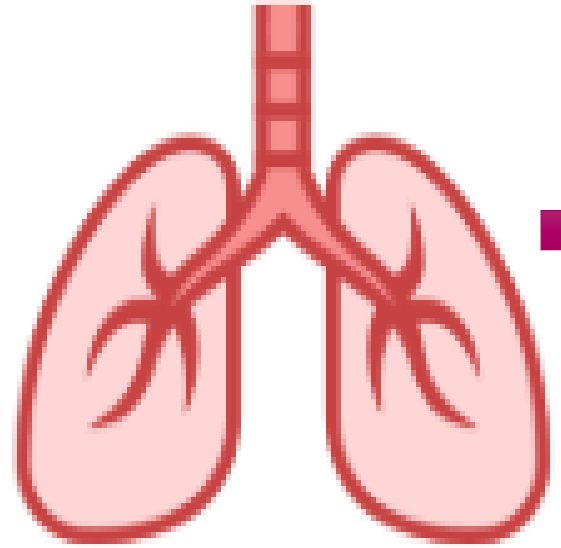
ENaC inhibition in the lung potentially provides a treatment for all CF mutations

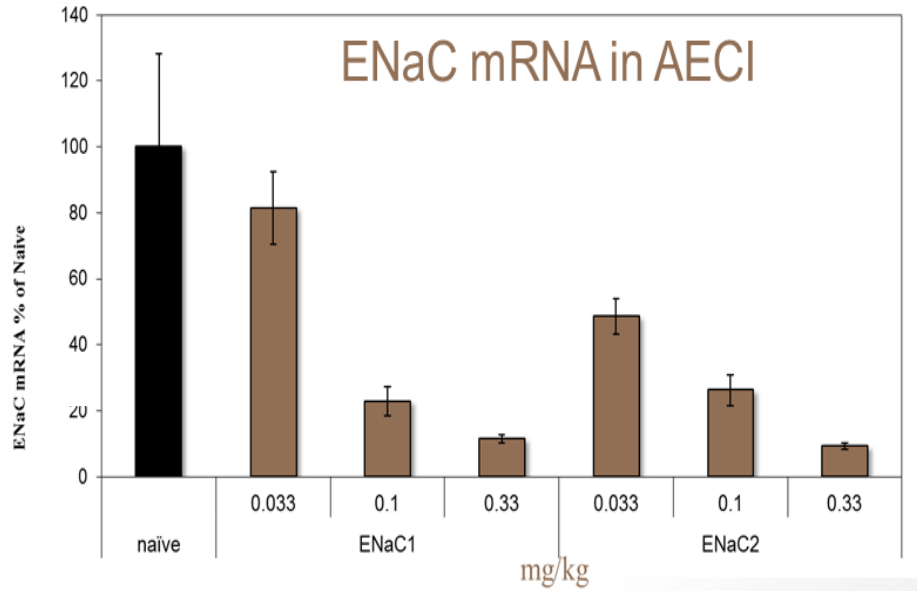
Aerosol Delivery

Potent Activity of ENaC ASO in Airway Epithelial Cells (AECs)

Cell Fractionation Demonstrates ENaC Expression and ASO Activity in AECs







AECs are sensitive to ASO activity

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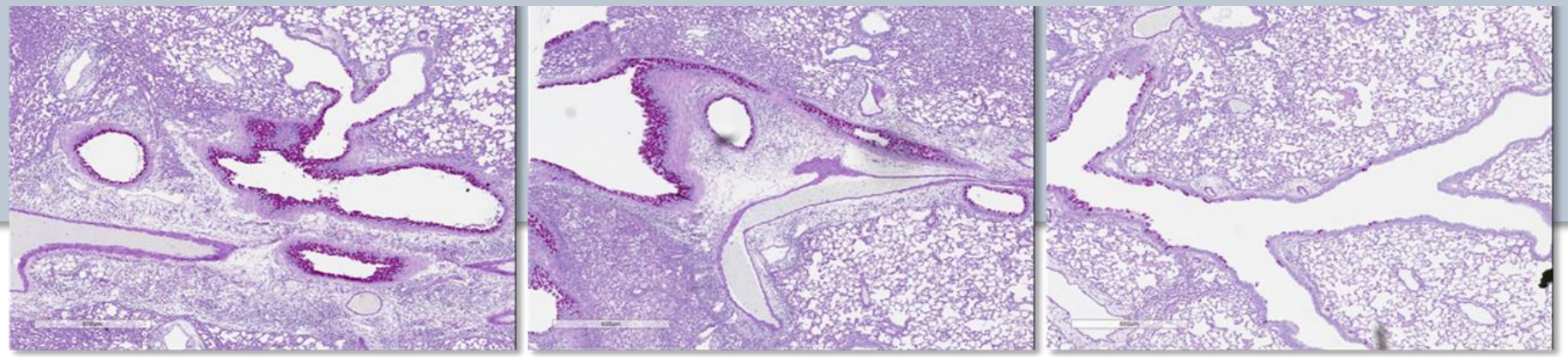
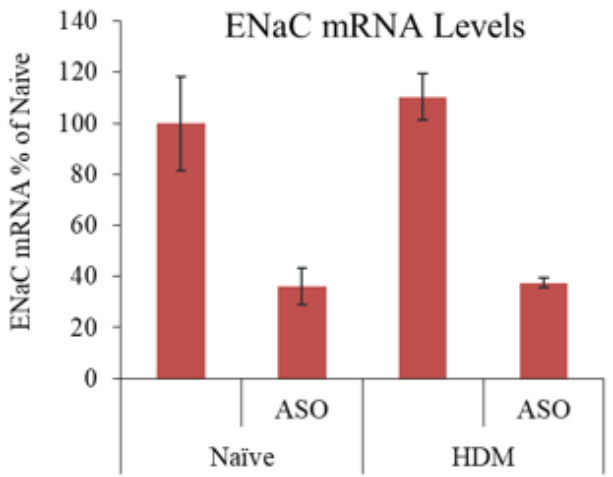
Aerosol Delivery

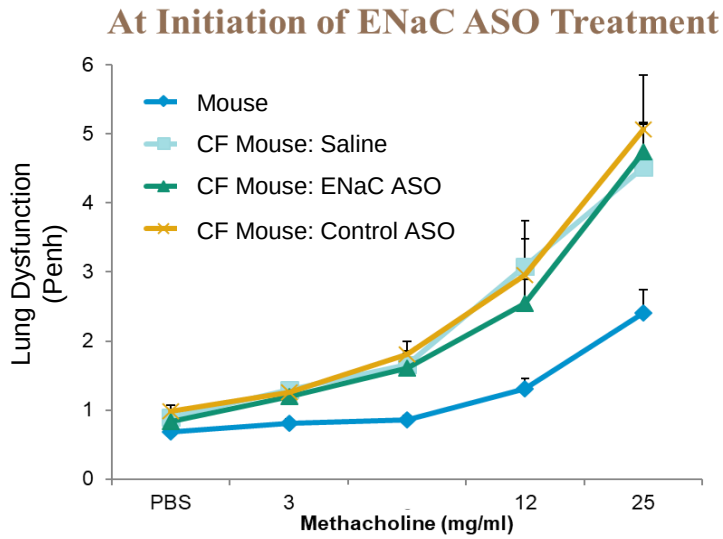
ENaC ASO Reverses Hypersecretion, Inflammation & Airway Hyperresponsiveness

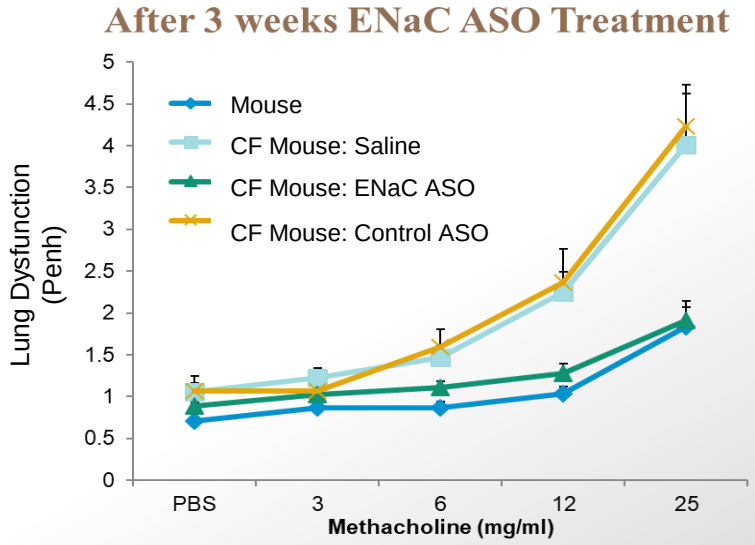
Adult onset CF-like lung disease in mice

Saline Control (6 Wks Baseline) | **ASO Control (at 9 Wks)** | **ENAC ASO (at 9 Wks)**









Aerosol Delivery

Pulmonary Pipeline

Target	Mechanism	Stage	Indication
ENaC	Improves Airway Hydration	Phase 1/2a	Cystic Fibrosis, other indications
Undisclosed	Goblet Cell De-Differentiation	Preclinical Development	COPD, Severe Asthma
Undisclosed	Epithelial Cell Differentiation	Late Discovery	COPD
Undisclosed	Modulation of ER Stress, Inflammation and Mucin Production	Late Discovery	IPF

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Aerosol Delivery
Lessons Learned

ASO delivery to the **lung** appears **safe** and well tolerated

Medicinal chemistry enhances potency after aerosol administration pays important dividends to enable ASO pulmonary delivery for drug discovery

- Gen 2.5 chemistry easily administered in modern devices
- Weekly or less frequent administration feasible

ASOs **active** in the presence of mucus

ASOs **distribute broadly** in lung after aerosol delivery with minimal systemic exposure

New medicinal chemistry efforts may further **enhance** pulmonary performance

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Aerosol Delivery

Importance

- **Broadens** the application of **antisense** technology for drug discovery
- **Opens** the **opportunity** to **treat** many diseases of the **lung**
- **Antisense** technology ideally suited to **create transformative medicines** for currently poorly treated diseases e.g. COPD

Oral Delivery

Brett Monia, Ph.D.

Chief Operating Officer

Oral Delivery
Past Experience (1 of 2)

Previously demonstrated 6-10% oral bioavailability and target reduction in man

- Enteric coating optimized to avoid ASO precipitation due to stomach acid
- Optimized penetration enhancer shown to be necessary for enhanced bioavailability
- Oral safety and tolerability demonstrated

Absorption is limiting

- First pass metabolism is not an issue

Generation 2 chemistry potency was insufficient

- Multiple large capsules were required daily
- Cost was prohibitive

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Oral Delivery
Past Experience (2 of 2)

Oral bioavailability declines as the size of animal increases

- Man is the species with the lowest bioavailability

Oral bioavailability declines as ASO size increases

Oral Kynamro (daily capsule) was tested in dyslipidemia patients

- Utilized proprietary formulation designs
- Gen 2.0 ASO

6-10% bioavailability achieved with significant pharmacology

- Up to 15% lowering of LDL and ApoB
- Variability: “High and Low responders”

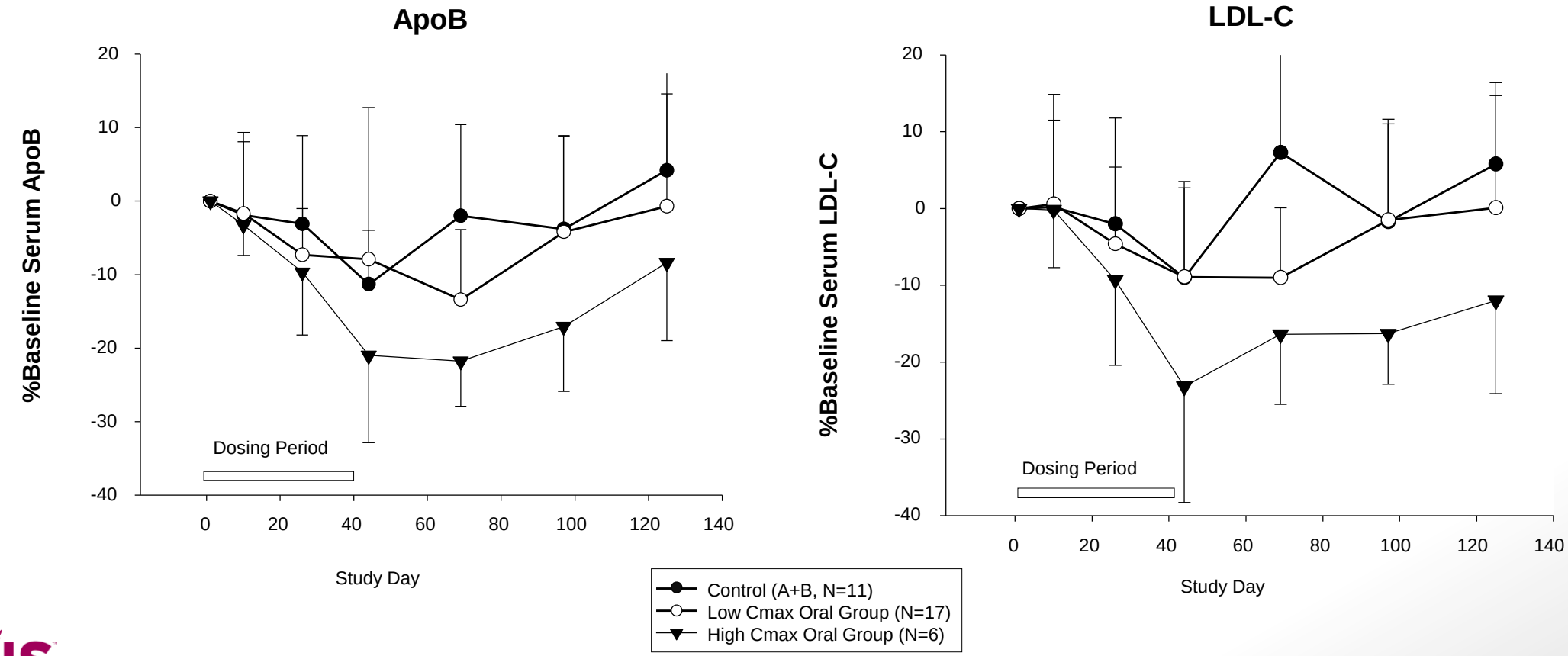
Good safety and tolerability

Oral delivery of Kynamro was achieved

Oral Delivery

Significant Reductions in Plasma ApoB & LDL-C with Daily Oral Kynamro

Daily 500 mg orally-administered Kynamro achieved significant reductions in plasma ApoB and LDL-C



Tillman et al. *Journal of Pharmaceutical Sciences.* **2008**, 97, 1, 225-236

Oral Delivery
General Concepts

Lower bioavailability results in **greater variability**

- For low bioavailability to be tolerable, the drug must be safe enough that variations in systemic drug levels do not produce problems

Larger the **animal**, **lower** the **bioavailability**

Dog is often the **preferred** preclinical species

Lower the **dose**, **better** the **oral absorption**

Minimum target **oral bioavailability** in **man** is typically **5%** if the drug is safe

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Oral Delivery

Recent Goals

Significantly enhance potency and/or the absorption of ASOs

Demonstrate that more potent ASOs are orally bioavailable

Evaluate the performance of enteric coating and penetration enhancers in combination with new more potent chemistry in preclinical species & in man

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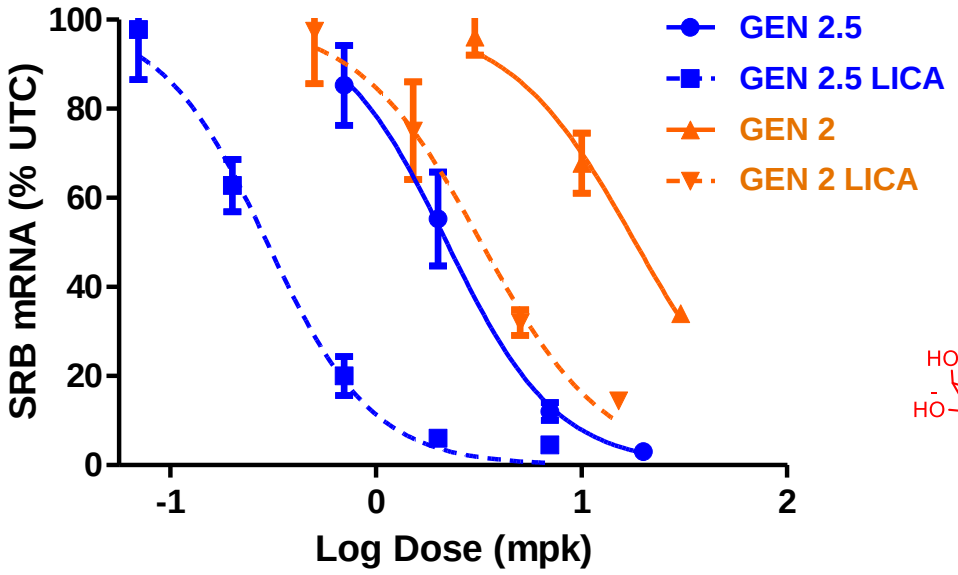
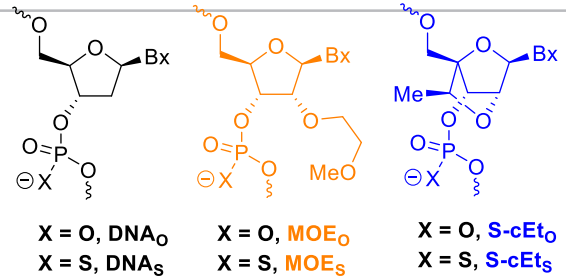
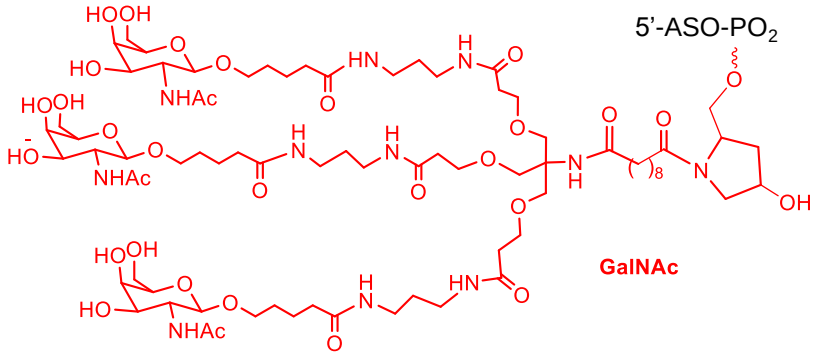
Oral Delivery

Generation 2.5 LICA Solves the Potency Issue







ISIS #	Single Dose (mpk)	ASO (5'-3')	SRB1 mRNA ED_{50} mg/kg	SRB1 mRNA* IC_{50} nM
GEN 2	3, 10, 30	$G_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sC_sT_sT$	18.3	175 ± 100
GEN 2 LICA	0.5, 1.5, 5, 15	$G_sC_sT_sT_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_sC_sT_sT_oA_o$GalNAc	3.3	25 ± 21
GEN 2.5	0.7, 2, 7, 20	$T_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC$	2.2	138 ± 56
GEN 2.5 LICA	0.07, 0.2, 0.7, 2, 7	$T_sC_sA_sG_sT_sC_sA_sT_sG_sA_sC_sT_sT_sC_oA_o$GalNAc	0.3	1.8 ± 0.84

DNA, MOE, *S*-cEt, Phosphorothioate (s), Phosphodiester (o), Triantennary GalNAc

*Mouse hepatocytes free uptake

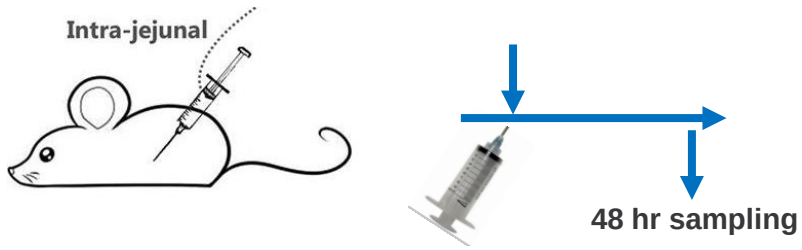
Prakash et al. *Nucleic Acids Res.* **2014**, *42*, 8796-8807.

Oral Delivery

Gen 2.5-GalNAc ASO Proof-of-Concept Studies in Rats and Dogs

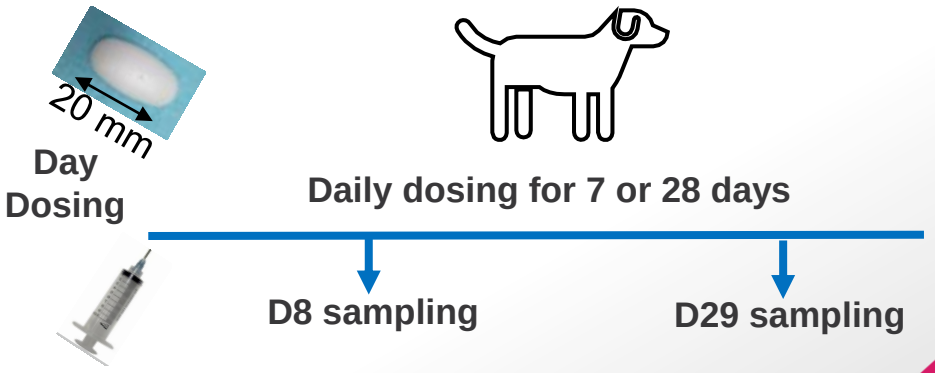
Intestinal absorption of formulated GalNAc GEN 2.5 (cEt) ASO in rat

- GalNAc-conjugated 16mer cEt ASO (GEN 2.5)
- Objectives: PK, liver tissue uptake and mRNA knockdown following a single IJ or SC dose in rats



Repeat-dose oral tablet study of GalNAc-conjugated cEt ASO in dog

- GalNAc-conjugated 16mer cEt ASO (GEN 2.5), in a tablet formulation
- Objectives: PK, liver tissue uptake and safety evaluation following multiple daily oral and SC doses in dogs for 7 or 28 days



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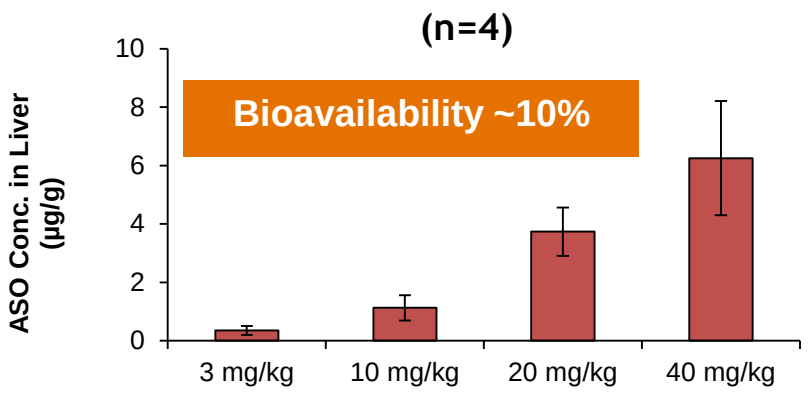
Oral Delivery

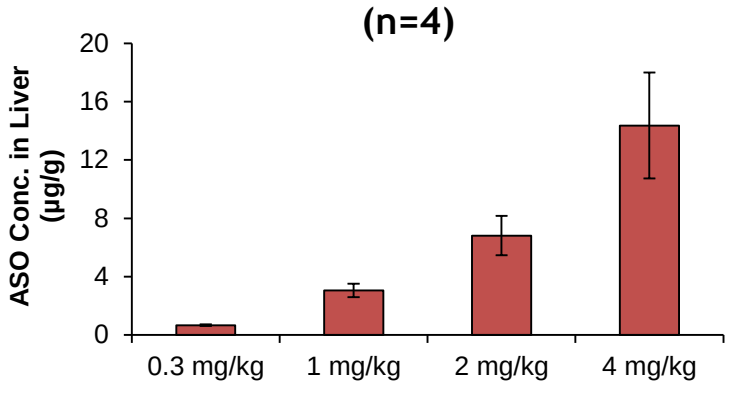
Improved Activity for Gen 2.5 GalNAc ASO as a Single Oral Dose in Rat

Liver ASO Uptake

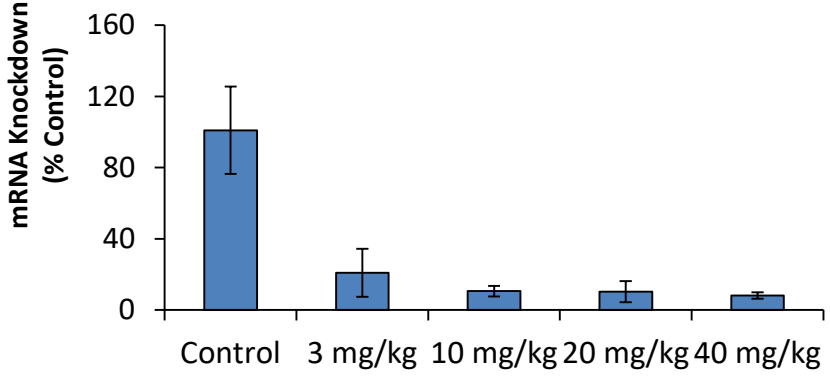
Enteral Dosing

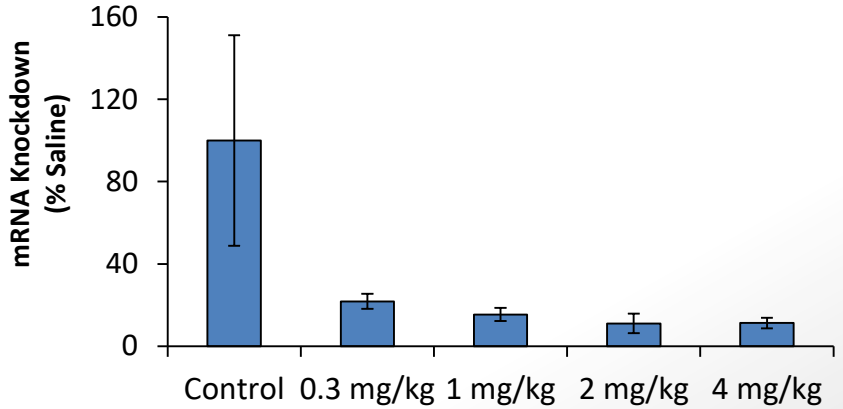


SC Dosing



Liver Target mRNA Reduction





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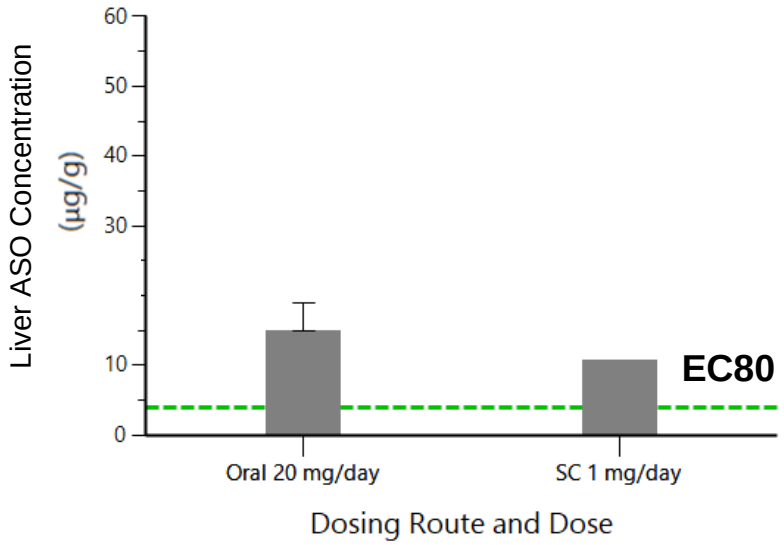
Oral Delivery

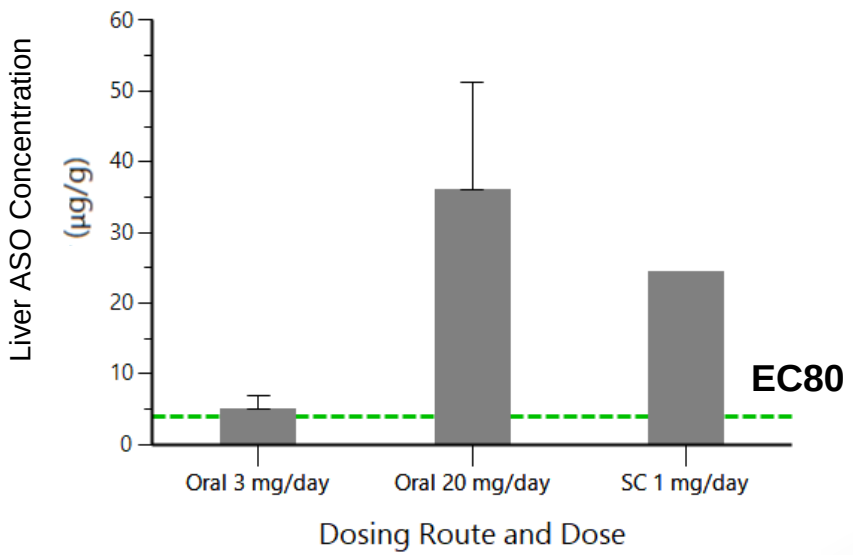
Enhanced Oral Bioavailability of Tablets Demonstrated in Dog

Greater than 10% bioavailability achieved

Daily dosing for 7 Days



Daily dosing for 28 Days



Oral Delivery

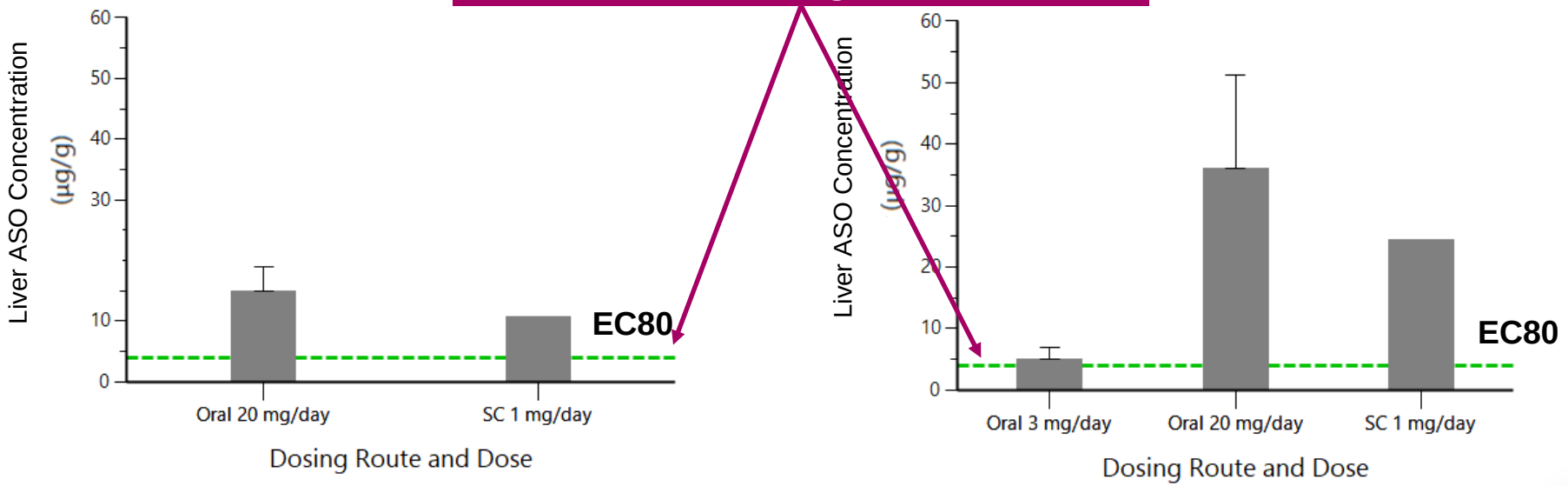
Enhanced Oral Bioavailability of Tablets Demonstrated in Dog

Greater than 10% bioavailability achieved

Daily dosing for 7 Days

Daily dosing for 28 Days

ASO concentration predicted to achieve 80% target reduction



- Liver concentrations increase with repeated administration
- Effective liver concentrations achieved with 20 mg/day oral tablet in dogs
- Replicated in non-human primates with corresponding pharmacodynamic activity

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Oral Delivery

Recent Accomplishments

Generation 2.5 GalNAc ASO can be enteric coated and formulated for oral administration

Generation 2.5 GalNAc ASO appears to provide potency necessary for commercially attractive ASO oral delivery

Oral generation 2.5 LICA formulation well tolerated in all three pre-clinical species

Oral bioavailability study in man in progress

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Oral Delivery

Importance

- **Advances** in **medicinal chemistry** and oral formulations support commercially attractive **oral** administration in humans
- **Commercially attractive oral dosing** expands scope of ASO therapeutics for broad, chronic diseases via enhanced patient convenience
- This will **enable** an entirely **new** set of **opportunities** in which subcutaneous dosing is less attractive
- Another example of the productivity of **Ionis research** to **expand** scope of **antisense** therapeutics
- Research to further **optimize ASO** oral delivery continues

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Bridging the Gap Between Human Genomics and Therapeutics with Ionis Antisense Technology

Expanded Opportunities for ASO Therapeutics by Capitalizing on Advancements in Human Genomics

Causes of human disease are being re-defined based on a revolution in human genomics research

Non-ASO drug platforms struggle to "Bridge-The-Gap" between genomics research and human therapeutics

Antisense technology is the most direct route from the gene to the patient

Advancements in antisense technology coupled to progress in human genomics will further ensure expanded and long-term therapeutic success for the Ionis antisense platform

IONIS

Bridging the Genomics Gap with Antisense Technology

Goals

- Exploit **advancements** in **human genomics** to **broaden** scope of **antisense** therapeutics
- **Focus** on **molecular causes** of disease to deliver more **transformational medicines** for the near and long term
- **Utilize genomic** information to select **transformative** targets
- **Better** define patient populations for clinical trials thereby **enhancing** the **efficiency** of clinical trials
- **Better** understand factors that influence the clinical course of disease
- **Better** understand potential for drug disease interactions

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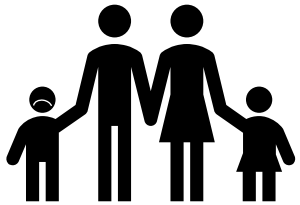
Bridging the Genomics Gap with Antisense Technology

Genomics is Changing The Way We Define Both Rare and Common Diseases

Genetically defined rare and common disease

Sick family member



Individual or Family Genome Sequencing

Identify causal mutation

Most have no therapeutic options

Population Genomics

- The genetic causes of thousands of rare diseases are known
- The molecular causes of many common diseases are being defined genetically (e.g. NASH, Neurodegenerative, Cardiovascular)
- Patient populations are being defined based on the molecular cause of disease
- Newly defined molecular causes of disease are largely undruggable with traditional drug discovery approaches

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Ionis ASO Platform Leading the Way

Converting Genomics Discoveries Into Transformational Medicines for Rare and Broad Populations

Genetic Target	Medicine	Disease	Patient Population
APOCIII	IONIS-APOCIII-L_{Rx}	Cardiometabolic	Many millions
APO(a)	AKCEA-APO(a)-L_{Rx}	Lp(a) CVD	Many millions
C3H (Complement)	IONIS-FB-L_{Rx}	AMD	Many millions
Angiopoietin-Like 3	AKCEA-ANGPTL3-L_{Rx}	Cardiometabolic	Many millions
Factor XI	IONIS-FXI-L_{Rx}	Thrombosis	Many millions
Huntingtin	IONIS-HTT_{Rx}/RG6042	Huntington's Disease	~200K
PNPLA3	ION839	NASH	~200K
Transthyretin	Tegsedi/TTR-L_{Rx}	ATTR	~50K (Hereditary)
SMN1/SMN2	Spinraza	SMA	~50K
C9ORF72	IONIS-$C9_{Rx}$	C9-ALS	~15K
SOD1	Toferson	SOD1-ALS	~3K
Lipoprotein Lipase	Waylivra	FCS	~3K
RHO	IONS357	adRP	~3K

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Bridging the Genomics Gap with Antisense Technology

Future Investments

Access genomics data through internal and external investment to maximize drug discovery & development success

- **Identify novel high-quality targets most attractive for Antisense technology**
  - Ensure for a rich and evergreen pipeline of “First-in-Class” medicines well into the future
- **Improve clinical trial efficiency**
  - Understand patient journey to support payor negotiations
  - Identify relevant patient factors
  - Biomarker identification and validation
- **Improve clinical trial outcomes**
  - Identify patient subpopulations that will benefit most from each medicine
  - Redefine causes of heterogeneous diseases
  - Sequence and profile our patients to better understand drug performance/patient outcomes

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Bridging the Genomics Gap with Antisense Technology
Importance

- **Coupling** Ionis ASO technology will help ensure delivery of more transformational medicines well into the future
- **Novel** targets
- **Selection** of most appropriate patients for clinical trials
- **Better** understand disease course
- **Better** understand drug disease interaction
- **Establish** biomarker-disease relationships
- **Opportunities for preventative medicine**

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New Directions for Core Antisense Research

Stanley Crooke, M.D., Ph.D.

Chief Executive Officer and Chairman

Core Antisense Research

Post RNA Binding Mechanisms

In principle, binding a tiny ASO to a very large RNA molecule should have little effect

- In fact binding of ASOs to many sites in RNAs has little effect on the RNA
- By harnessing cellular mechanisms that recognize ASO RNA duplexes, binding of an ASO to an RNA can cause pharmacologic effects

The more post binding mechanisms we can choose from, the more versatile and broadly useful is antisense technology

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Core Antisense Research

Post RNA Binding Mechanisms: Goals

To understand the mechanisms of action of ASOs in sufficient detail to support optimal ASO design

- Understand each step involved in each antisense mechanism
- Use the knowledge to design better ASOs and to identify optimal ASOs for therapy more efficiently

To broaden the number of mechanisms of action available to Ionis

- Increase the versatility of the technology

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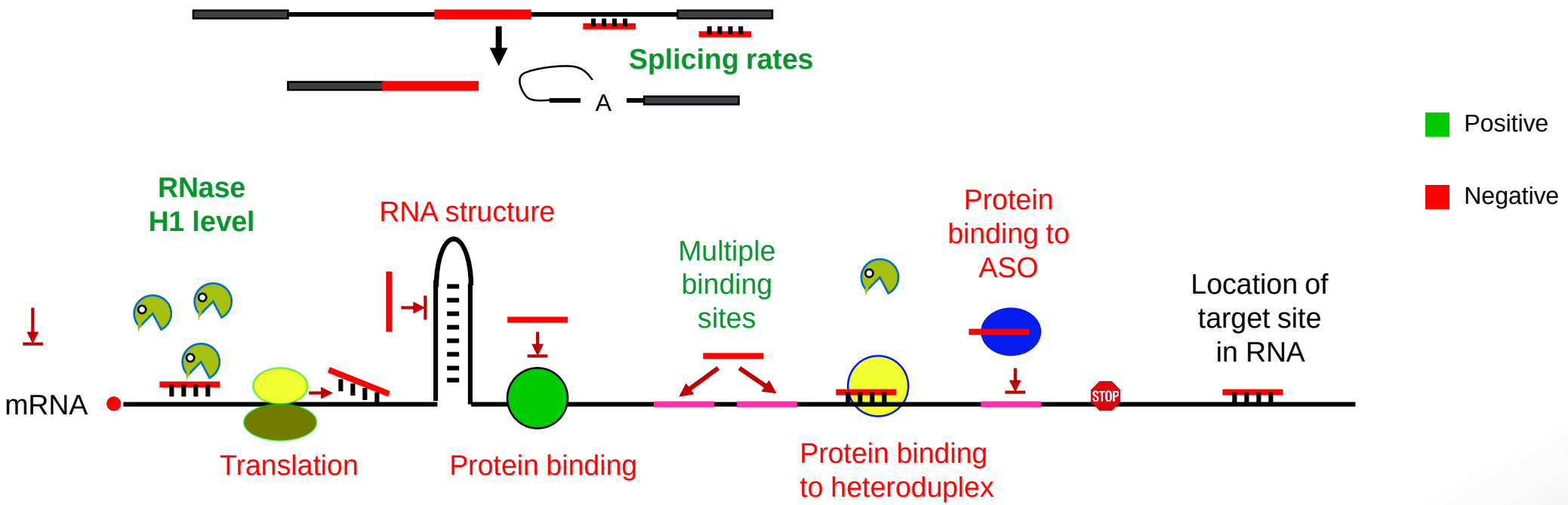
Core Antisense Research

Post RNA Binding Mechanisms

RNA features can affect ASO activity



- No or minor effects:
  - mRNA copy number
  - Transcription rate(except very rapid transcription rates (e.g., cMyc)
  - Half-life

Crooke et al., Nat. Biotech. 2017, 35(3):230-237; Lima WF et al., PLoS One. 2014;9(7):e101752; Vickers TA et al., PLoS One. 2014 Oct 15;9(10):e110615; Vickers TA et al. ,NAR, 2011;39(10):e71; Vickers TA et al., NAR 2015;43(18):8955-63; Liang XH et al., Mol Ther. 2017;25(9):2075-2092; Liang XH et al., 2018 NAR;46(1):293-313; Liang XH et al., unpublished; Pollak A et al., unpublished;

Multiple Post-Binding Mechanisms

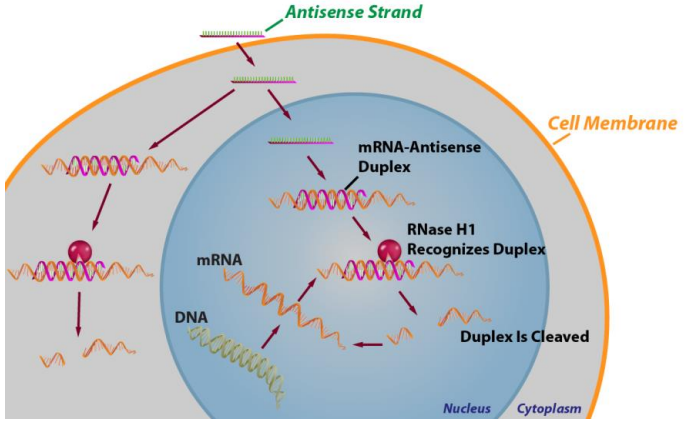
Core Antisense Research

Post RNA Binding Mechanisms

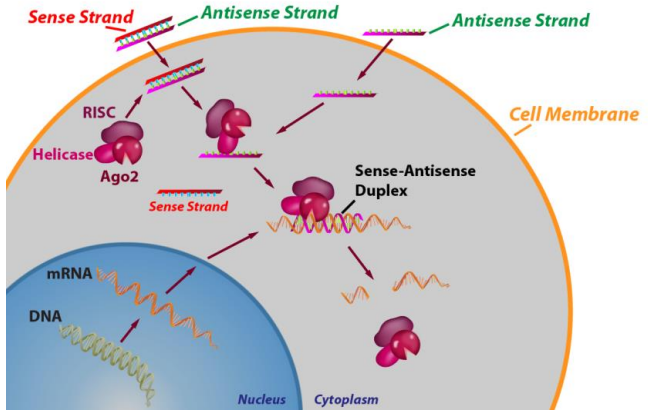
Simplified Antisense Mechanisms

Enzymatic RNA Degradation

RNase H1

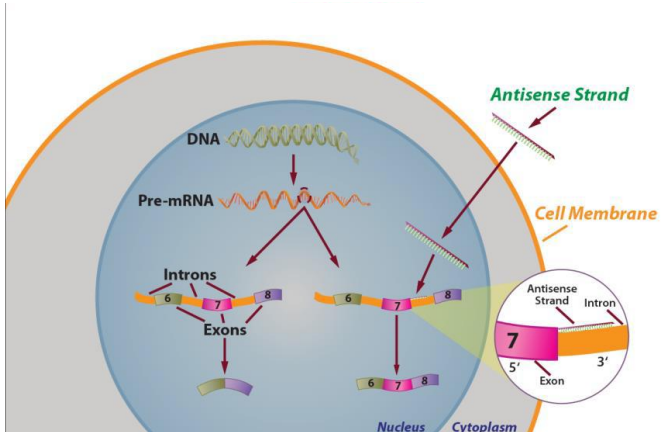


siRNA

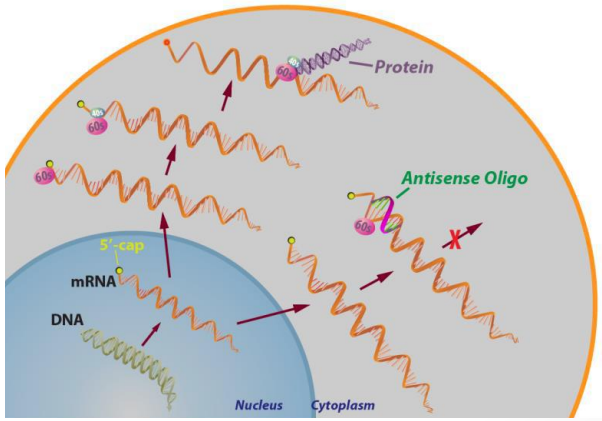


Occupancy Only Mechanisms

Splicing Modulation



Translation Arrest



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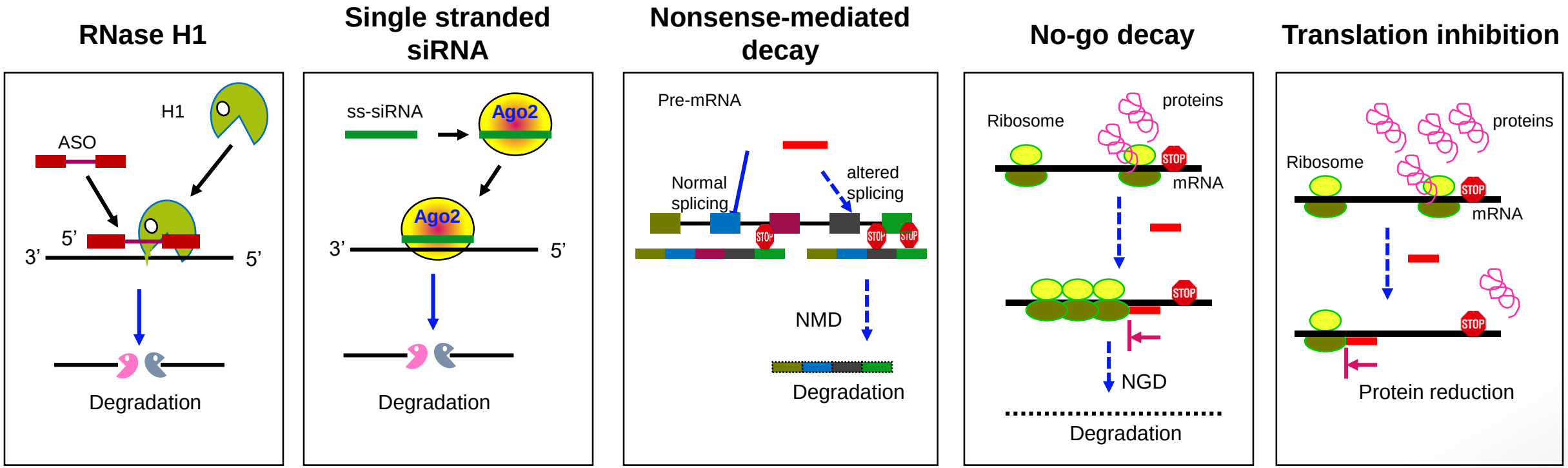
In Antisense Drug Technology: Principles, Strategies, and Applications, Second Edition, Crooke, ST. (ed), Baco Raton, FL, CRC Press, Chapter 1, 3-46, 2008.

Reducing Target Protein and RNA Levels

ASOs Can Reduce Target Gene Expression via Different Mechanisms



Crooke ST. Curr Mol Med. 2004;4(5):465-87; Crooke ST. NAT, 2017;27(2):70-77; Crooke et al., Nat. Biotech. 2017, 35(3):230-237; Lima WF et al., Cell. 2012;150(5):883-94; Yu D. et al., Cell. 2012 150(5):895-908; Hanecak R et al., J Virol. 1996, 70(8):5203-12; Ward AJ., et al., NAR 2014;42(9):5871-9; Rigo F et al., NCB, 2012;8(6):555-61; Liang XH et al., NAR, 2019;47(13):6900-6916;

Reducing Target Protein and RNA Levels

Potential Value of No-go Decay and Other Non-RNase H1 Mechanisms

Increased versatility for antisense technology

Potential solutions “for RNase H1-difficult” RNAs

Potential solution to RNAs that may develop “tolerance” to RNase H1 induced degradation

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RNA Degradation Mechanisms

Factors That Influence ASO Activity Translation Efficiency Affect ASO Activity

Why be interested?

- Understanding the factors that influence the activity of ASOs is the first step in developing approaches to better designs of ASOs

RNAs are composed of several domains

- The 5’ untranslated region
- Introns
- Exons
- The open reading frame
- 3’ untranslated regions

ASOs designed to bind to each of these different domains differ slightly in effects

Understanding the causes of these slightly different behaviors, we can better design ASOs to different domains of RNAs

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Selectively Increasing the Level of Specific Proteins

Identifying Potential Mechanisms to Selectively Increase Specific Protein Levels

In principle a significant limitation of antisense is that it can only be used to cause reduction of RNAs and proteins

- The equivalent of antagonist therapy

It would add substantial versatility to the platform and broaden the reach of the technology if we could design ASOs to be “agonists”

- On occasion (like Spinraza) we can use splicing ASOs to do this, but we need more

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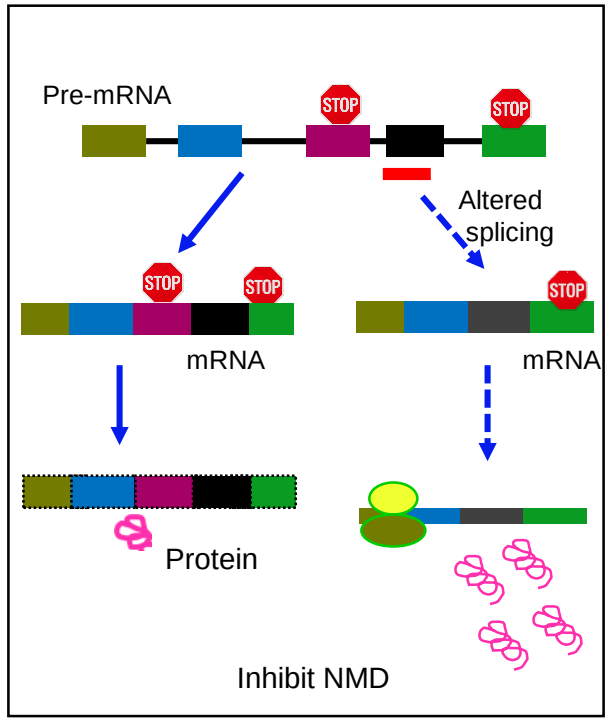
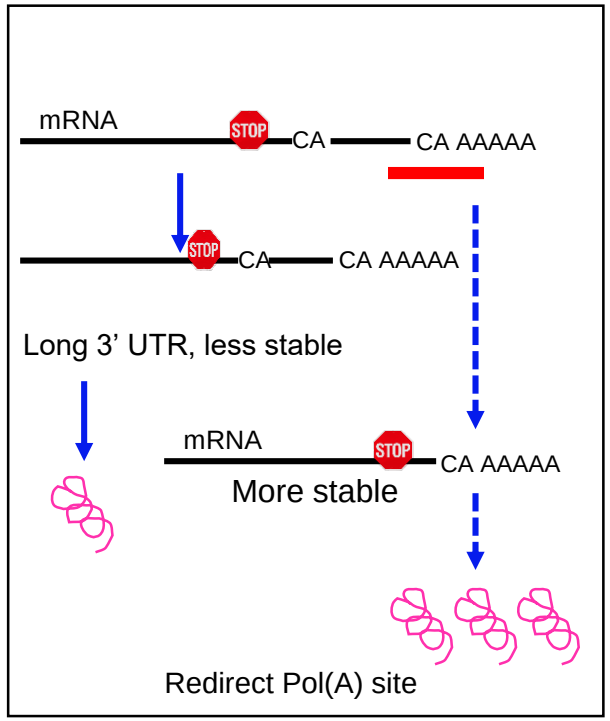
Selectively Increasing Level of Specific Proteins

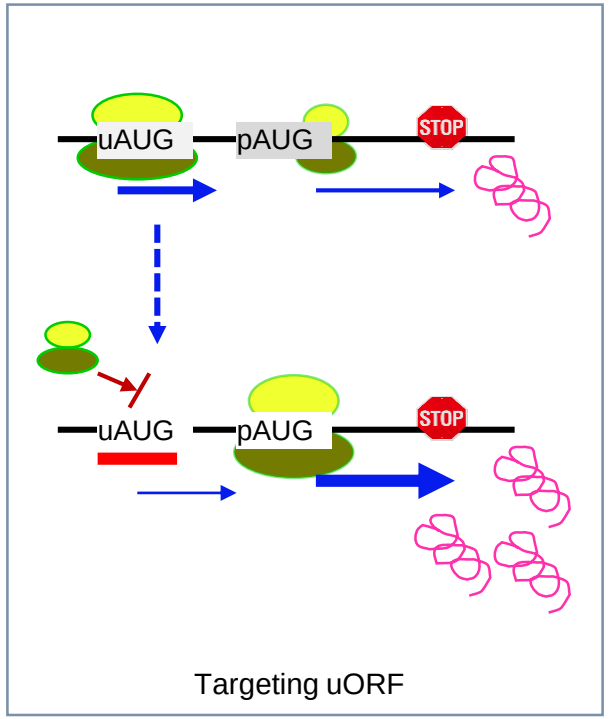
ASOs Can Increase Protein Levels via Different Mechanisms

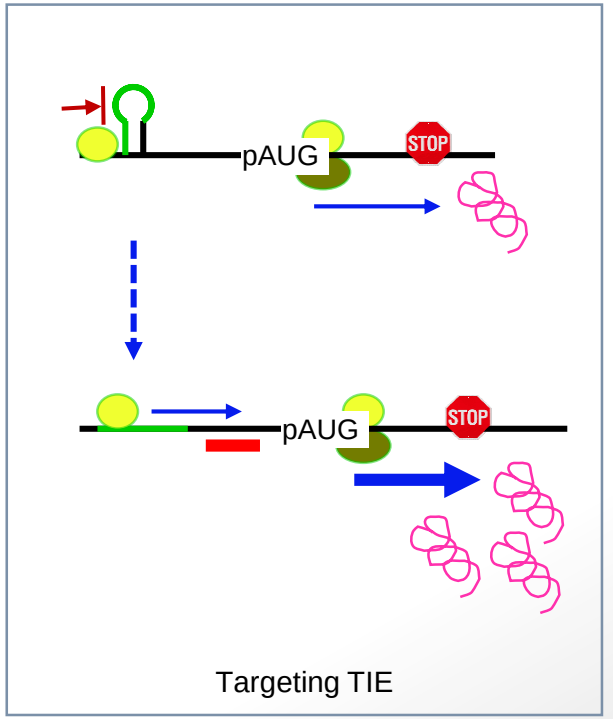
To increase mRNA levels





To enhance translation





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Liang et al. NBT (2016): 34, 875-882, Liang et al, NAR (2017): 45, 9528-9546

Selectively Increasing Level of Specific Proteins

Lessons Learned

ASOs may be designed to take advantage of many post binding mechanisms

Multiple mechanisms to reduce the levels of target RNAs enabled

Multiple mechanisms to increase the levels of specific proteins enabled

Factors that alter the activity of ASOs designed to work via specific mechanisms better understood

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Selectively Increasing Level of Specific Proteins

Importance

The versatility of Antisense technology substantially enhanced

Multiple choices of mechanisms facilitate selection of the optimal approach to address a broad range of biological problems and diseases

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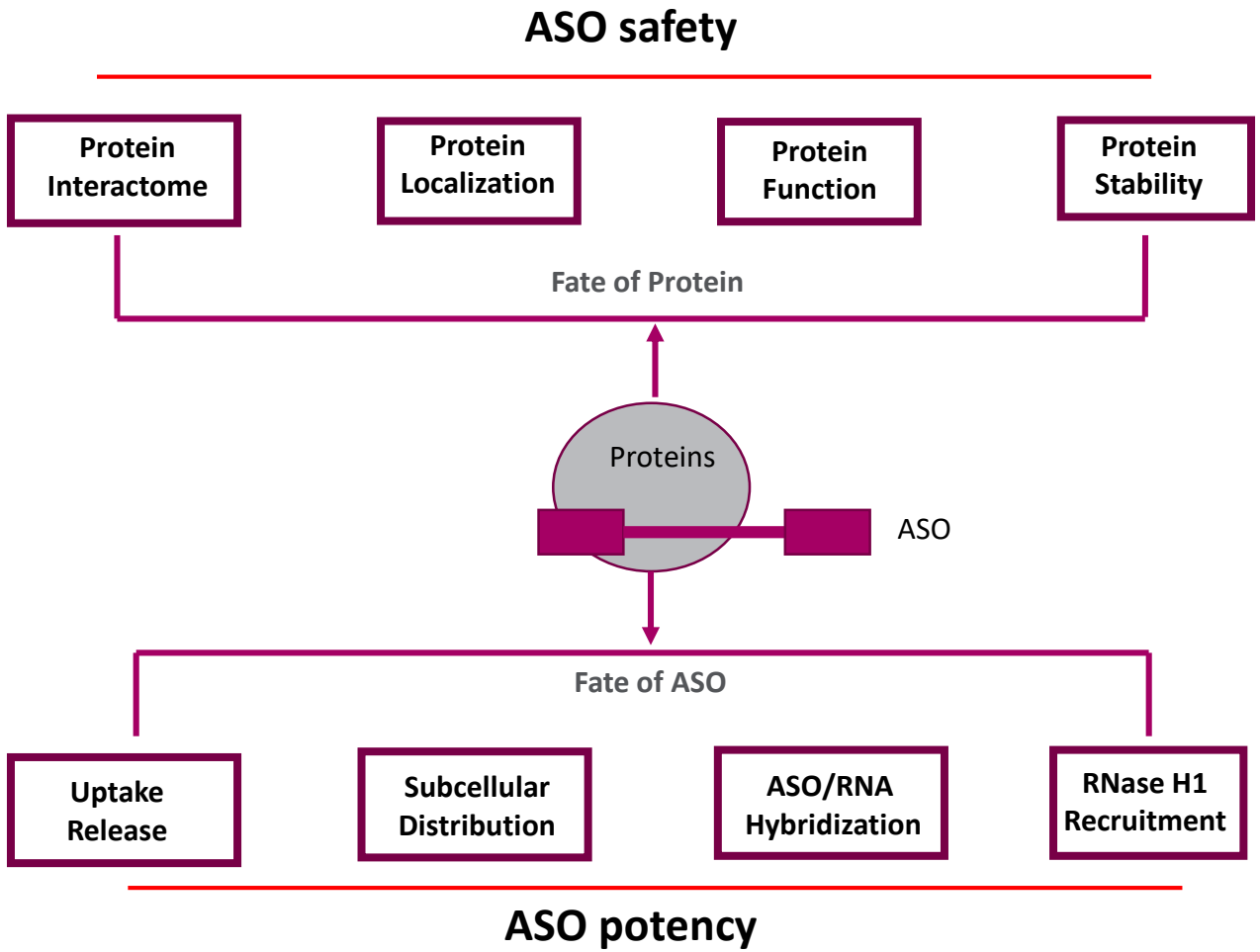
Proteins Determine the Fate of PS-ASOs In Vitro and In Vivo

Proteins Determine the Fate of PS-ASOs

ASO - Protein Interactions Play Important Roles in ASO Performance



Crooke, ST., et al., (2017) NBT 35:230-237; Liang, XH., et al, (2015) NAR, 43:2927-2945;Shen, W., et al, (2015) NAR, 43(9):4569-4578; Shen W., et al., (2014) NAR, 42(13):8648-62
Liang XH et al., (2014) NAR, 42(12):7819-32; Shen W., et al., (2017) NAR, 45(18):10672-10692; Liang XH., et al., (2018) NAR 46(19):10225-10245
Vickers, T.A., et al., (2019) NAR epub; Shen W., et. al., (2019) NBT 37(6):640-650.; Magawa, M., et. al., (2019) NAR, 47(11):5465-5479; Wang Y., et al., (2019) NAT. In press

Proteins Determine the Fate of PS-ASOs

To Understand the Molecular Mechanisms of ASOs, We Must Now Consider Two Codes

Nucleic Acid

Oligonucleotides

- Sequence
- Charge
- Phosphorothioates
- 2’modifications
- Orientation of 2′ modified wings
- Structure
  - Duplexes
  - G quartets, et al
  - Lattice works

RNA

- Sequence
- Atypical bases (A to I editing, for example)
- Structure
- 2′ modifications
- Base modifications
- RNase H1 site and sequence preferences
- Protein binding sites

IONIS

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Amino Acid

Oligonucleotides

- Phosphorothioates
  - Number
  - Placement
- Charge
- 2′ modifications
  - Hydrophobicity
  - Number
  - Orientation (5′ or 3′)
- Sequence (partial)
- Base modifications
- Pendant groups(partial) (conjugates)

Protein

- Domains (for key proteins)
- Structures
- Charge
- Hydrophobicity
- Modifications
  - Acylation
  - Phosphorylation
  - Glycosylation
  - Lipidation
  - Ubiquitinylation

IONIS

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Proteins Determine the Fate of PS-ASOs

Lessons Learned

The fates of PS-ASOs throughout biological systems are defined by proteins

Key proteins involved in ASO distribution, activity and toxicities identified and characterized

Structure activity relationships for ASOs interacting with proteins are becoming very well understood

Structure activity relationships of proteins interacting with ASOs are becoming very well understood

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Enhancing Productive Distribution Inside Cells

Enhancing Productive Distribution Inside Cells

Predicate

> 99% of intracellular ASO is wasted

Known intracellular sites in which ASOs are active

- Nucleus
  - Transcription complex
  - Spliceosomes
  - Nucleoplasm
- Endoplasmic reticulum
- Polysomes

Known intracellular sites in which ASOs are inactive

- Lysosomes
- Early endosomes

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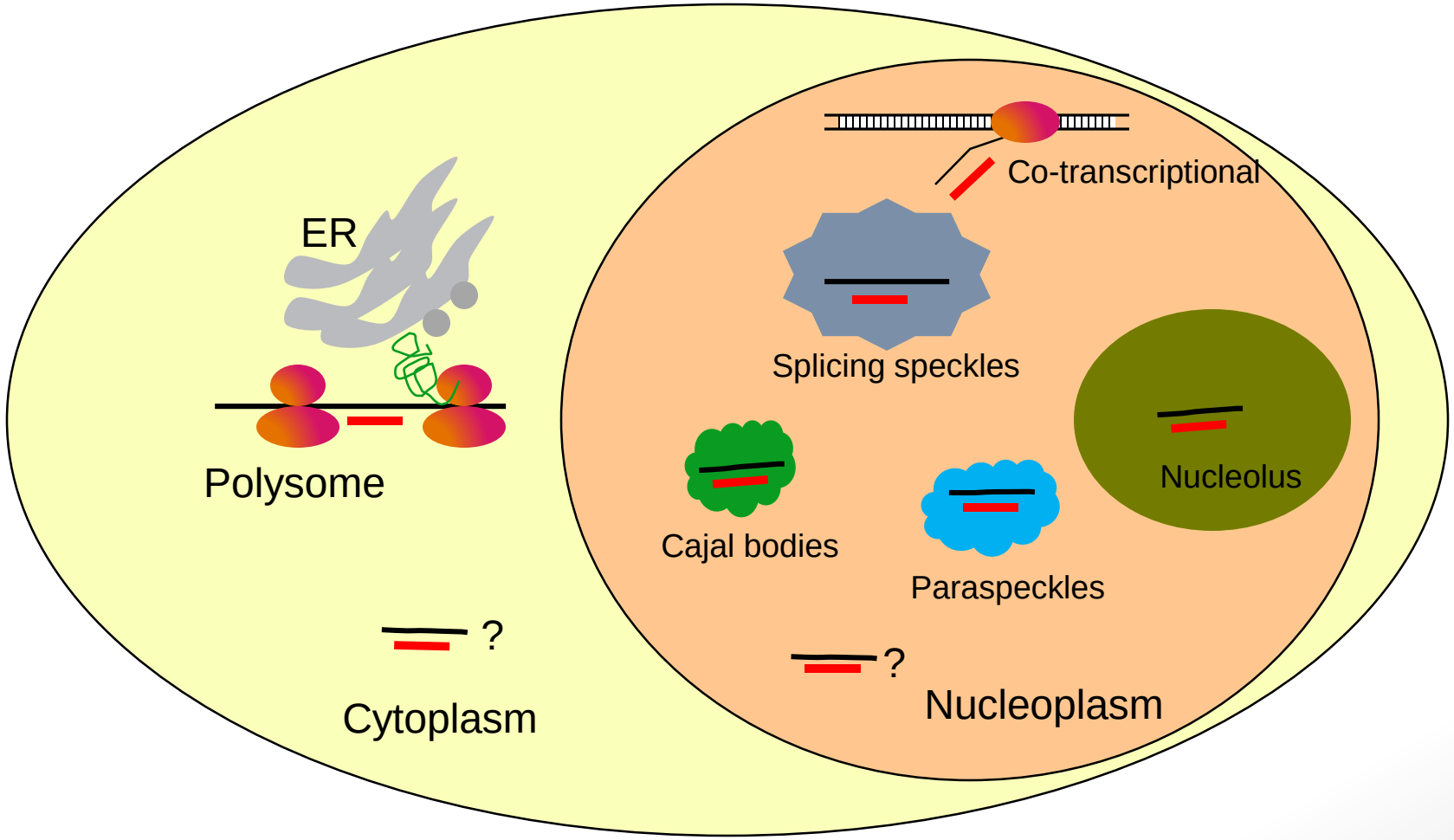
Enhancing Productive Distribution Inside Cells

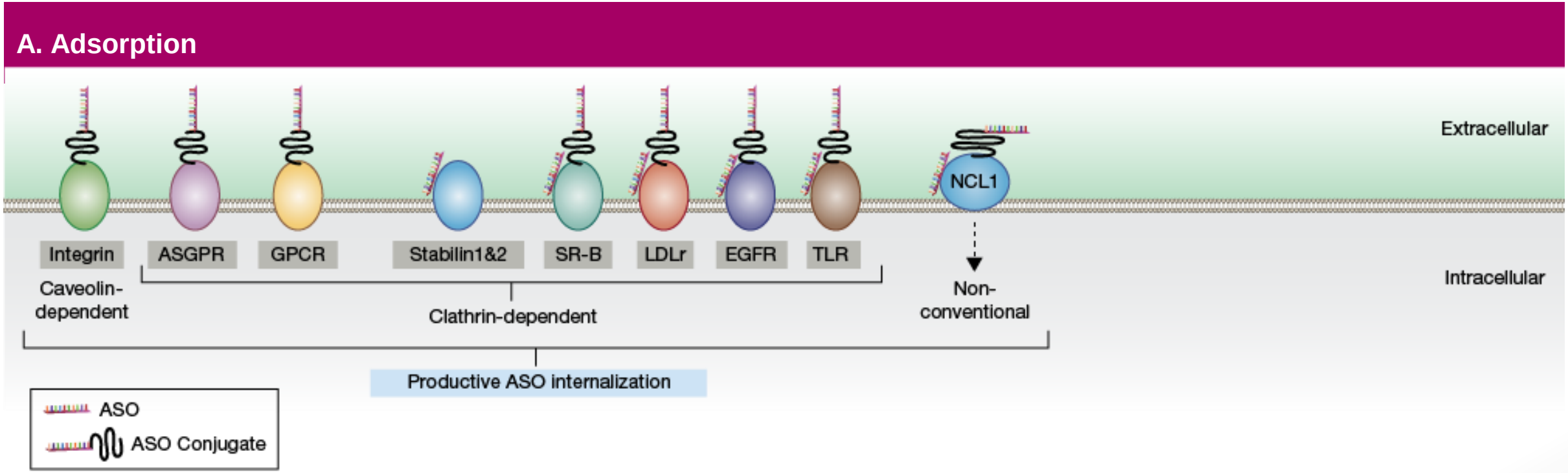
Confirmed and Probable Subcellular Sites Where ASOs Are Active



Crooke et al., Nat. Biotech. 2017, 35(3):230-237; Liang et al, NAR 2011, 39(3):e13; Wheeler TM et at., Nature, 2012;488(7409):111-5; Lima WF et al., NAR 2016, 44(7):3351-63; Vickers TA et al., NAR 2015;43(18):8955-63; Liang XH et al., Mol. Ther. 2017;25(9):2075-2092; Liang XH et al., 2018;46(1):293-313.

Enhancing Productive Distribution Inside Cells

Mechanisms of Cellular Uptake and Distribution of PS ASOs



Crooke ST et al., (2018) Cell Metab. 27(4):714-739.,Crooke ST et al., (2017) NBT 35(3):230-237
Dowdy SF. et al., (2017) NBT 35(3):222-229; Wang S., et al., (2018) NAR 46(7):3579-3594
Prakash TP. et al., (2014) NAR 42(13):8796-807; Juliano R. et al., (2015) Adv Drug Deliv. Rev. 87:35-45
Juliano R., et al., (2016) NAR 2016, 44(14):6518-48; Wagenaar TR et al., (2015) NAR 43(2):1204-15
Liang XH., et al., (2018) NAR 49(19):10225–10245; Wang S et al., (2018) Mol. Therapy Nucleic Acids, (13) 686-98.

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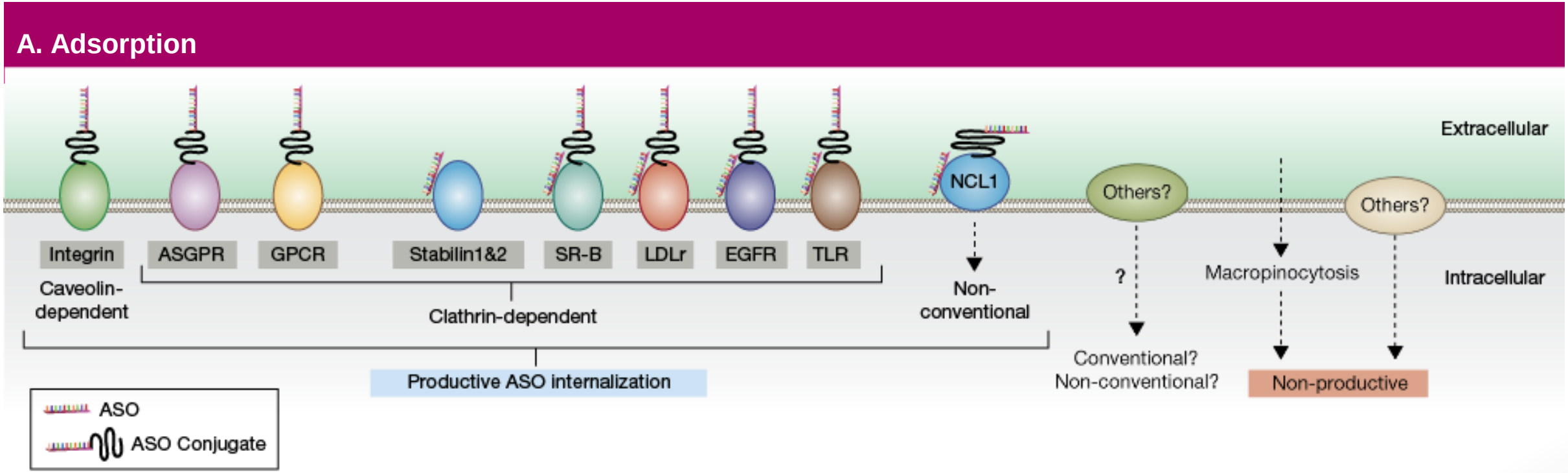

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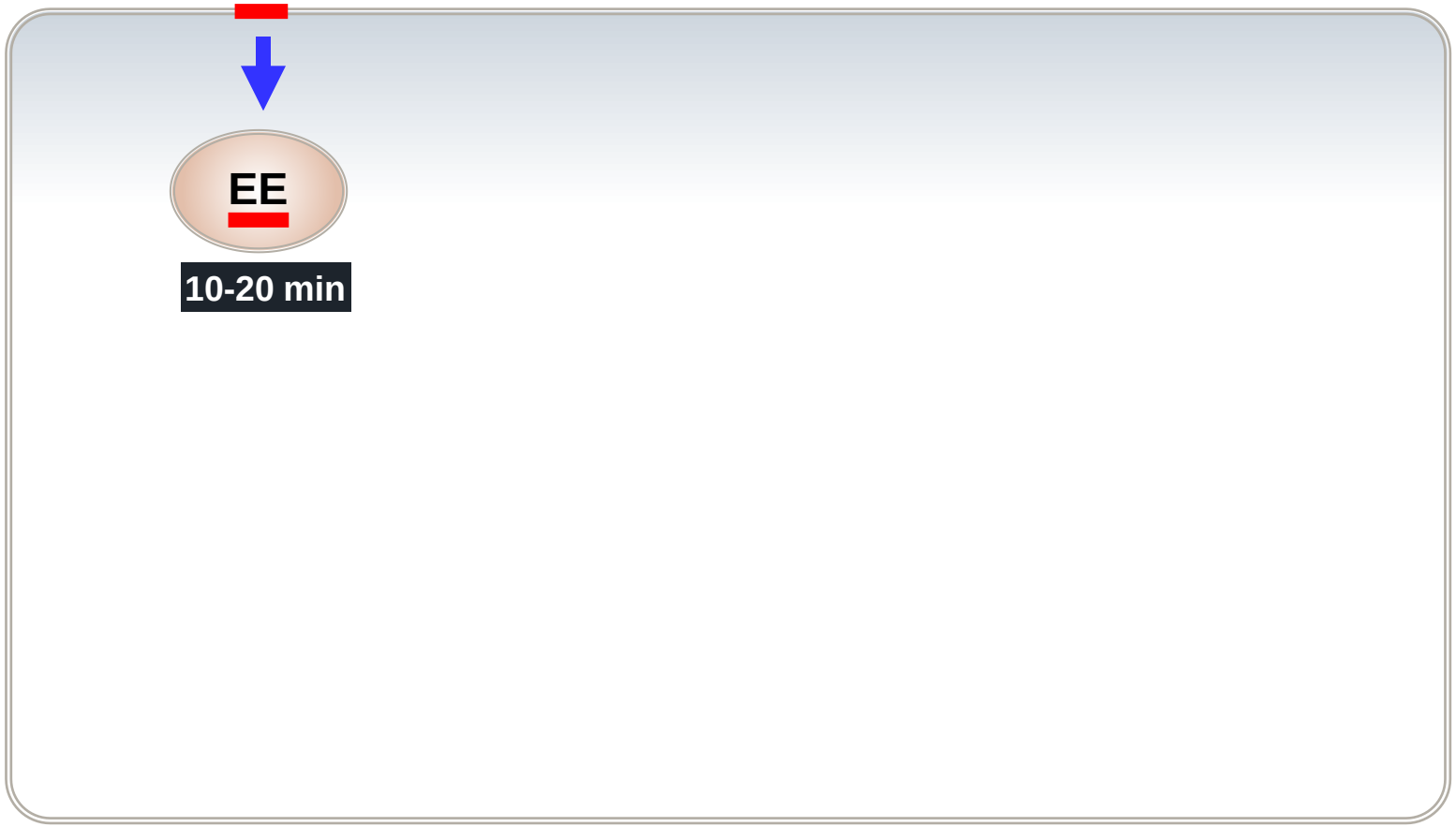
Enhancing Productive Distribution Inside Cells

Mechanisms of Cellular Uptake and Distribution of PS ASOs



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EE, early endosome; LE, late endosome

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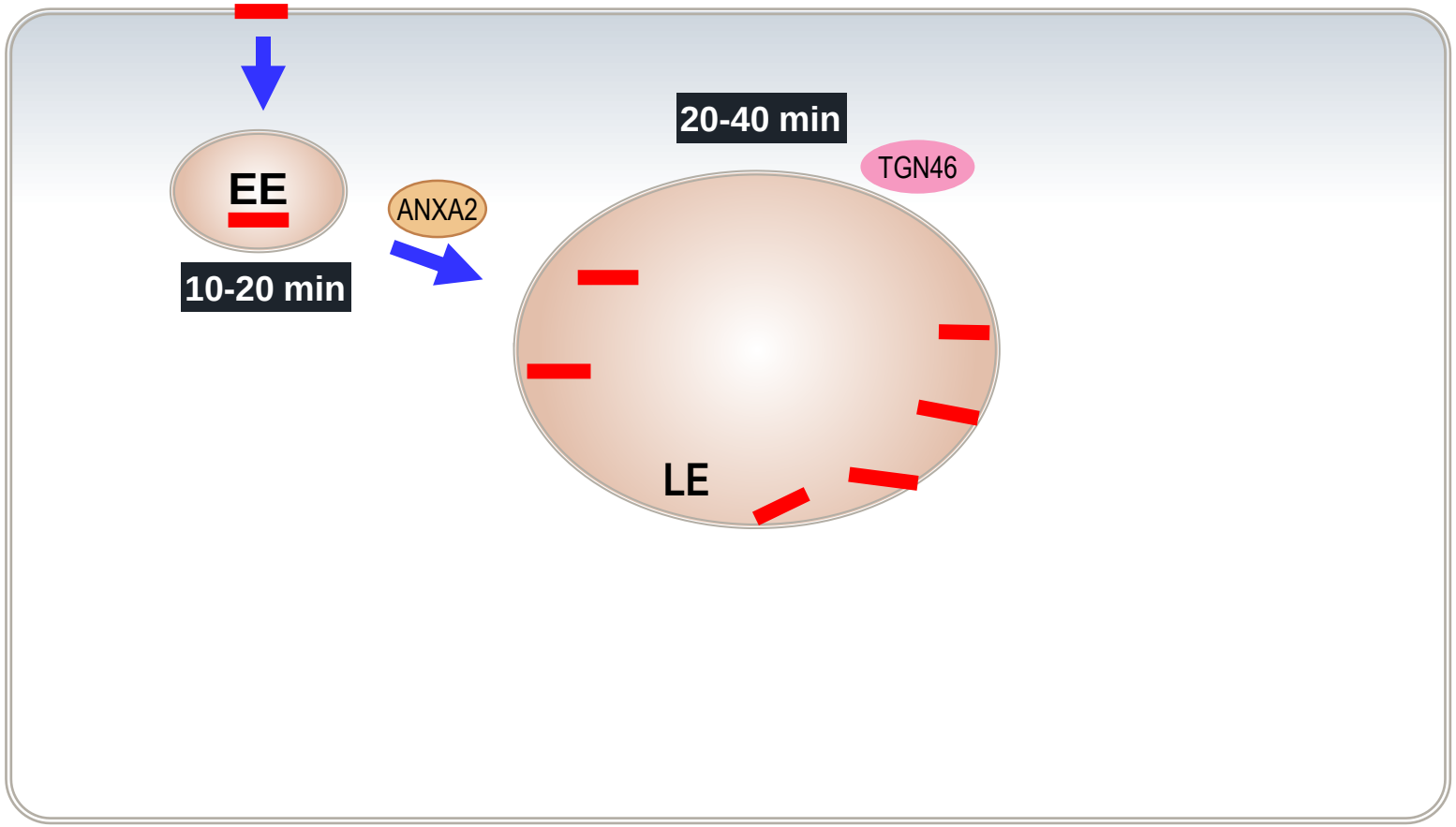

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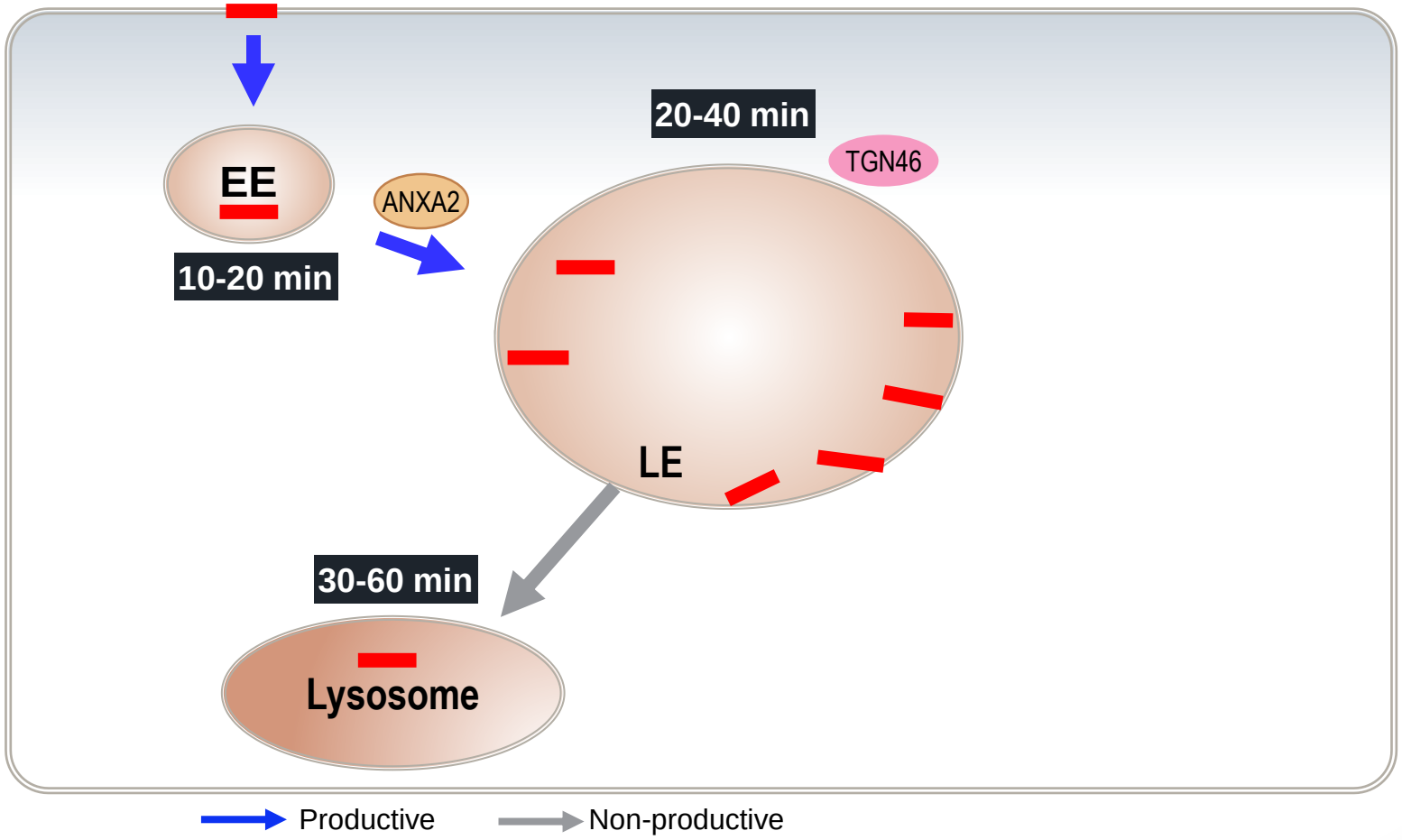

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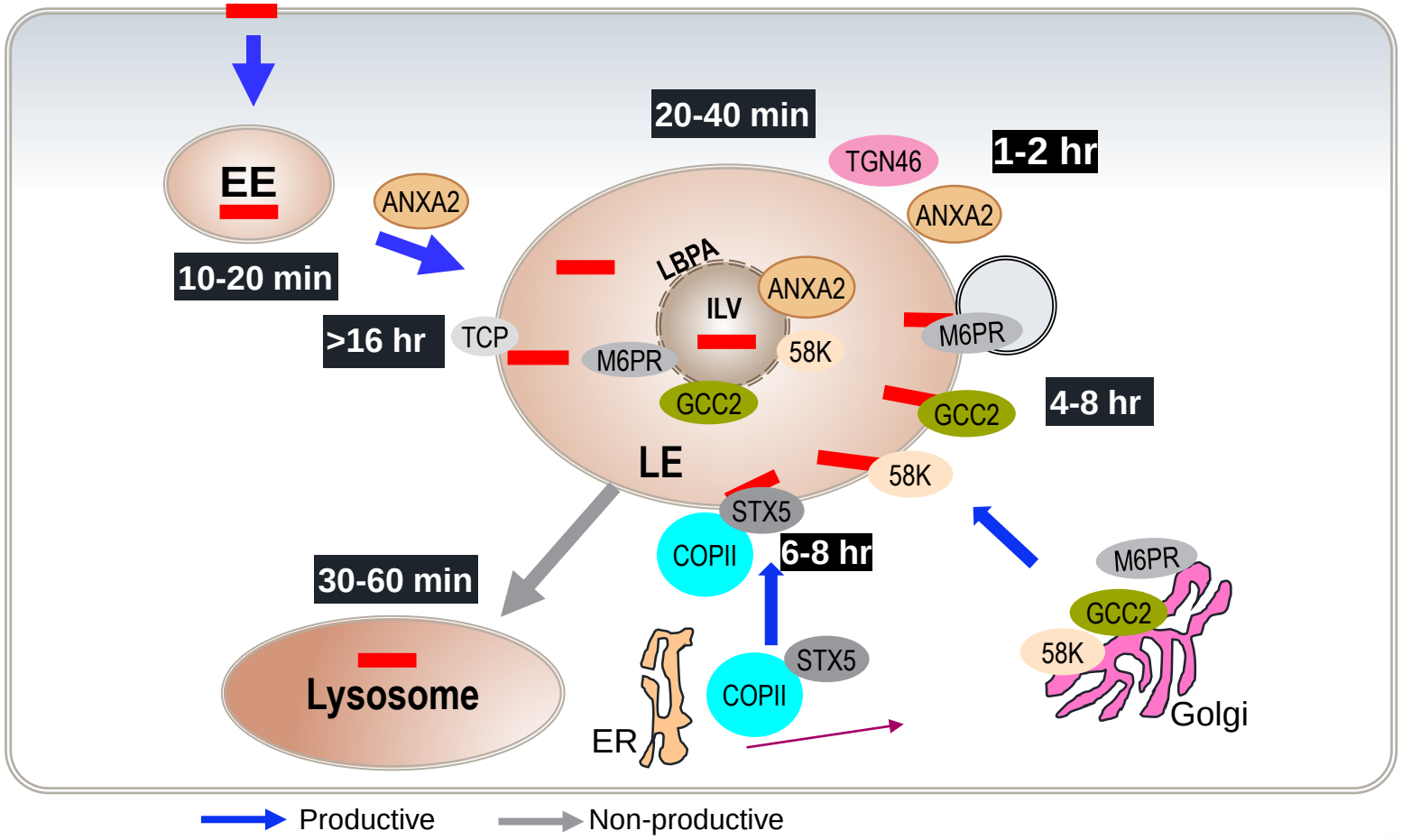

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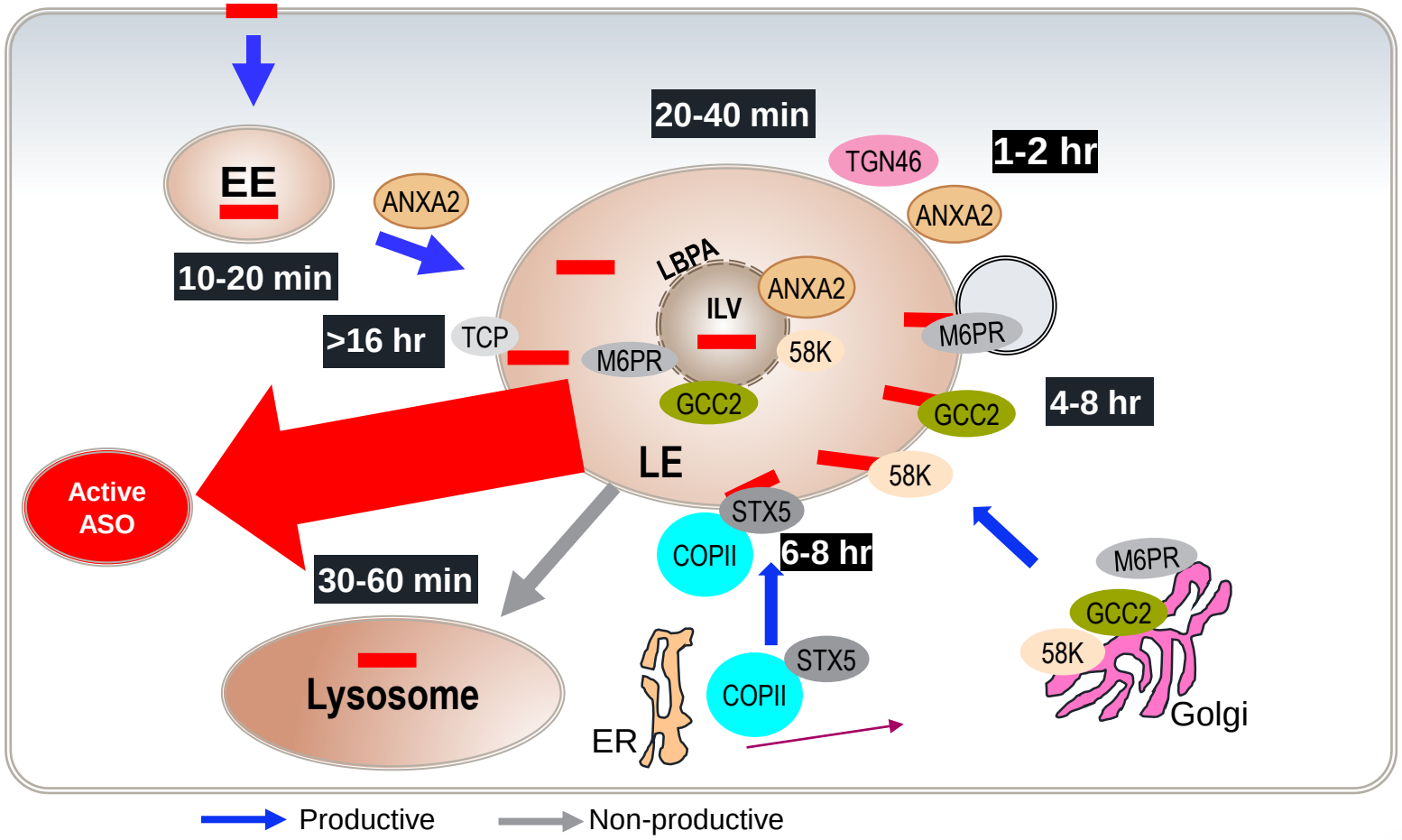

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Enhancing Productive Distribution Inside Cells

Lessons Learned

- **We** have identified most of the major sites in cells at which ASOs are active
- **We** have identified several sites in cells at which ASOs are inactive
- **We** know that the choice of whether an ASO enters a productive or nonproductive pathway is made at the cell surface
  - Micropinocytosis for example is a nonproductive pathway producer
- **We** know the major pathways and the key proteins involved
- **We** are learning new medicinal chemical approaches

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Enhancing the Therapeutic Index of PS-ASOs by Altering the Proteins with Which They Interact

Enhancing Therapeutic Index
Key Observations

In a screen of 100 2′MOE second generation ASOs, 1-5 ASOs maybe toxic

- Since all 100 ASOs are the same except for the sequence, the toxicity must be affected by sequence

In a screen of 100 higher affinity more potent generation 2.5 ASOs (e.g., 2′cEts or LNAs), 30-40 or more are toxic and the toxicities are worse

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Enhancing Therapeutic Index
Questions

Why are some ASO sequences toxic and others not toxic?

Why do 2′ modifications enhance potency?

- Result in a much higher function of ASO sequences being toxic
- Why are the toxicities worse?

What is the mechanism of the toxicities?

Can we better understand the mechanism of toxicity?

Can we enhance the safety and performance of ASO medicines by answering key questions about therapeutic index?

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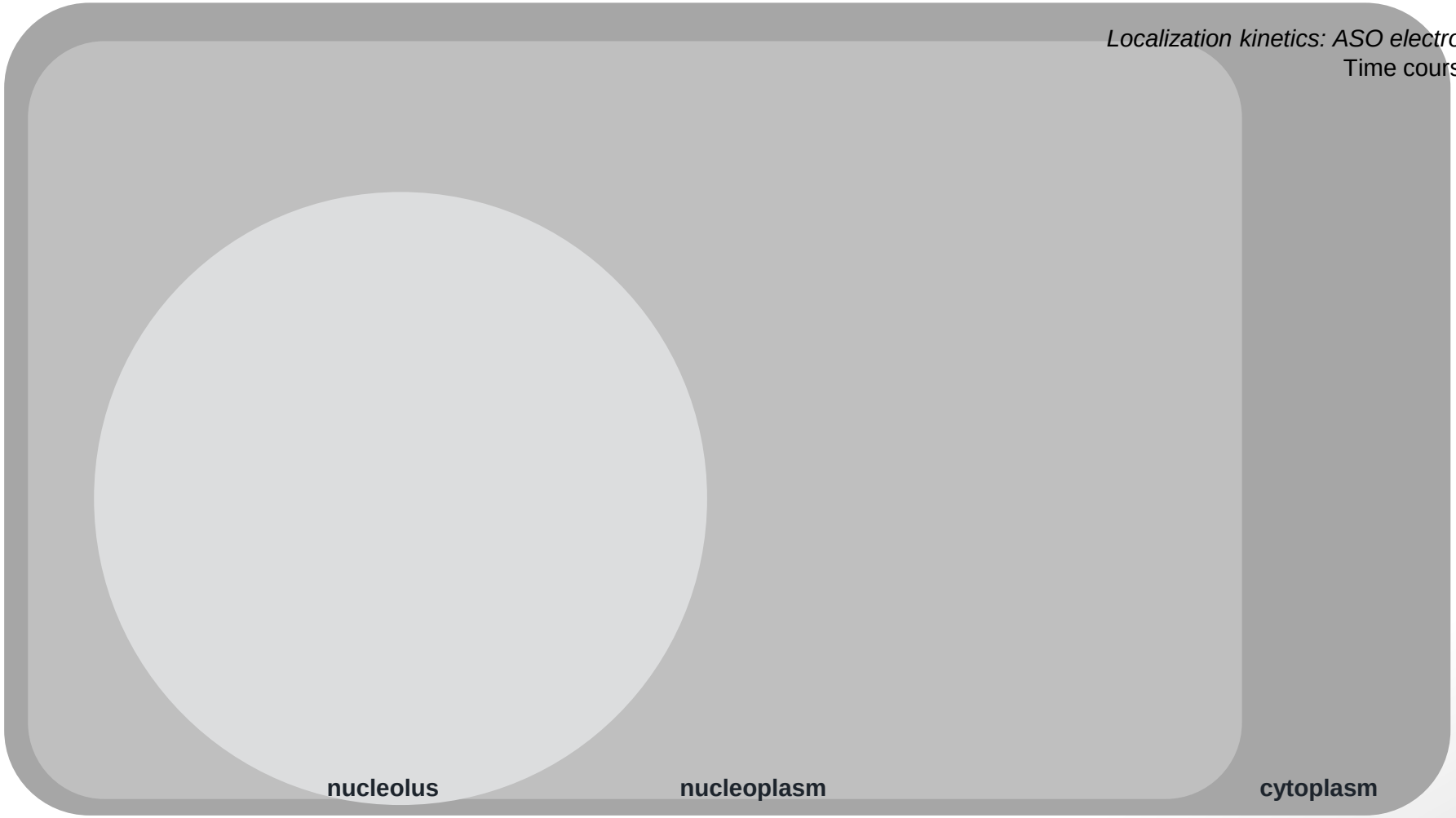
Enhancing Therapeutic Index

A Path to ASO Toxicity

Nucleolar mislocalization of cellular proteins by toxic cEt ASOs mediated by RNase H1

Localization kinetics: ASO electroporation in HeLa

Time course: **Electroporation**



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Enhancing Therapeutic Index

A Path to ASO Toxicity

Nucleolar mislocalization of cellular proteins by toxic cEt ASOs mediated by RNase H1

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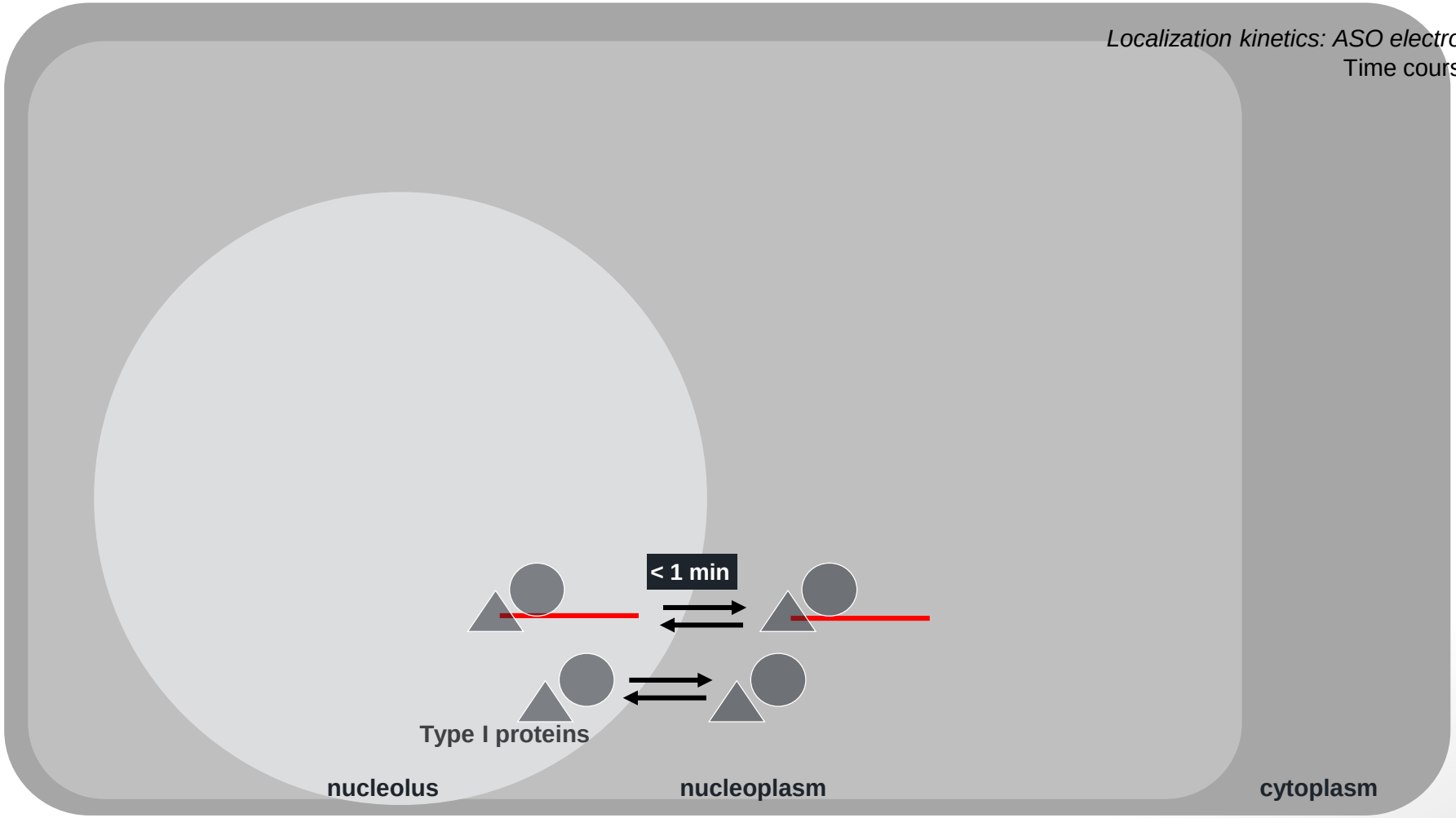
Time course: **Electroporation**



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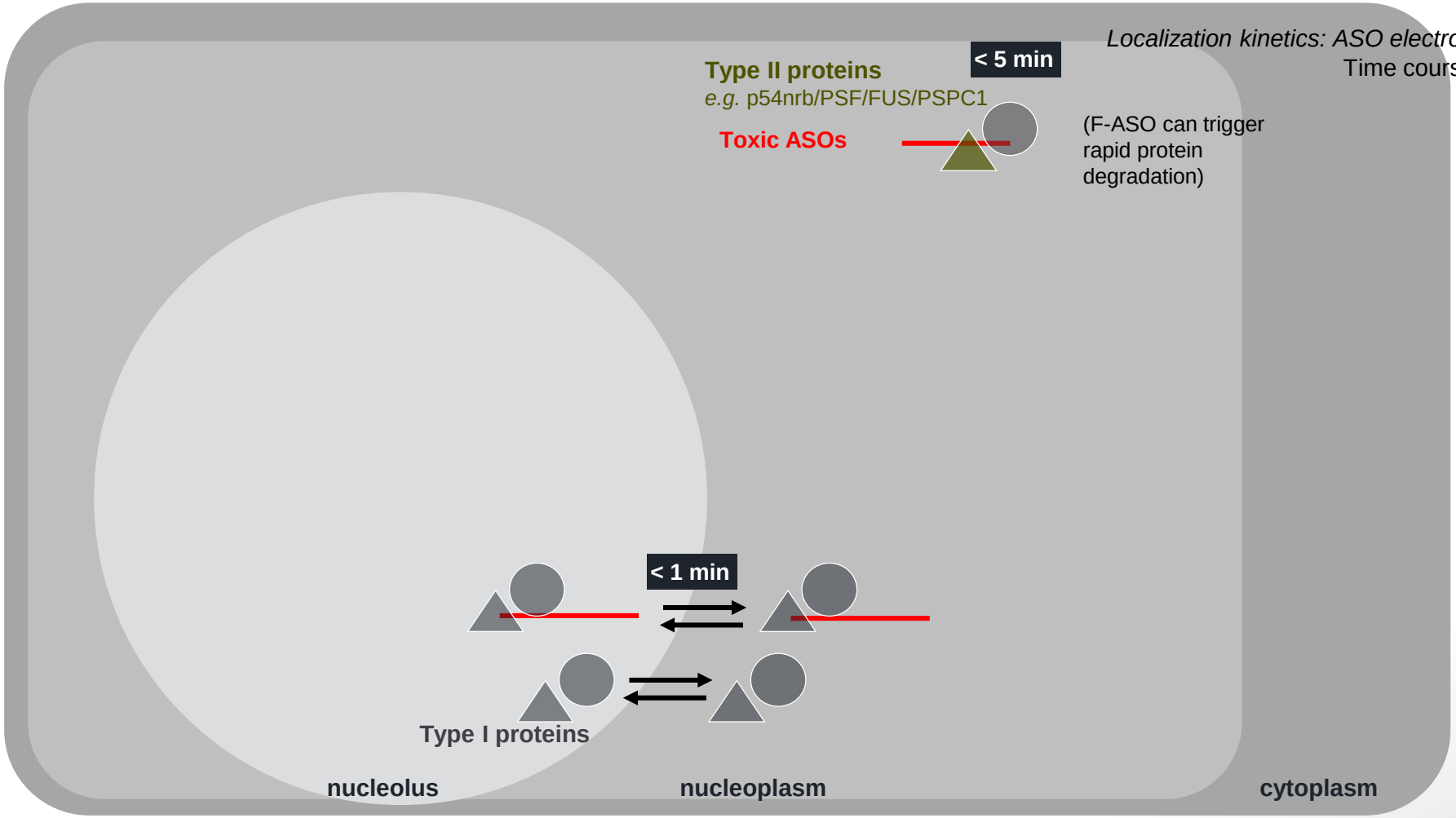

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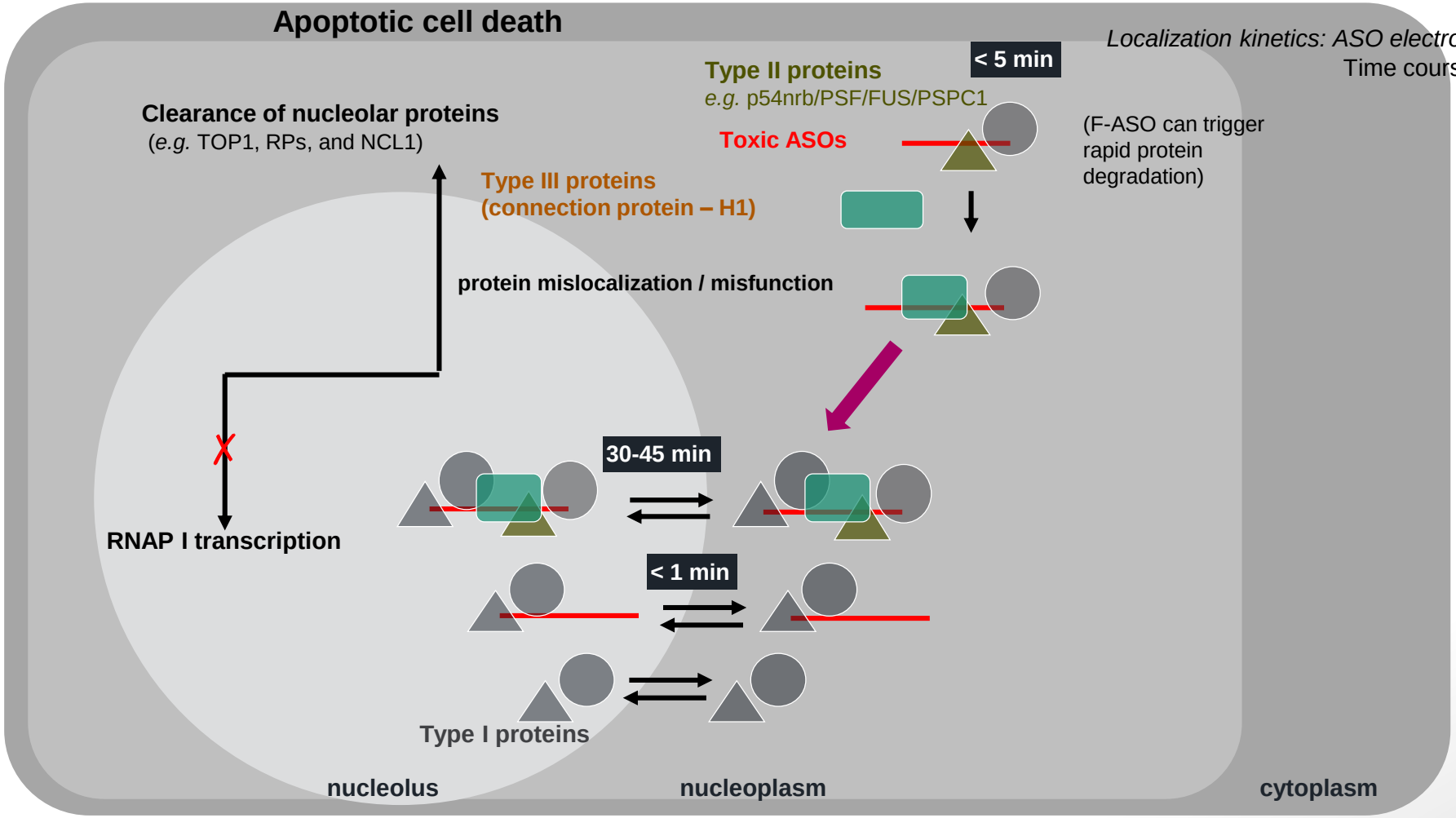

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Toxicities Can Be Ablated or Reduced Without Significant Loss of Potency by Gap 2 Position Modifications

Gap 2 Position Modifications Reduce Toxicities Without Significant Loss of Potency

Examples of toxic PS-ASOs and improvement in therapeutic index by 2’ methoxy at gap position 2

	Parent				2'-OMe (gap2)			
Target	Ion #	TD_{50} (mg/kg)	ED_{50} (mg/kg)	TI (TD_{50}/ED_{50})	Ion#	TD_{50} (mg/kg)	ED_{50} (mg/kg)	TI (TD_{50}/ED_{50})
HDAC 2	546108	57	3.2	18	1133122	>10000	5.5	>1818
HDAC 2	546110	318	6.4	50	1133123	>10000	6.8	>1190
HDAC 2	546118	569	23.8	24	1133127	>10000	24.9	>401

	Parent				2'-OMe (gap2)			
Target	Ion #	TD_{50} (mg/kg)	ED_{50} (mg/kg)	TI (TD_{50}/ED_{50})	Ion#	TD_{50} (mg/kg)	ED_{50} (mg/kg)	TI (TD_{50}/ED_{50})
FXI	464924	425	2.3	184	1133247	>10000	3.4	>2941
FXI	465172	>10000	6.7	>1492	1133326	>10000	16.7	>598
FXI	465174	>10000	4.3	>2325	1133328	>10000	4.8	>2083
FXI	465178	126	1.7	74	1133332	>10000	1.8	>5555

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Gap 2 Position Modifications Reduce Toxicities Without Significant Loss of Potency

Lessons Learned and Importance

Key Messages

Understanding the mechanisms of toxicity is broadly enabling

- All cell types studied to date
- All organs studies to date
- All species studied to date
- Accounts for substantially all of the toxicity

The straightforward solution to the problems means

- We are incorporating into the basic design of ASOs and this will substantially enhance efficiency
- We are incorporating into protein RTS activities and enhancing once again the efficiency of Ionis drug discovery

These and other patents extend our control of the technology

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Overall Conclusions

Our leading RNA targeting technology is broadly enabling and delivering excellent **value today**

- A solid long-term plan to create and advance the technology executed
- Perseverance
- One step at a time

The momentum in advances in the technology is extraordinary today

In the aggregate the major new directions in core antisense research put Ionis in position to achieve the dream of "**designer medicines**"

The competitive advantage enjoyed by Ionis today is large and continued innovation increases our competitive advantage

Continued innovation extends our control of the technology

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IONIS™
IONIS: A FORCE FOR LIFE