Therapeutic Modalities: Nucleotide Therapeutics

Janaiah Kota, Ph.D.

Executive Director, Molecular Therapeutics & Head of Nucleic Acid Platform

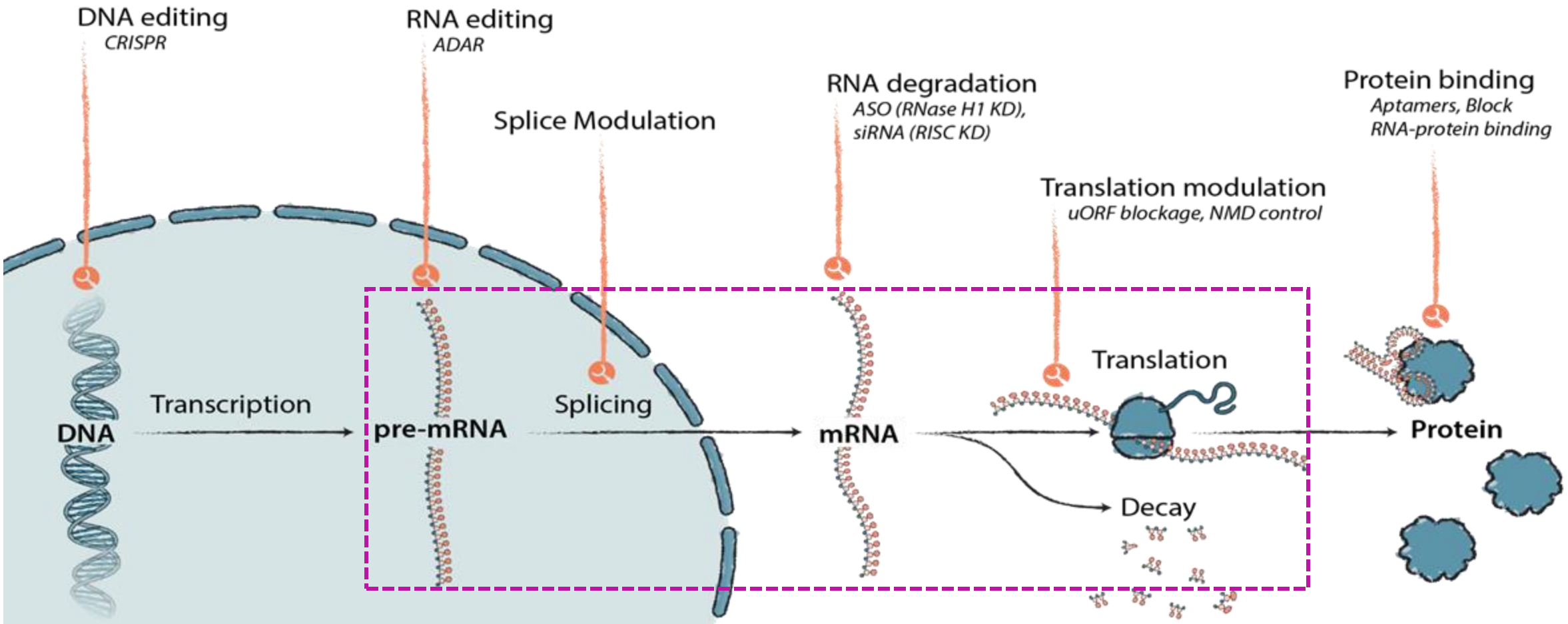
Research Department

Ultragenyx, Novato, CA, USA

Outline

- Leveraging the Central Dogma for creating nucleic acid therapeutics to treat genetic diseases
  - Overview of Antisense Oligonucleotide (ASO) and short inhibitory RNA (siRNA)
    - Mechanism of action of ASOs & siRNAs
  - Overview of mRNA therapeutics
- Conclusions and Future outlook

Central Dogma- Overview of Therapeutic Modalities

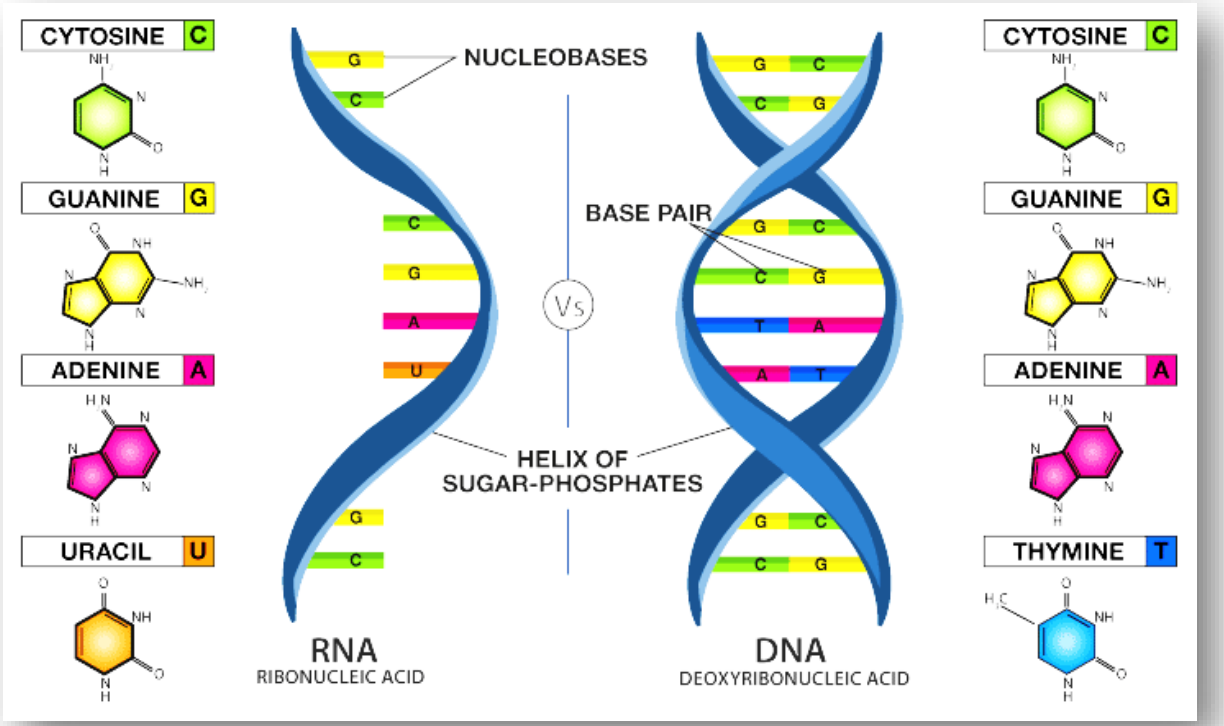


What are nucleotide therapeutics?

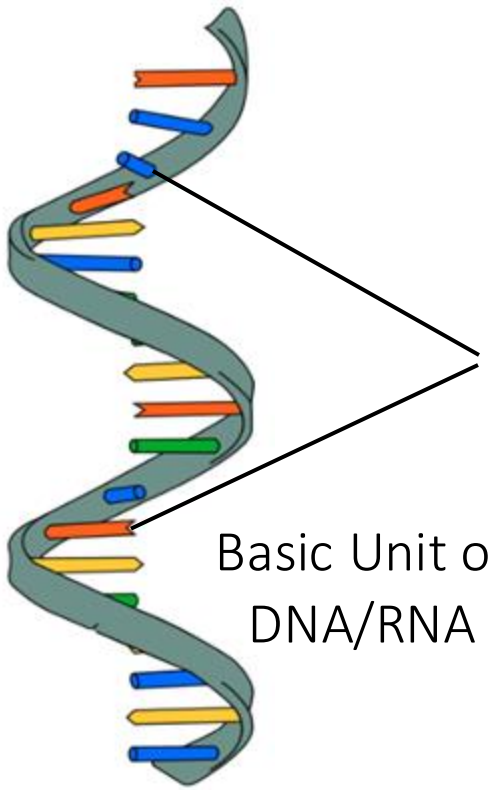
aka Oligonucleotide Therapeutics

- A broad class of genomic medicines that are designed to target specific RNA sequences to modulate gene expression
- Synthetic, single stranded DNA or RNA molecules.
- Typically, 14-22nt always <100nt except mRNAs

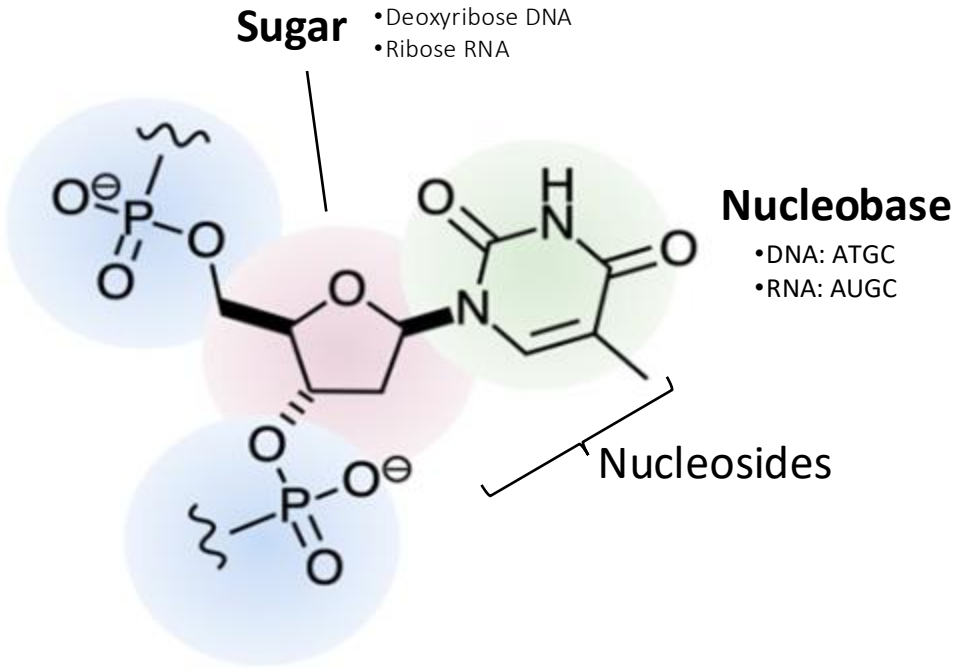
Cell Nucleic Acids



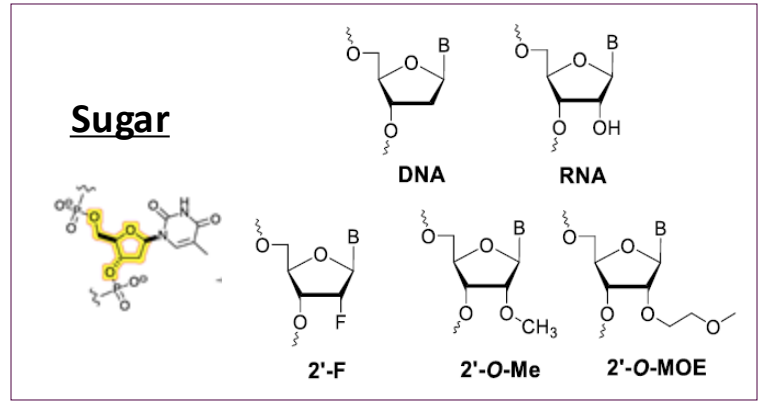
Chemically modified to improve pharmacological properties

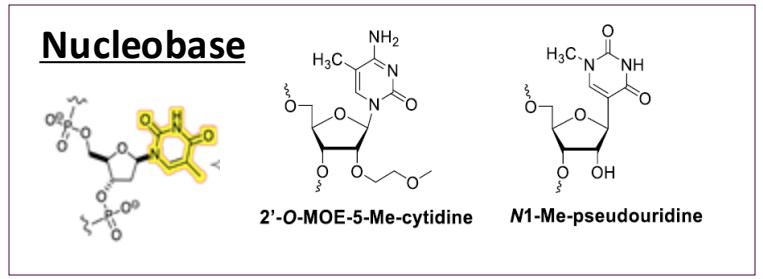


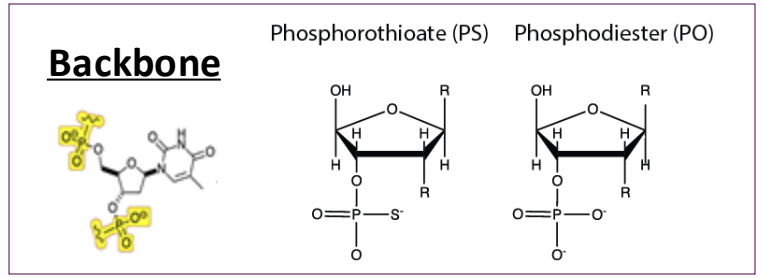
Basic Unit of DNA/RNA



Phosphate (PO) Linkages



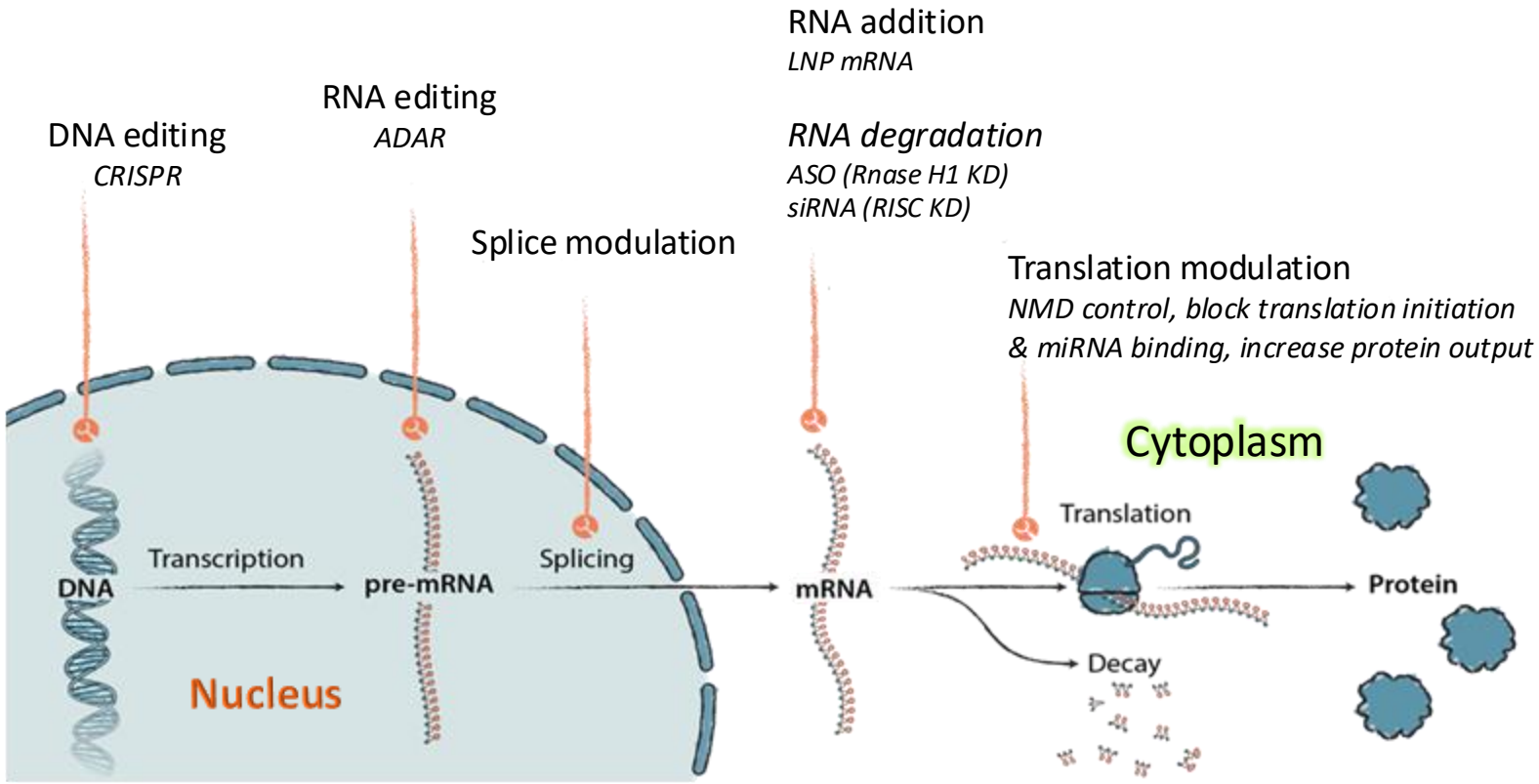




Different types of oligonucleotide therapeutics

Selection based on underlying genetic defect and disease context

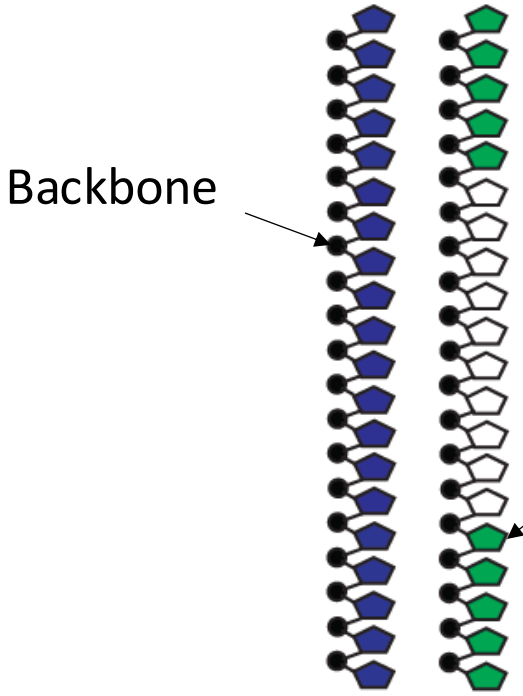
- Antisense Oligonucleotides (ASOs)
  - Splice modulation
  - RNA degradation (nucleus and cytoplasm)
  - Translation modulation
- Small interfering RNAi (siRNAs)
  - RNA degradation (cytoplasm)
- LNP mRNA
  - Gene replacement
- Other types
  - gRNAs, genome editing
  - saRNAs, tRNAs & aptamers etc.



Overview of Antisense Oligonucleotides (ASOs)

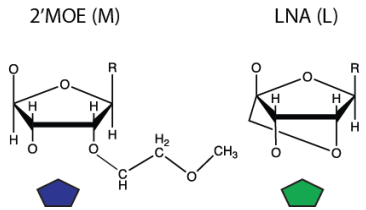
- Single-stranded oligonucleotide
  - Comprised of ribonucleosides and/or deoxyribonucleosides
  - 14-22 nucleotides long
  - Synthesized on a machine
- Chemically modified to improve pharmacological properties
  - Enhance metabolic stability
  - Protect from endonucleases
  - Determine the mechanism of action
- ASOs are specific to a target RNA via Watson-Crick base pairing

ASO

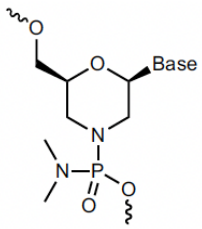


Nucleosides

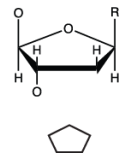
Ribonucleosides



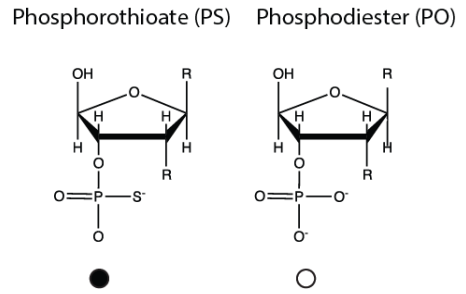
Morpholino



DNA



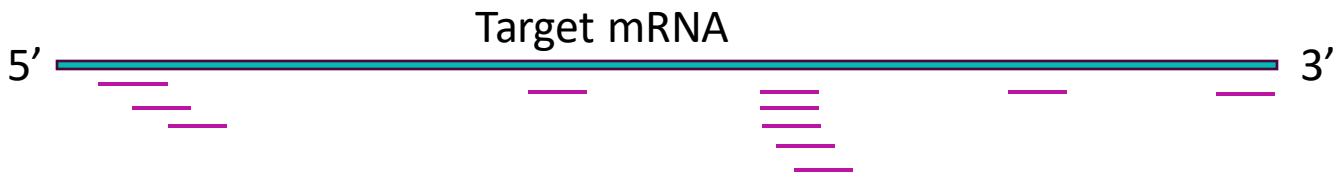
Backbone linkages

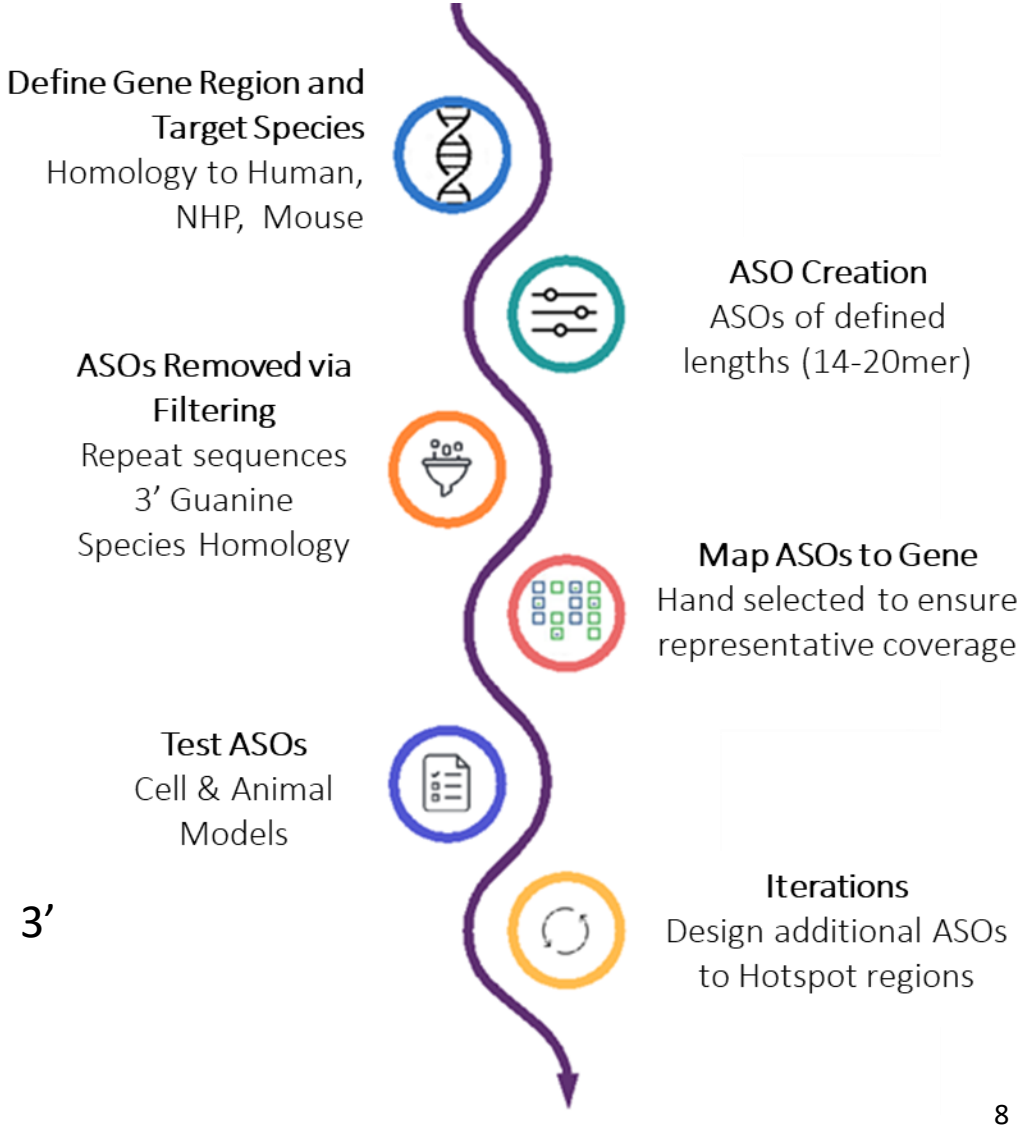


- Adenine : Thymine/Uracil
- Guanine : Cytosine

ASO design pipelines are enabled by informatic analysis

- Parameters:
  - Regions of interest
  - Species conservancy
  - Physical parameters
- Generate a list of potential ASOs for screening
- Iterative process



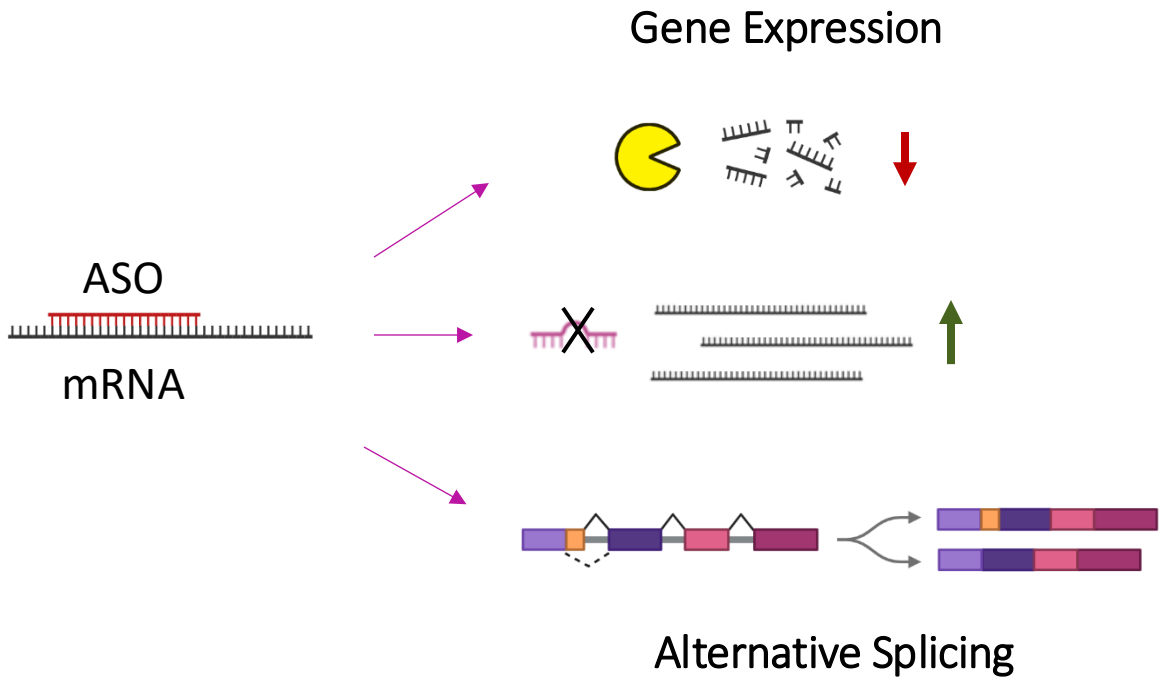


Mechanism of action differentiates the types of ASOs to be considered for therapeutic purposes

- Gamper ASOs
  - Downregulates the target gene expression
  - Degrade target mRNA

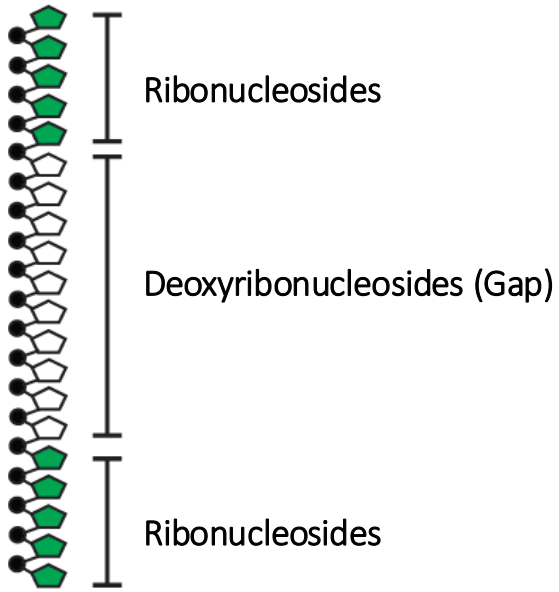
- Steric hindrance ASOs
  - Interfere with RNA regulator elements to upregulates target gene expression

  - Alters the splicing of a target gene to generate different RNA or protein isoforms



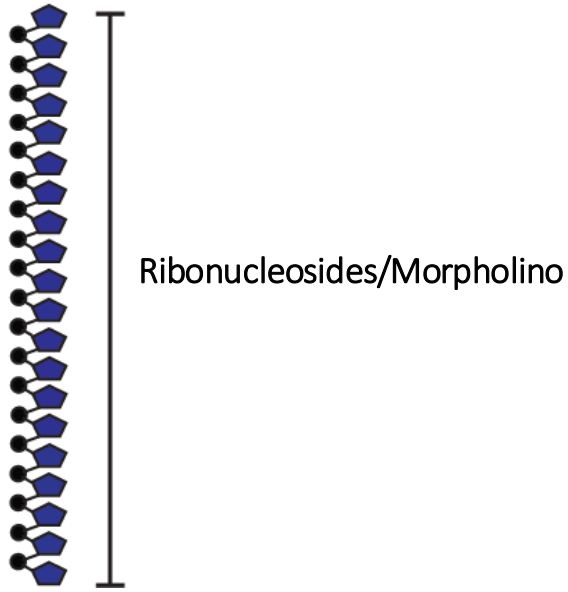
The chemical structure of an ASO determines its mechanism of action

Gapmer ASO



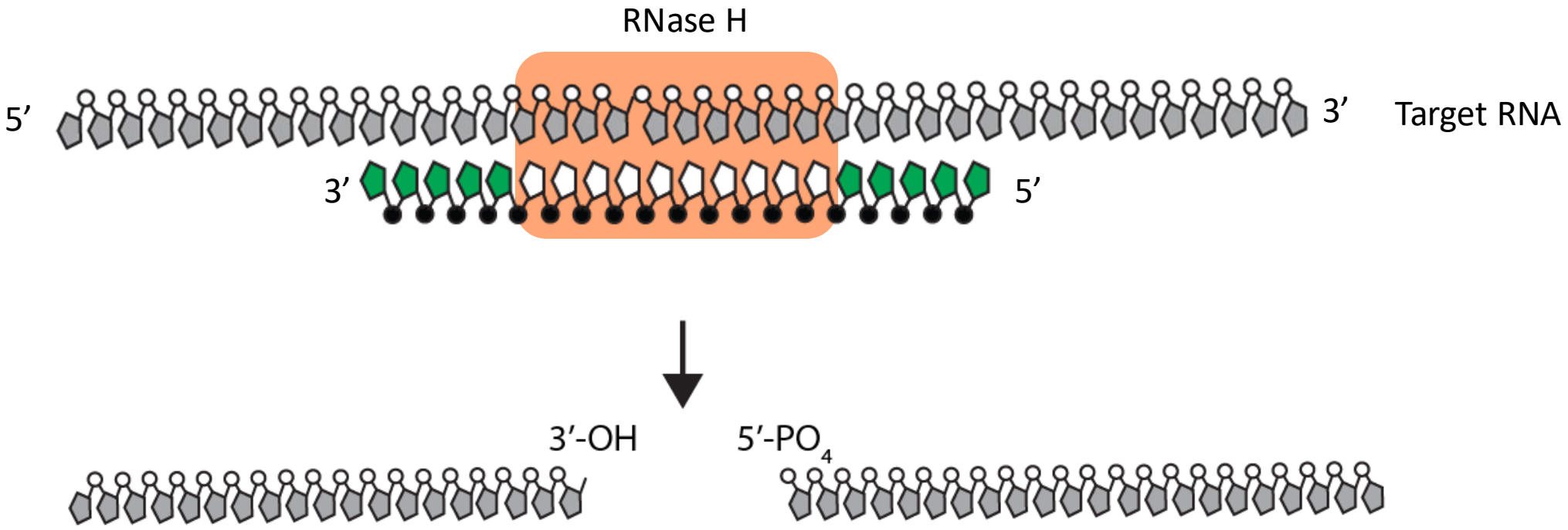
Induces degradation of target RNA

Steric Hindrance ASO



Blocks the binding of proteins or RNAs

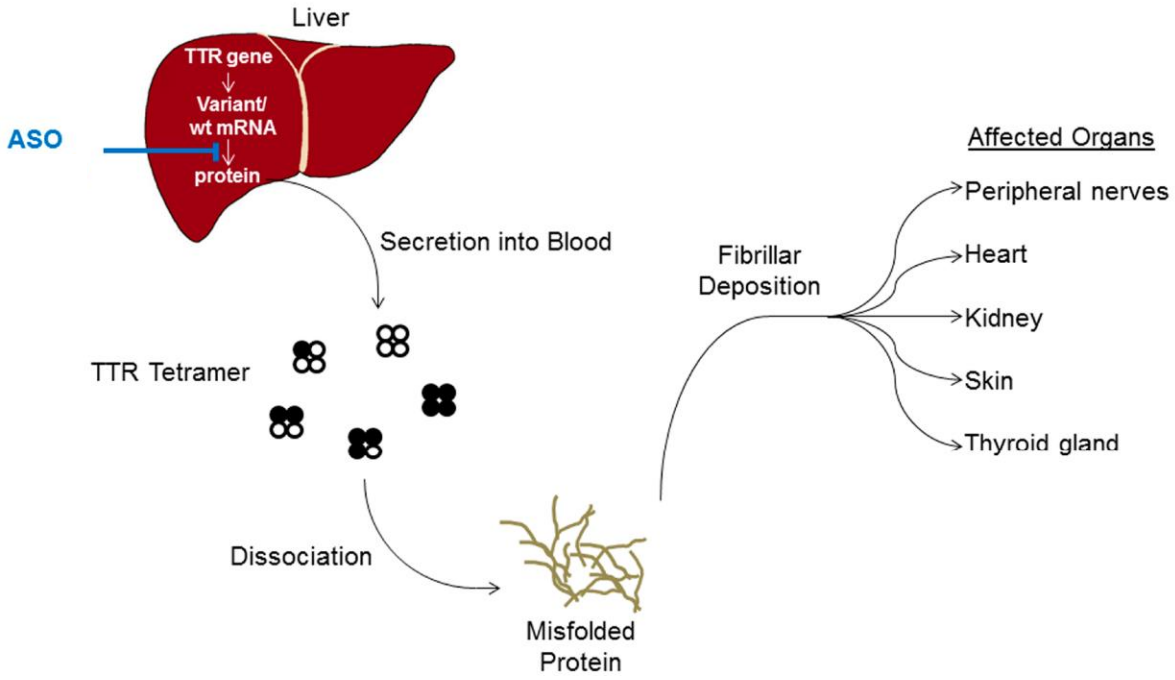
Gapmer ASOs induce the degradation of a target RNA

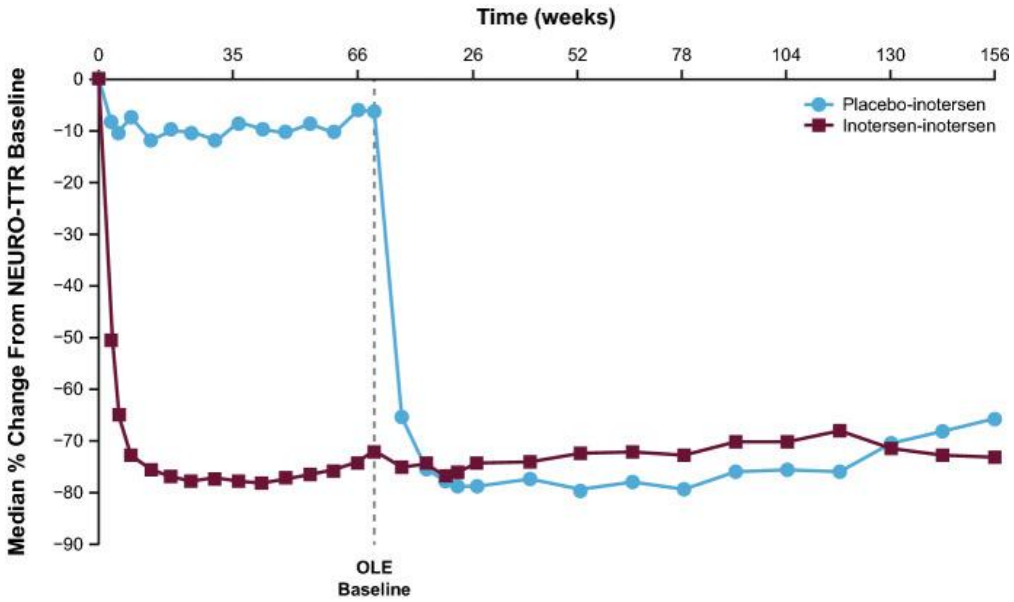


Approved Gapmer ASO drugs

- Kynamro for Homozygous Familial Hyperchlesterolemia
- Tegsedi for Polyneuropathy of Transthyretin (TTR) Amyloidosis
- Waylivr for Familial Chylomicronemia

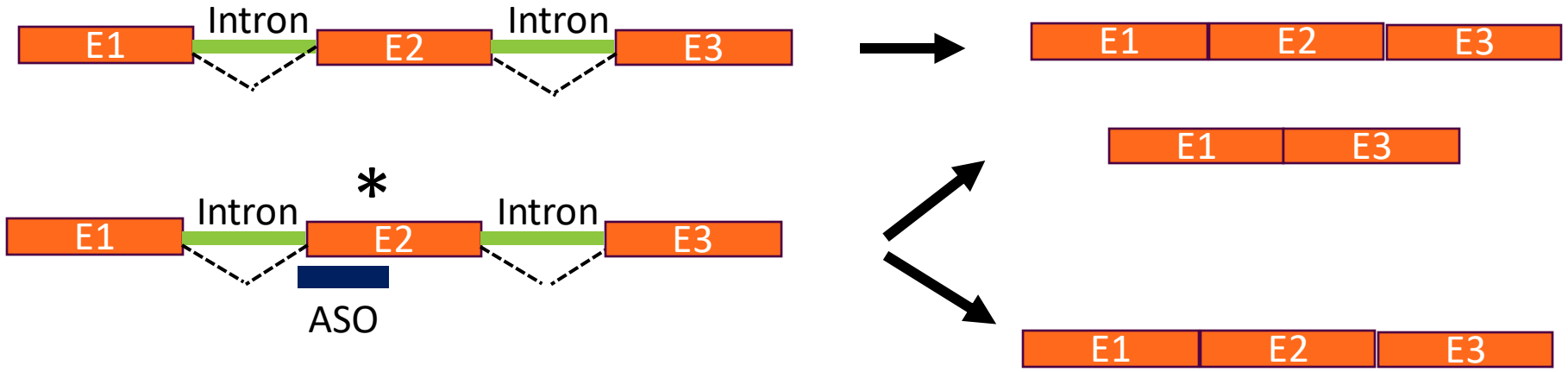
Approved Gamper ASO drug for Polyneuropathy of Transthyretin (TTR) Amyloidosis





Steric hindrance ASOs inhibit RNA-binding proteins and regulatory RNAs

For Therapeutic purposes both for downregulation or upregulation of gene expression



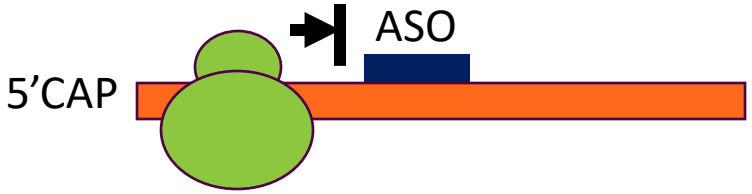
Normal Splicing

Exon Skipping

- Truncated Protein, DMD
- Upregulation by removing poisonous exons, Dravet

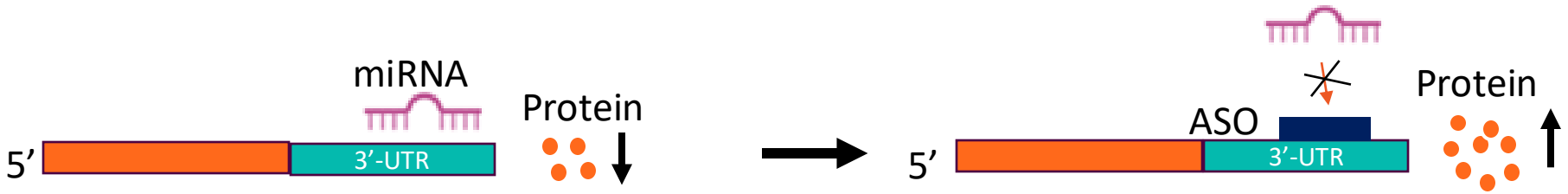
Exon Inclusion

- Suppress splice altering mutations, SMA



Translation Arrest

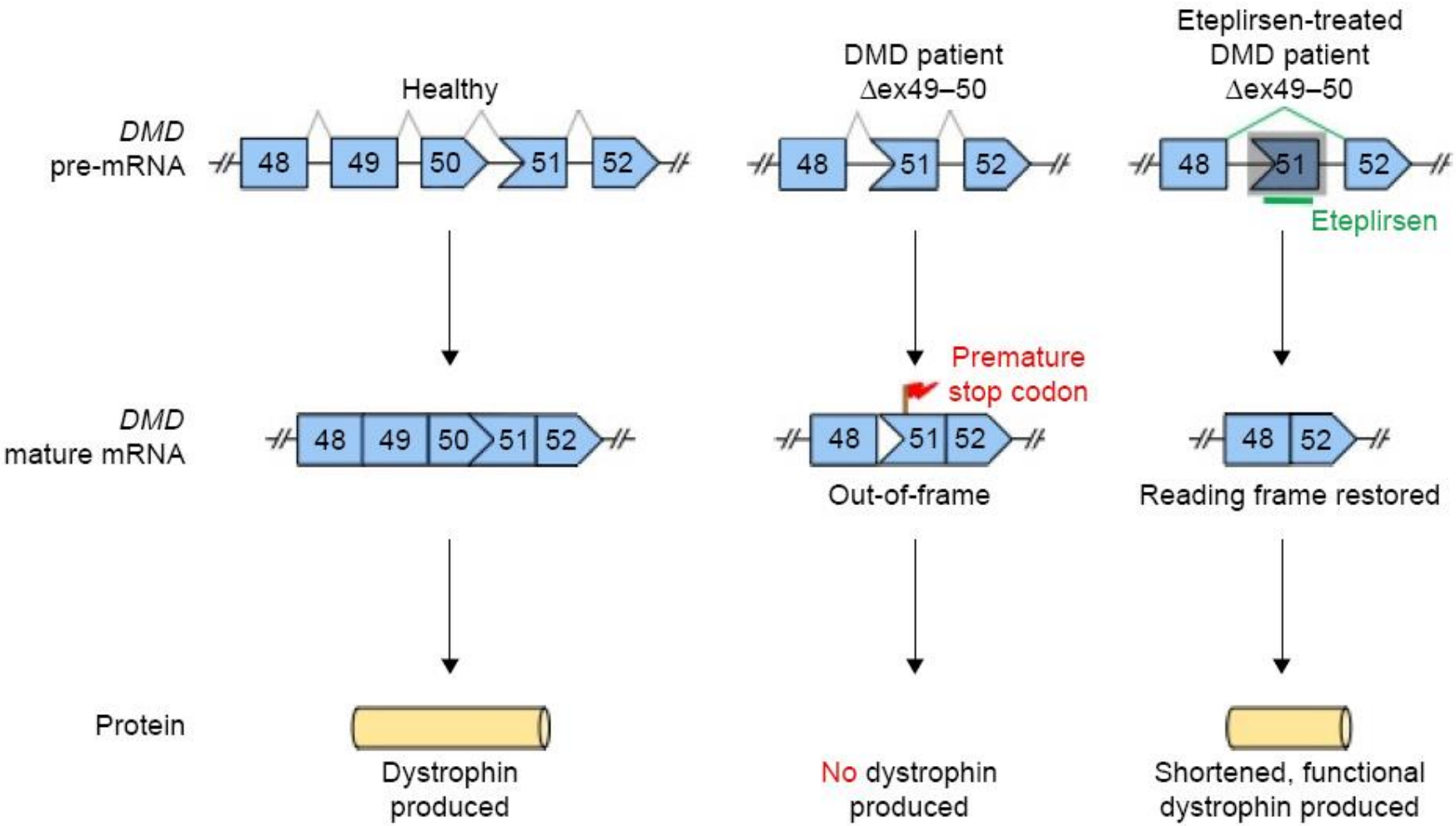
Pathogenic transcript, CMV Retinitis



Gene Upregulation

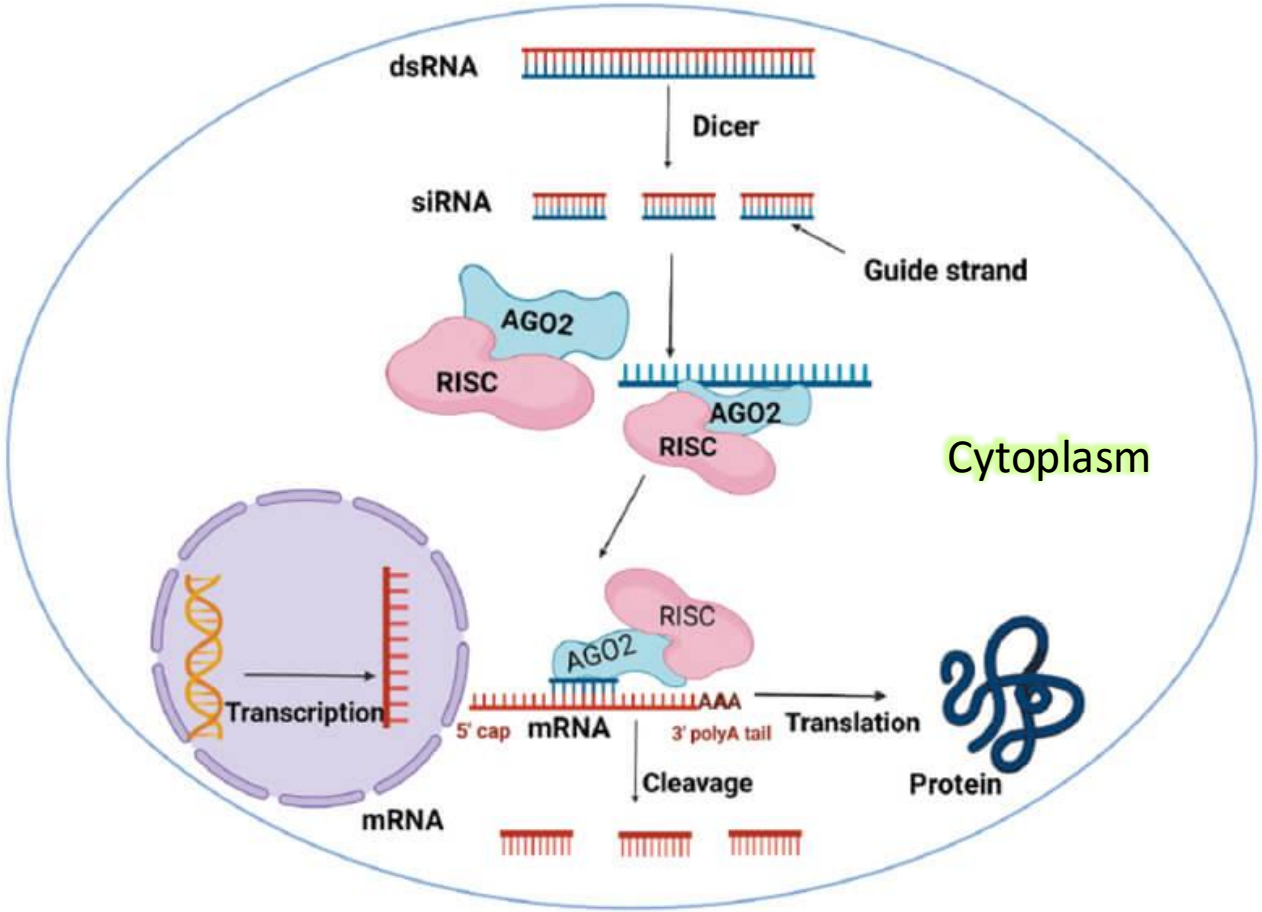
- Haploinsufficiency

Steric block ASO drug for Duchene Muscularly Dystrophy (DMD)



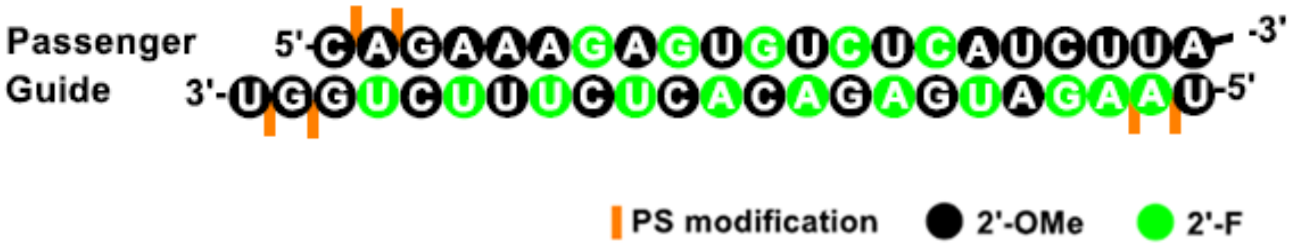
Small Interfering RNAs (siRNAs) knockdown target RNA

- Double-stranded oligonucleotide, comprised of ribonucleosides
- Typically, 20-25 nucleotides
- Downregulates gene expression
  - Degradation of target mRNA
  - Reduce protein
- Mechanism of action in the cytoplasm

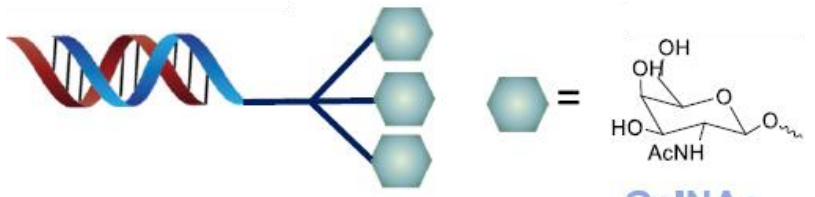


siRNA requires a delivery vehicle

- Poor metabolic stability
  - Rapidly degraded by nucleases
  - Fully modified ribonucleosides
- Requires a delivery vehicle to take to the cell and tissue
  - Conjugated with targeting moieties
    - GalNac for liver delivery
  - Package in a delivery vector
    - LNP particles



GalNac conjugated siRNA

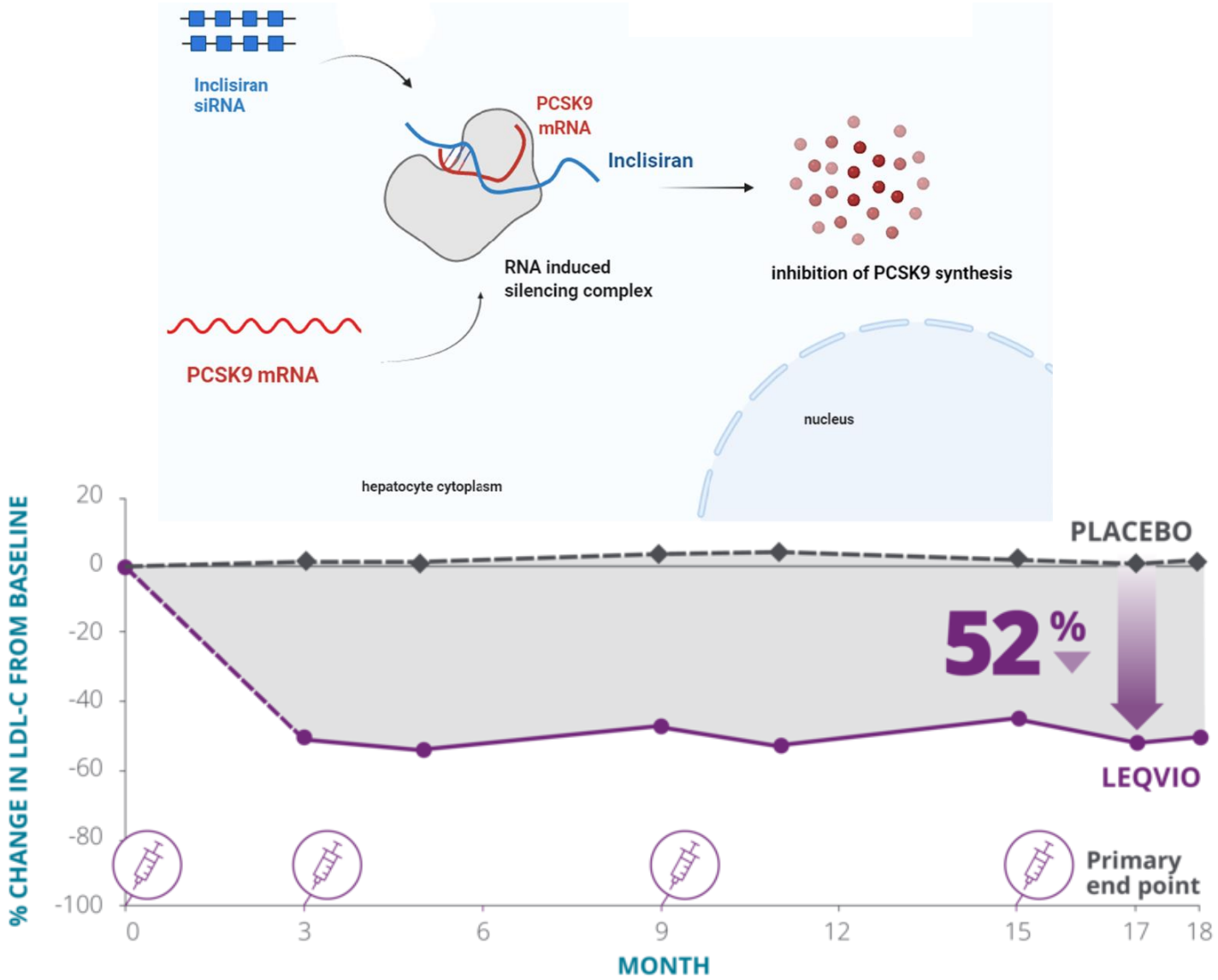


- Leqvio for LDL-C
- Amvuttra for TTR Polyneuropathy siRNA
- Givlaar, Hepatic Porphyria

LNP formulated siRNA

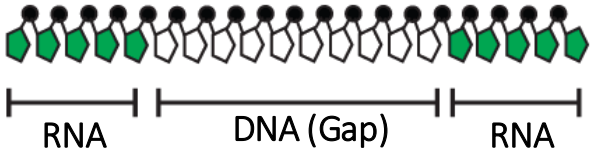
Onpattro
TTR Polyneuropathy

Durable effects of siRNA drug with single injection

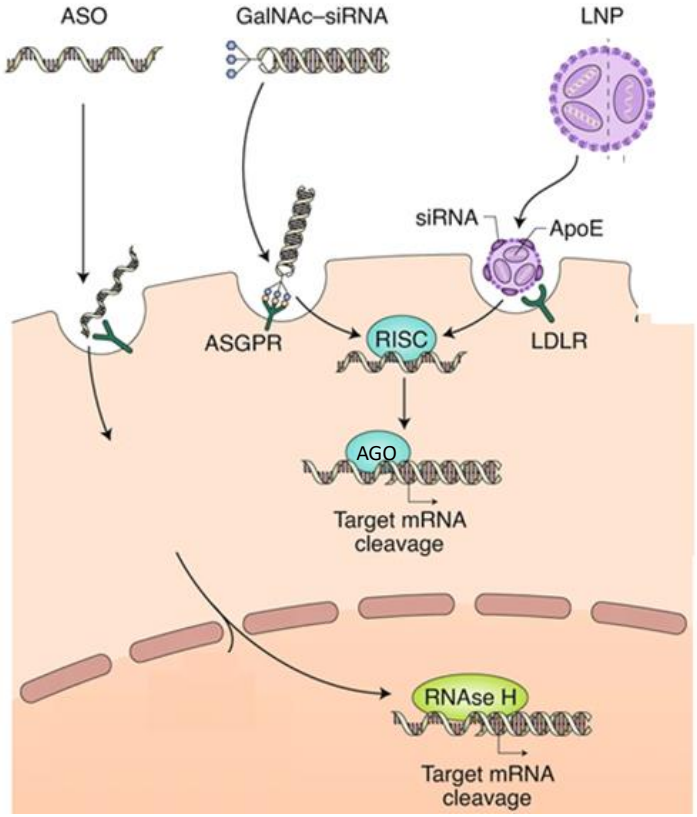


Gapmer ASO vs siRNA

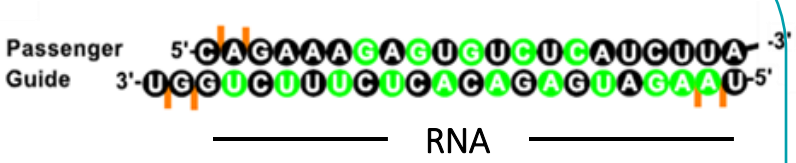
Gapmer ASO



- Single stranded DNA RNA hybrid, 14-21nt
- PS back bone
- RNAse H1 knockdown
- Both nucleus & cytoplasm
- Cell uptake naked ASOs
- Micromolar (uM) potency
- High dosing frequency

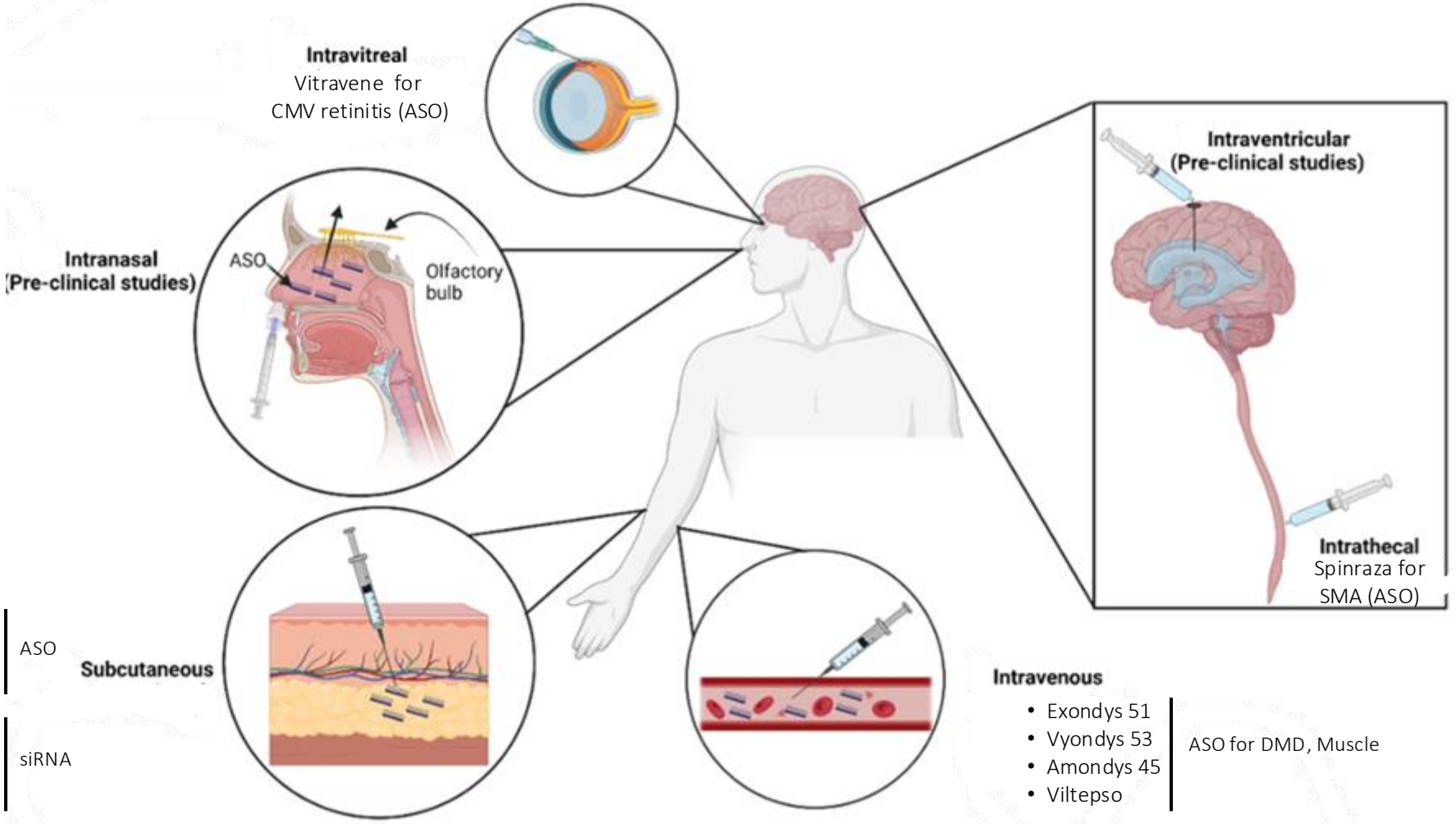


siRNAs



- Double stranded RNA, 20-25nt
- PS PO mixed backbone
- Argonaute knockdown
- Cytoplasm only
- Requires delivery formulation or conjugation
- Nanomolar (nM) potency
- Low dosing frequency

Routes of Administration of Oligonucleotide Therapeutics



- Kynamro for HoFH, Liver
- Tegsedi for TTR, Liver
- Waylivr for FCS , Liver

ASO

- Givlaar for Porphyria, Liver
- Oxlumo for PH1, Liver
- Amvuttra for TTR, Liver
- Leqvio for LDL-C, Liver

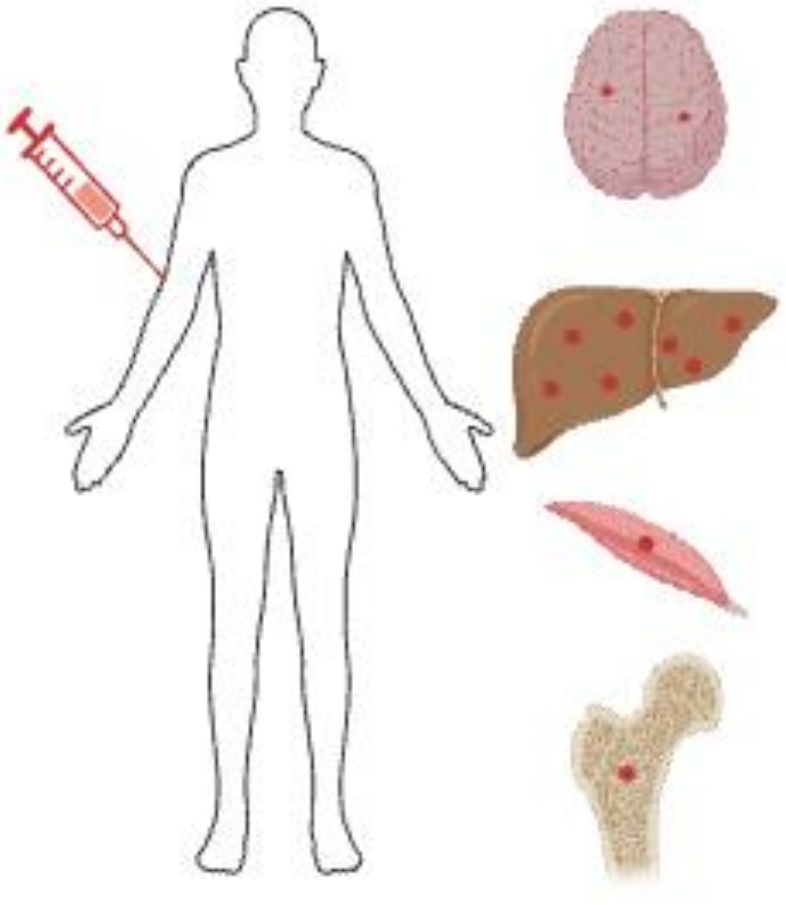
siRNA

- Exondys 51
- Vyondys 53
- Amondys 45
- Viltepso

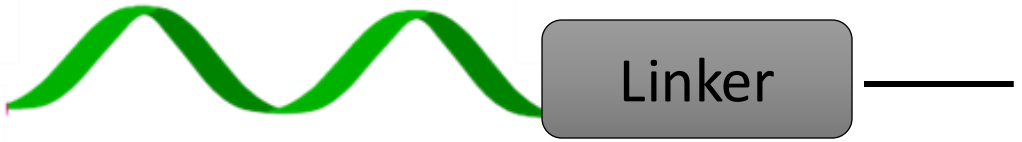
ASO for DMD, Muscle

- Onpattro for TTR (siRNA LNP), Liver

Tissue and Cell Specific Delivery



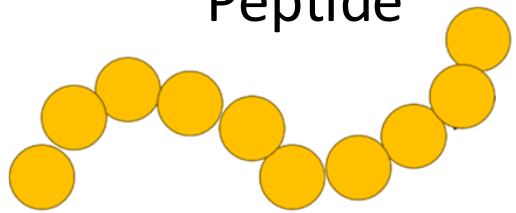
Conjugate ASO or siRNA to targeting moiety



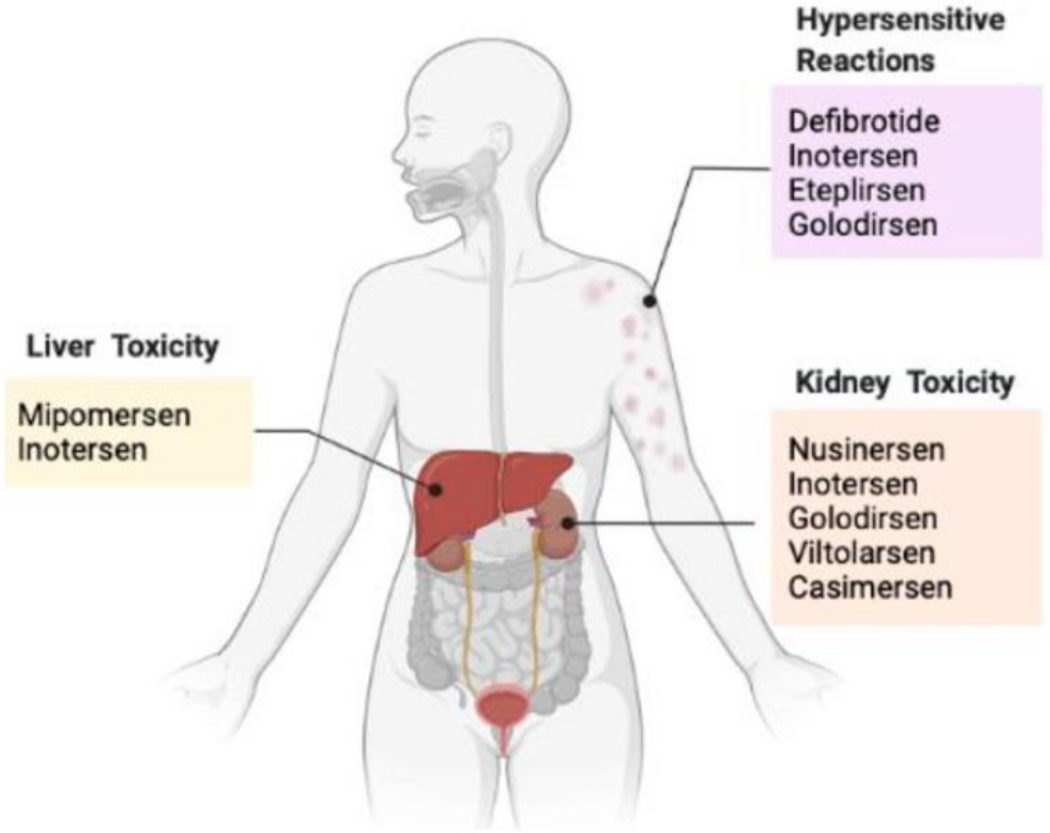
- Tissue & cell specific delivery
- Reduce Toxicity

Antibody

Peptide



ASOs are clinically validated modalities, but they are limited by tolerability issues

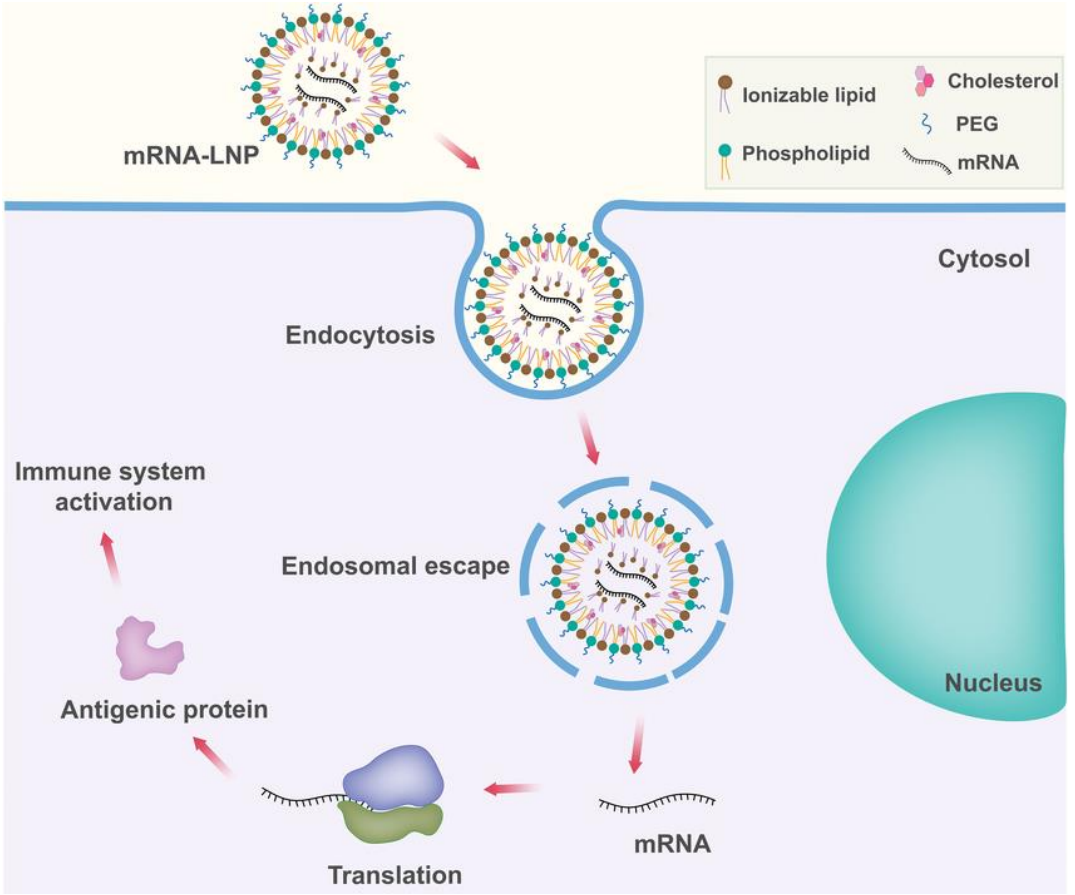


- Toxicity associated mechanisms are not clearly defined
  - Plasma proteins binding
  - Off-target hybridization
- Minor changes in ASOs can have massive effects
- Evaluate multiple ASOs to find optimal clinical candidate

Alhamadani F, et al. Adverse Drug Reactions and Toxicity of the Food and Drug Administration-Approved Antisense Oligonucleotide Drugs. Drug Metab Dispos. 2022 Jun;50(6):879-887. doi: 10.1124/dmd.121.000418.

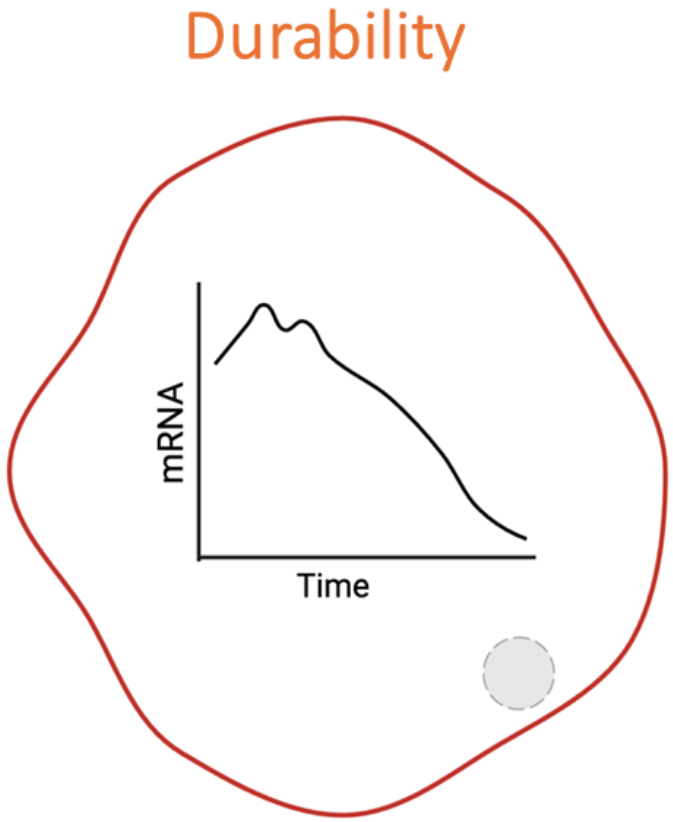
Lipid nanoparticles (LNPs) mRNA therapeutic modality

- Tiny lipid-based particles designed to encapsulate and deliver therapeutic RNA to cells and tissues.
- Pivotal role in COVID vaccine development.
- One FDA approved rare genetic disease drug
  - Onpattro- siRNA to treat polyneuropathy of hereditary transthyretin-mediated amyloidosis (hATTR)
- Potential alternative to gene therapy to restore functional copy of mRNA

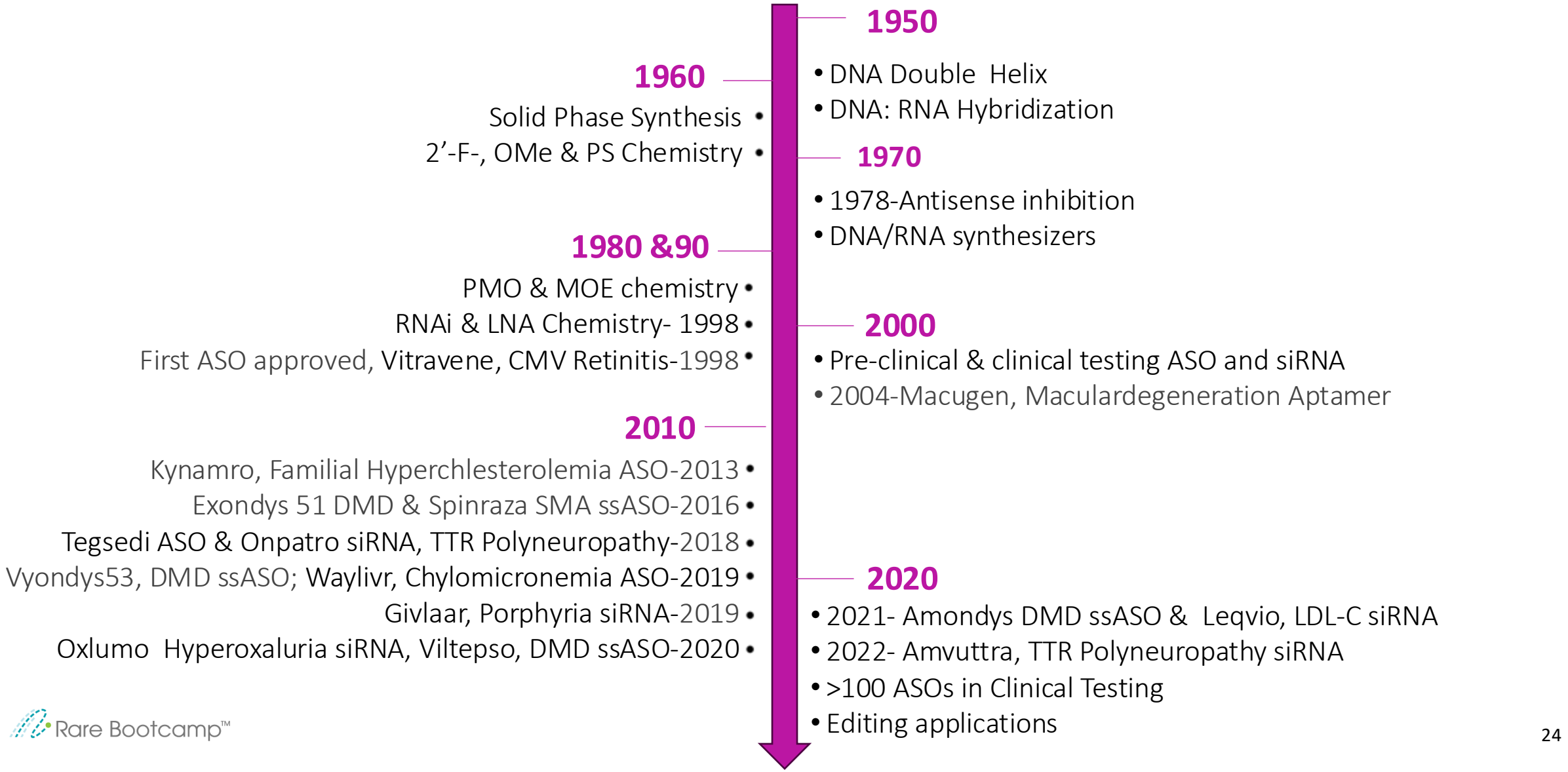


Current limitations of LNP mRNA therapy

- No limitations on gene size
- Transient in nature
  - Rapid mRNA turnover impact durability
  - Requires chronic dosing
  - Immunogenicity
- Appealing delivery vehicle for editing applications
  - Off-target toxicity



Oligonucleotide therapeutics have evolved and grown in number over decades

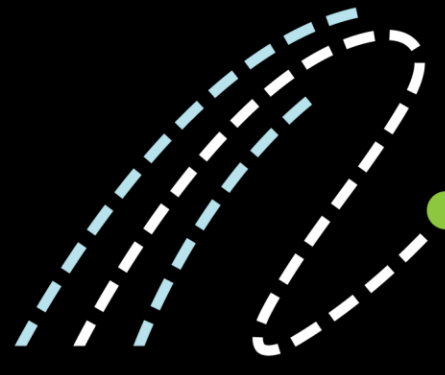


Conclusions

- Oligonucleotide- based drugs (ASO and siRNA) have revolutionized rare genetic disease space as transformative therapies.
- Chemical modifications of oligos have been pivotal in improving metabolic stability, tissue uptake and tolerability.
- Disease biology, target gene, and tissue determine oligonucleotide therapeutic modality choice and fit
- The oligonucleotide therapeutics landscape is rapidly growing, with 18 approved drugs and many more in late-stages of clinical development

Future Outlook

- Improve prediction models to enhance pharmacological and toxicology profiles
  - Bioinformatics and mechanize learning tools for ASO design
  - Gap between cell culture, animal models and clinical outcomes
- Continuous chemistry advancements to develop more potent drugs
  - Increase half life, reduce dosing frequency and toxicity
- Extra-hepatic and cell specific delivery remains a major challenge to broaden the scope and reduce off-target toxicity
  - Field testing a variety of targeting moieties
- High drug development costs and limited access.
  - Enhance knowledge-sharing efforts between patient advocacy, academia, and industry.



Rare Bootcamp™

Thank You