Therapeutic Modalities: Antisense Oligonucleotides

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Ultragenyx RARE Bootcamp
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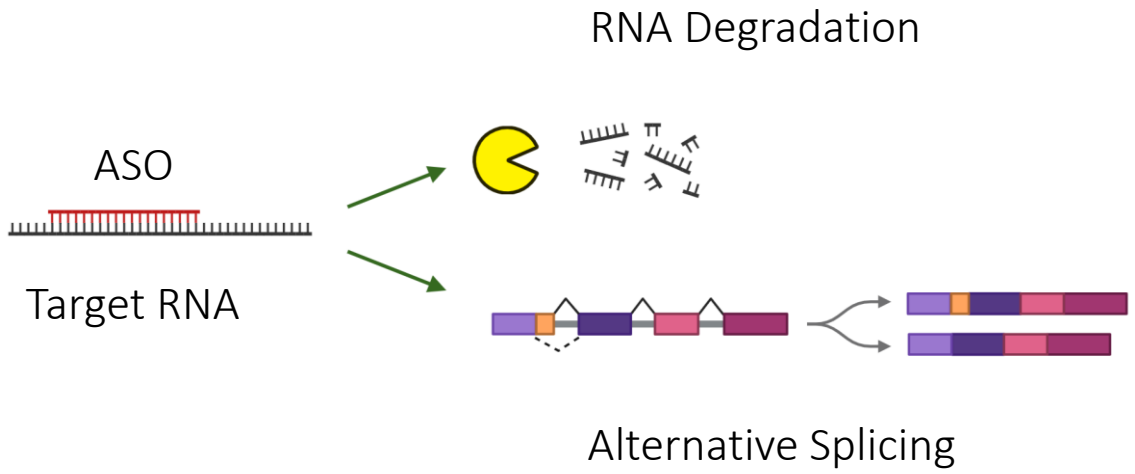
DINDOT LABORATORY

Nucleic Acid Therapies

- AAV-mediated gene therapy
- Messenger RNA (mRNA) therapy
- Antisense oligonucleotide (ASO) therapy
- Small interfering RNA (siRNA) therapy
- Genome modifying therapies (CRISPR/Cas)

Antisense Oligonucleotides (ASOs)

- Single-stranded oligonucleotide
  - Comprised of ribonucleosides and/or deoxyribonucleosides
    - 14-22 nucleotides long
- Chemically modified to protect the molecule from nucleases and provide drug-like properties.
  - Synthesized on machine
  - FDA considers ASO a drug (not biologic)
- Binds to a target RNA via Watson-Crick base pairing.
- Function
  - Downregulates or upregulates the expression of a target gene
  - Alters the splicing of a target gene to generate different RNA or protein isoforms



ASOs have been studied for decades

1978 - ASO approach proposed

1989 - ASO Medicinal Chemistry

1990 – Optimal ASO length identified

1996 – 2’MOE Chemistry

1998 – LNA chemistry

1998 – Formiversen Approved

2001 – IT, ID, and aerosol dosing

2011 – SMA Clinical Trials

2013 – Mipomersen Approved

2016 – Nusinersen Approved

2016 – Eteplirsen Approved

2018 – Inotersen Approved

2019 – Valenosorsen Approved

2019 – FDA allows N-of-1 (Milasen)

2019 – Golodirsen Approved

2020 – Angelman Syndrome Clinical Trials (GeneTx/Ultragenyx, Roche, Ionis)

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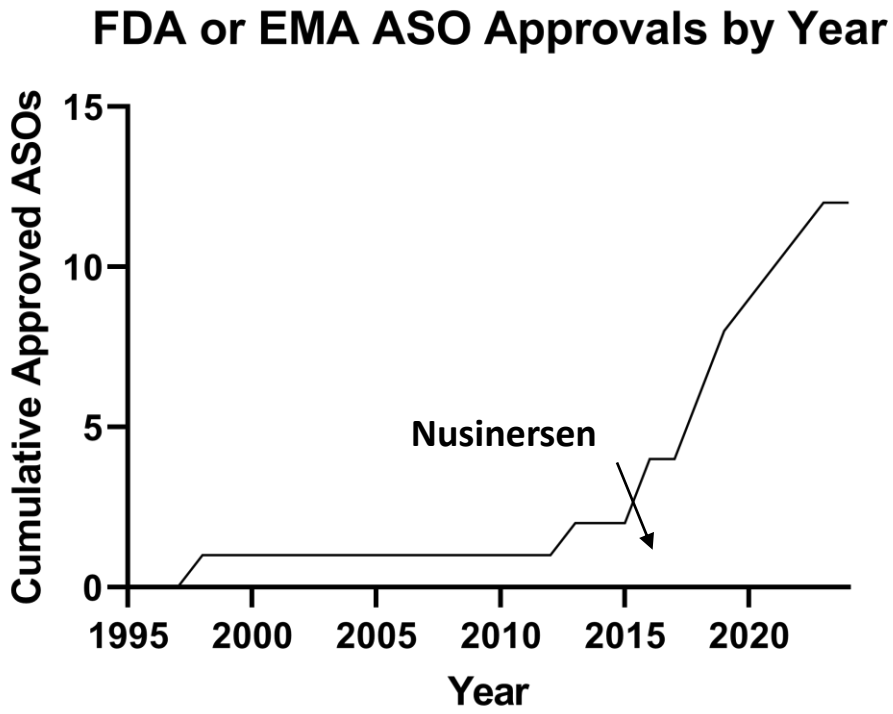
ASOs are an emerging drug modality

- 12 ASOs have been approved by the US Food and Drug Administration (FDA) or the European Medical Agency (EMA)

Organ Targeted	FDA Approved (2023)
Liver	5
Skeletal Muscle	4
Central Nervous System (CNS)	3
Eye	1



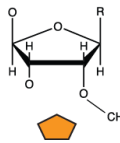
ASO Overview

ASOs are chemically modified

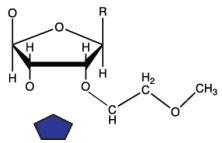
Nucleosides

Ribonucleosides

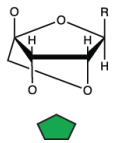
2'OMe (O)



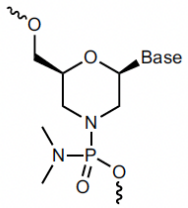
2'MOE (M)



LNA (L)

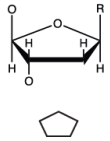


Morpholino



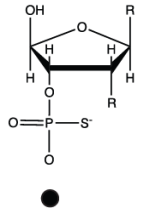
Deoxyribonucleoside

DNA

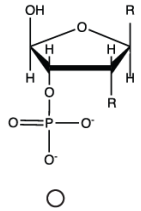


Backbone linkages

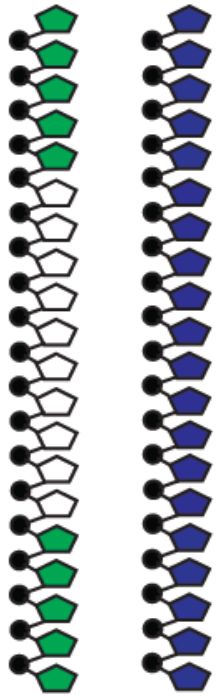
Phosphorothioate (PS)



Phosphodiester (PO)



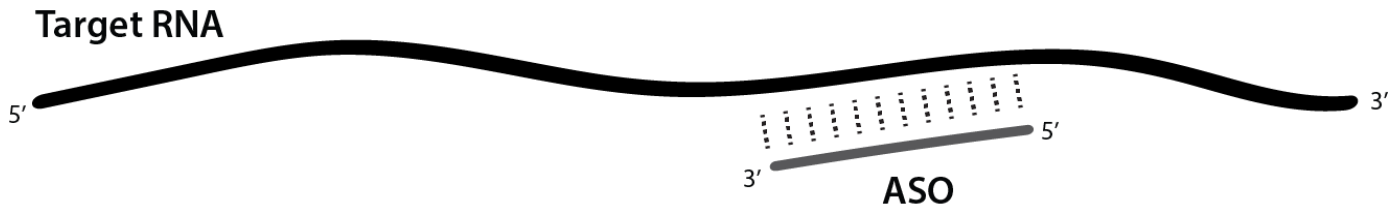
Chemically Modified ASOs



Chemical Modifications

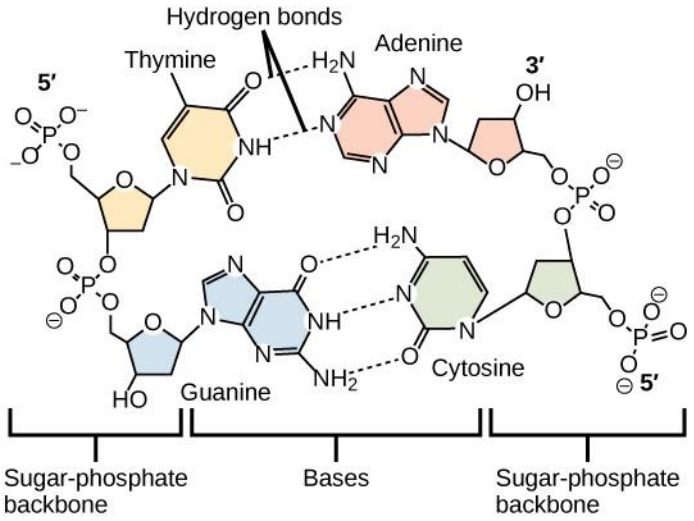
- Increase stability
- Enhance pharmacological and pharmacokinetic properties

ASOs are specific to a target RNA via Watson-Crick base pairing



Bioinformatic analyses, likely replaced by artificial intelligence

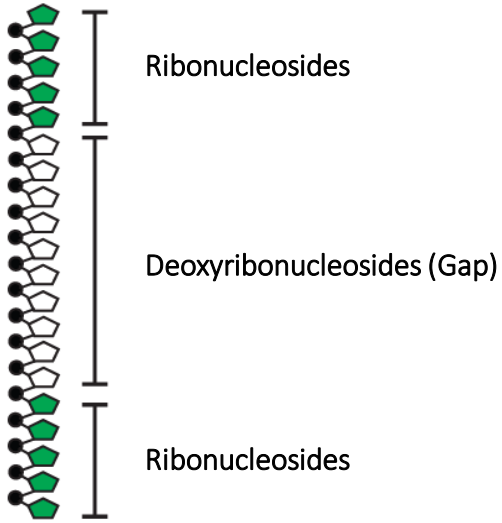
Watson-Crick Base Pairing



Adenine : Thymine/Uracil
Guanine : Cytosine

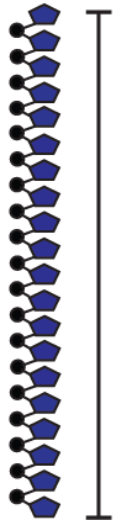
The chemical structure of an ASO determines its mechanism of action

Gapmer ASO



Induce RNase H-mediated degradation of target RNA

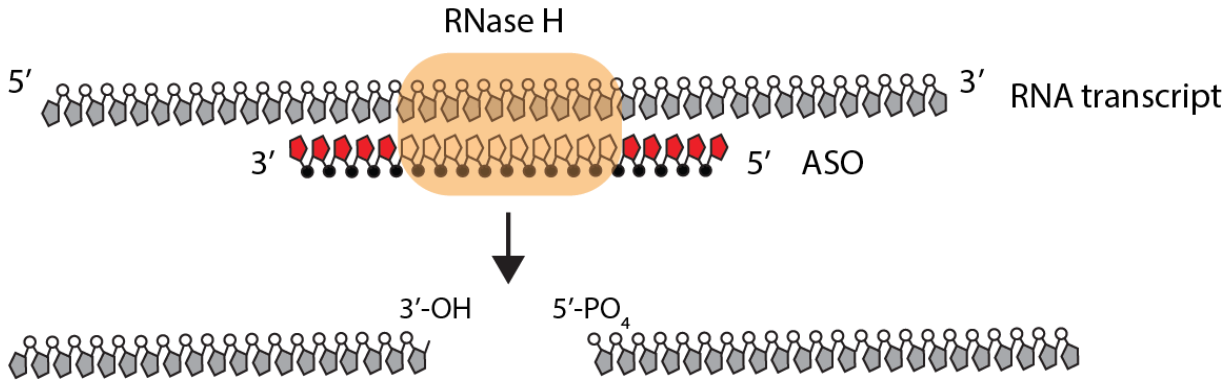
Steric Hindrance ASO



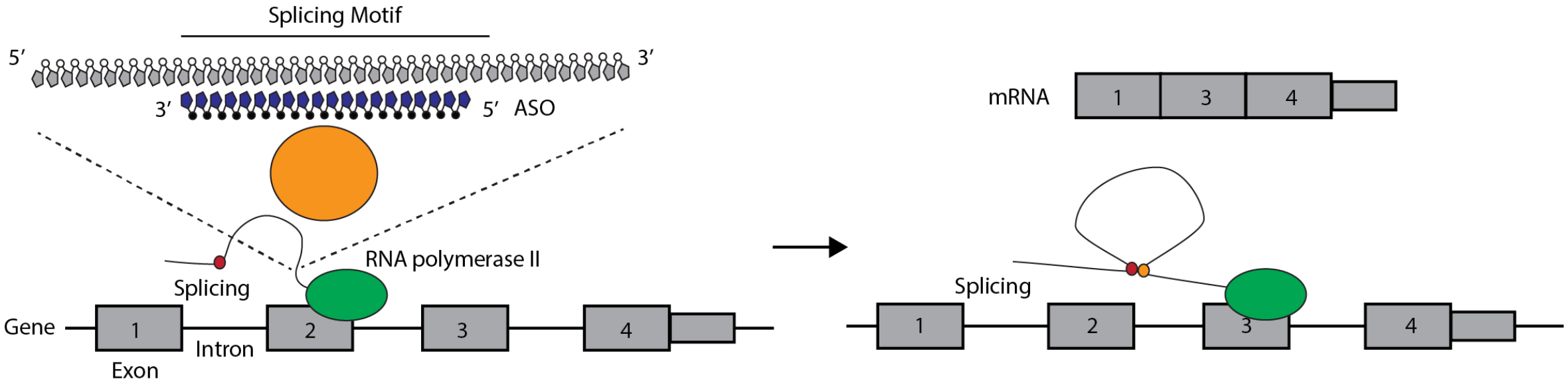
Blocks the binding of proteins or RNAs to a target RNA

Gapmer ASOs induce the degradation of a target RNA

- ASO binds to target RNA via Watson-Crick base-pairing
- RNA/DNA hybrid recruits the RNase H1 enzyme, which cuts the RNA through its endonuclease activity
- RNase H1 mediated cleavage – nucleus
  - Premature transcriptional termination
- RNase H2 mediated cleavage – cytoplasm
  - mRNA degradation

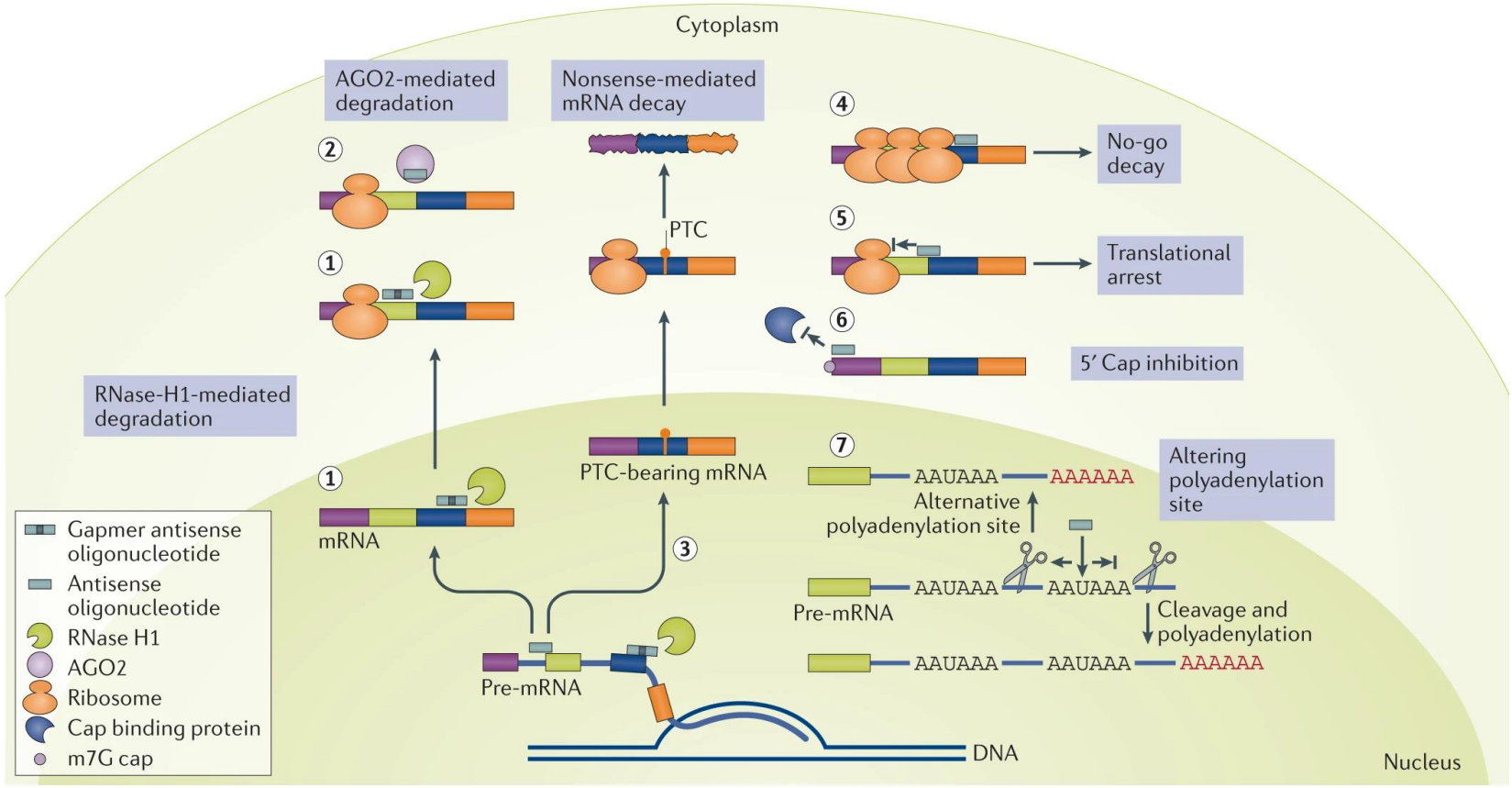


Steric Hindrance ASOs inhibit RNA-Binding Proteins and RNAs



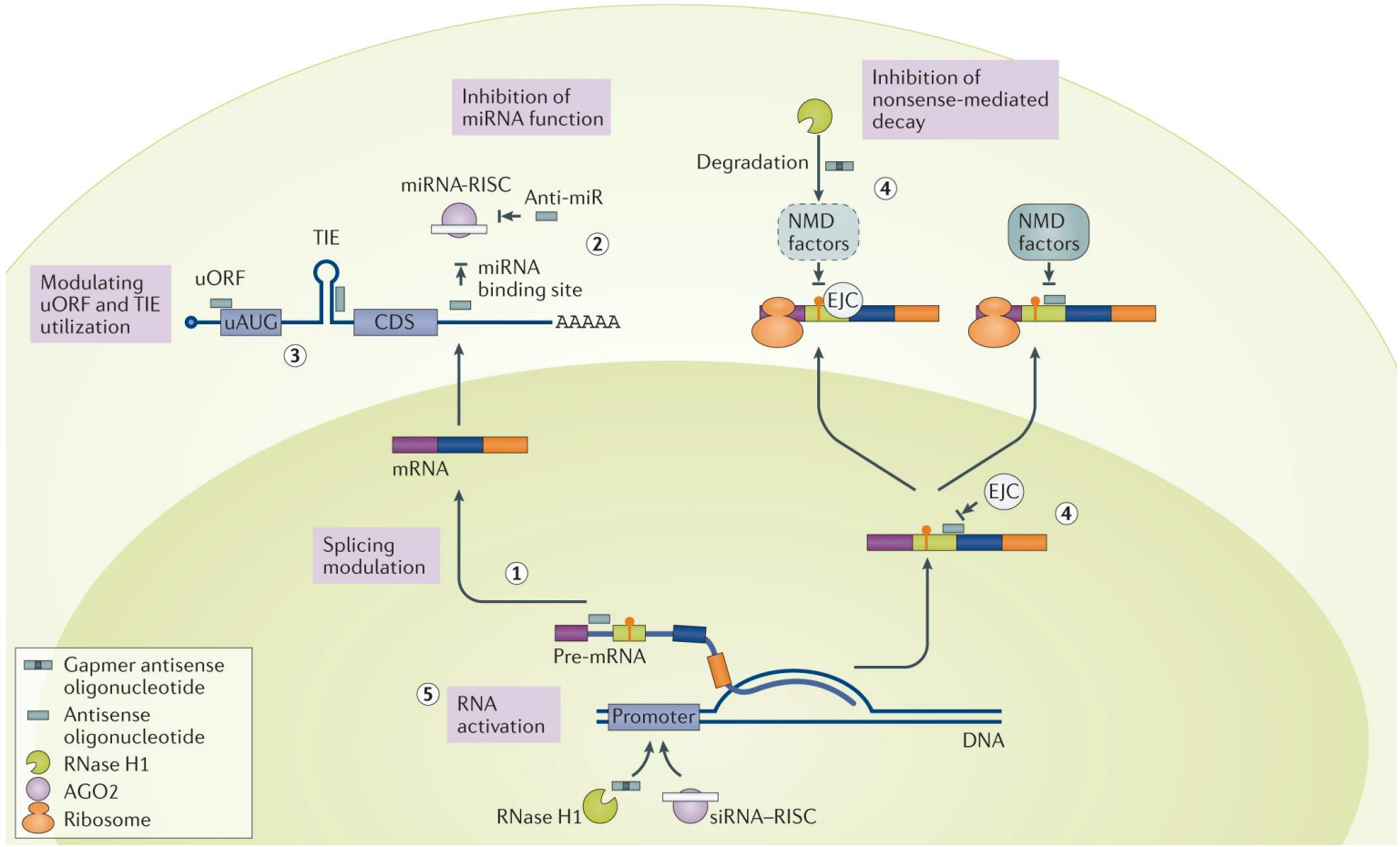
The therapeutic approach of an ASO can vary in many ways…

ASO-Mediated Repression of Gene Expression



Crooke ST, Baker BF, Crooke RM, Liang XH. Antisense technology: an overview and prospectus. Nat Rev Drug Discov. 2021 Jun;20(6):427-453. doi: 10.1038/s41573-021-00162-z. Epub 2021 Mar 24. PMID: 33762737.

ASO-Mediated Upregulation of Gene Expression



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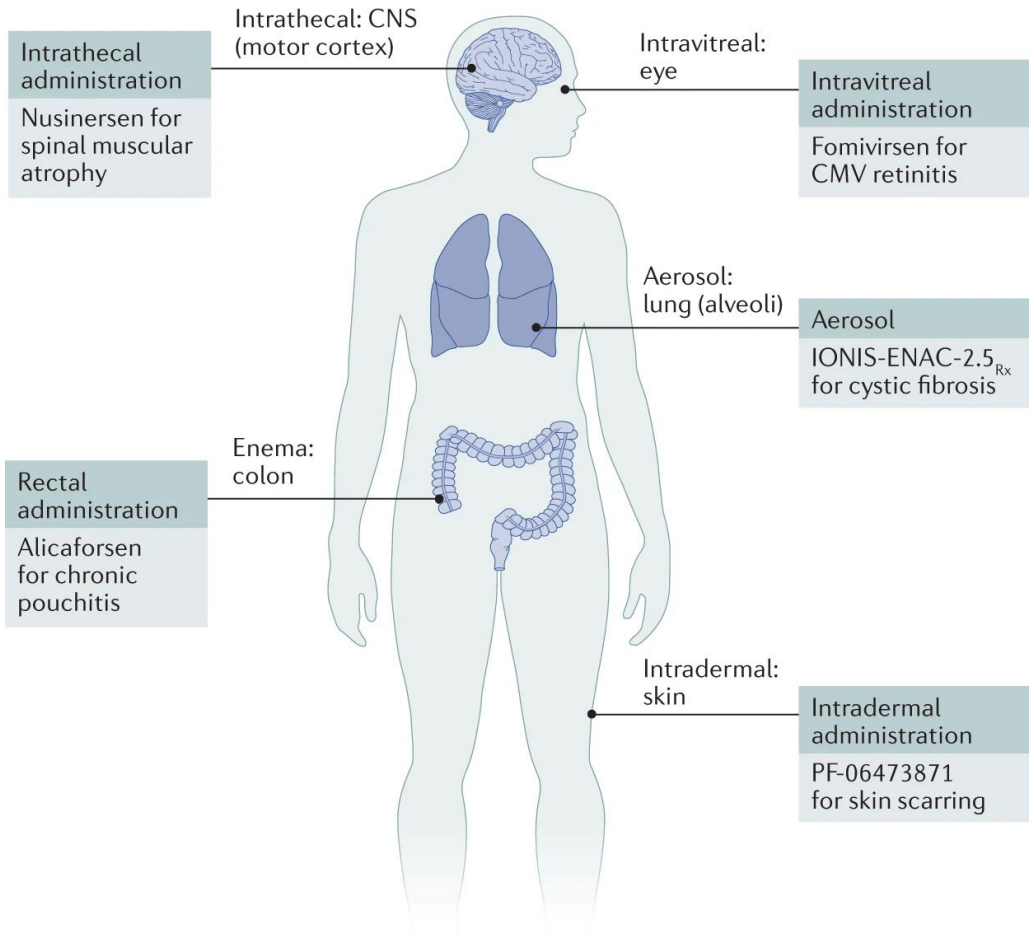
ASOs can be administered by different routes



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ASO Pharmacokinetics

- IV administered ASOs distribute throughout the body, following the flow of blood.
  - ASOs do not cross the blood-brain barrier.
- ASOs are water soluble and do not require a lipofection agent (gymnosis [naked delivery]).
- ASOs are taken up by all cell types (endocytic pathway).
- ASOs can be conjugated for targeted organ delivery (GalNac [liver]).
- ASOs have a relatively long half-life (weeks – months [chemistry dependent]).

ASO Overview Summary

- ASOs are a powerful modality for developing disease-modifying therapies.
  - Every gene and approach is different.

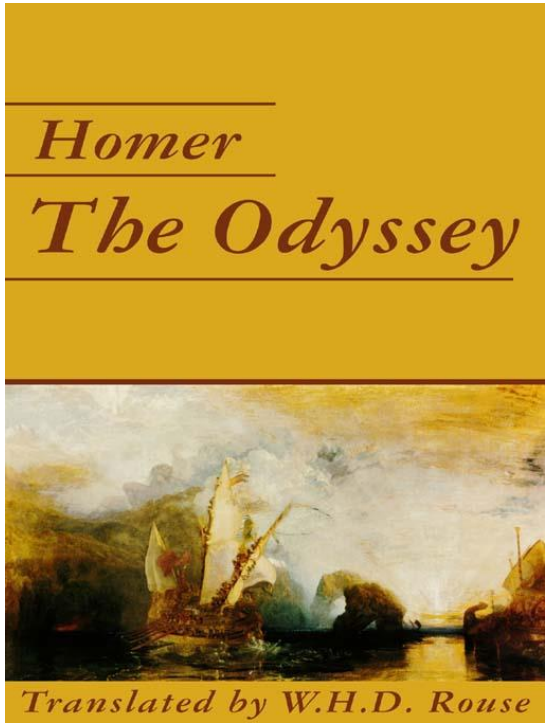
- The ASO research and clinical enterprise is expanding.

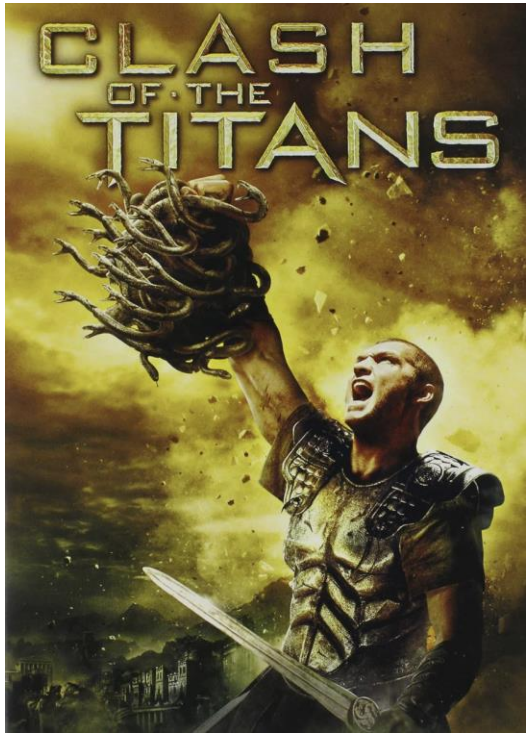
- The landscape of ASO therapies is rapidly evolving, with the number of both approved and developing ASO therapies increasing at an exponential rate.

ASO Nuances & Challenges

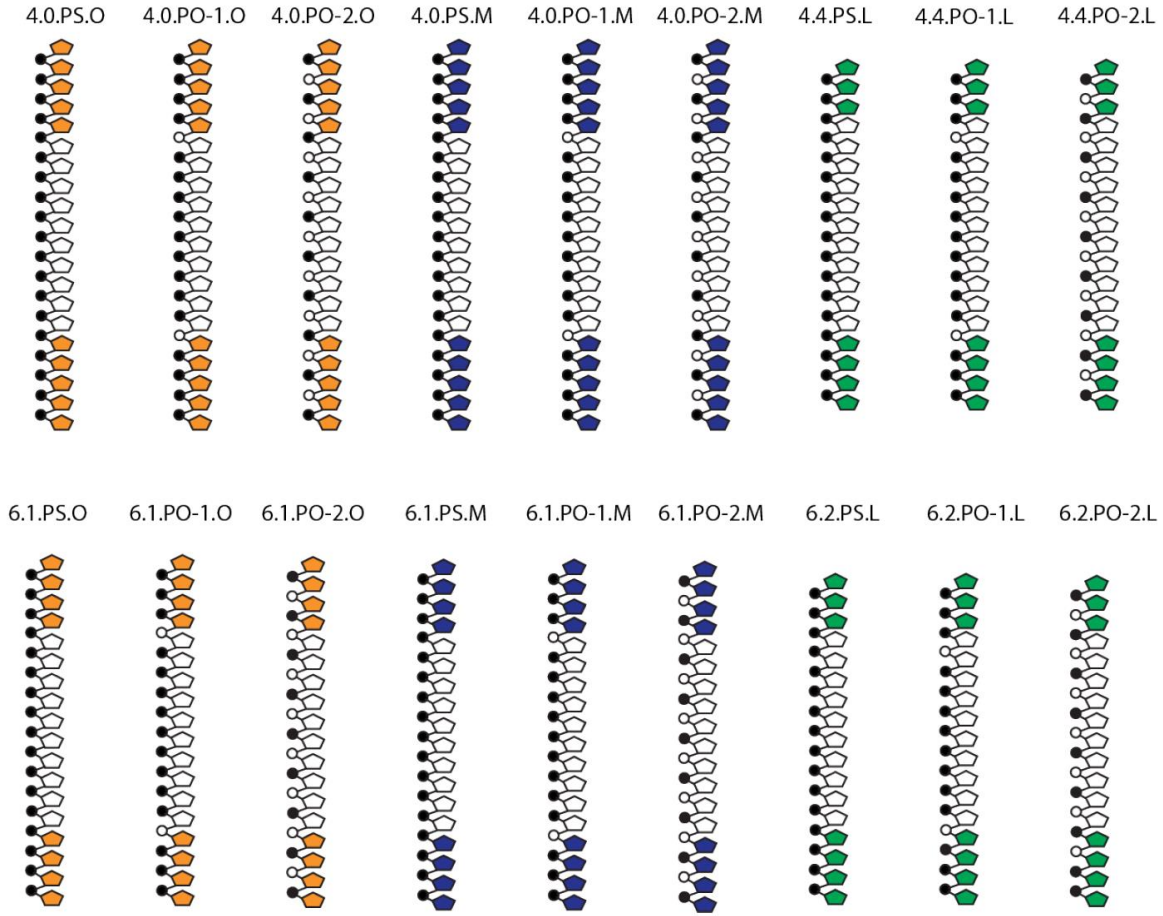
Developing an ASO is a journey

The pharmacological properties of an ASO are dependent on many factors and largely unknown.

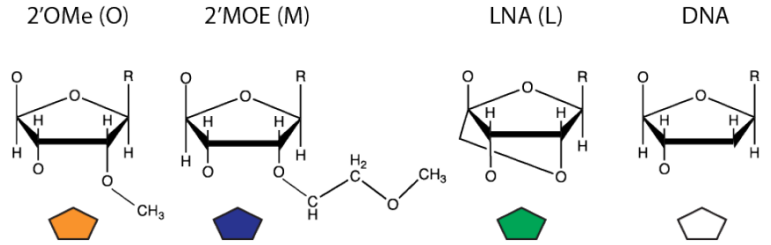




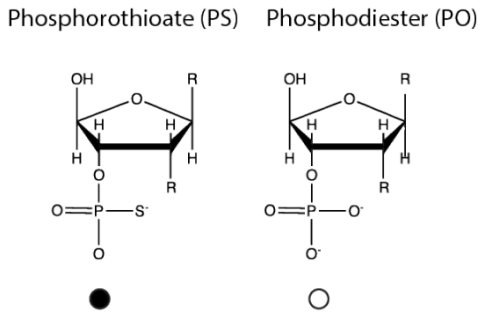
Designing ASOs is complicated by an exponential number of sequence and chemistry combinations



Nucleosides



Backbone Linkages



Dindot SV, et al. An ASO therapy for Angelman syndrome that targets an evolutionarily conserved region at the start of the *UBE3A-AS* transcript. Sci Transl Med. 2023 Mar 22;15(688):eabf4077. doi: 10.1126/scitranslmed.abf4077. Epub 2023 Mar 22. PMID: 36947593.

Minor changes to ASOs can have massive effects

In Vivo Evaluation of Candidate Allele-specific Mutant Huntingtin Gene Silencing Antisense Oligonucleotides

Amber L Southwell[1], Niels H Skotte[1], Holly B Kordasiewicz[2], Michael E Østergaard[2], Andrew T Watt[2], Jeffrey B Carroll[3], Crystal N Doty[1], Erika B Villanueva[1], Eugenia Petoukhov[1], Kuljeet Vaid[1], Yuanyun Xie[1], Susan M Freier[2], Eric E Swayze[2], Punit P Seth[2], Clarence Frank Bennett[2] and Michael R Hayden[1]

For each of these properties: SNP position, wing modification, and oligo length, we were unable to define governing principles of ASO design and recommend evaluation of multiple molecules to identify optimal ASO candidate drugs.

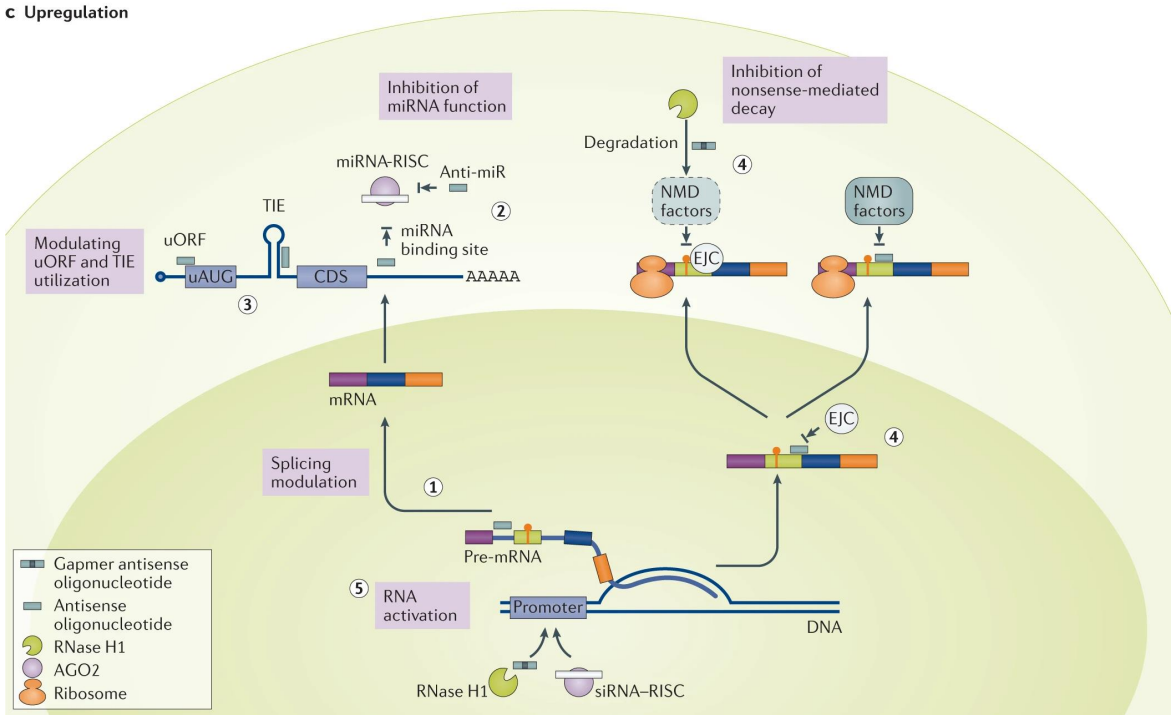
The Future of ASOs

- The principles governing the pharmacological and toxicological properties of ASOs are unclear.
  - Need for better bioinformatics/algorithms/artificial intelligence to design ASOs
- There is a need for vehicles and/or conjugates for targeted delivery of ASOs to organs and cells.
- New ASO-based or derivative modalities are needed.
  - Longer half-life, more potent, less toxic

Thank You!

Appendix

ASOs: Upregulation



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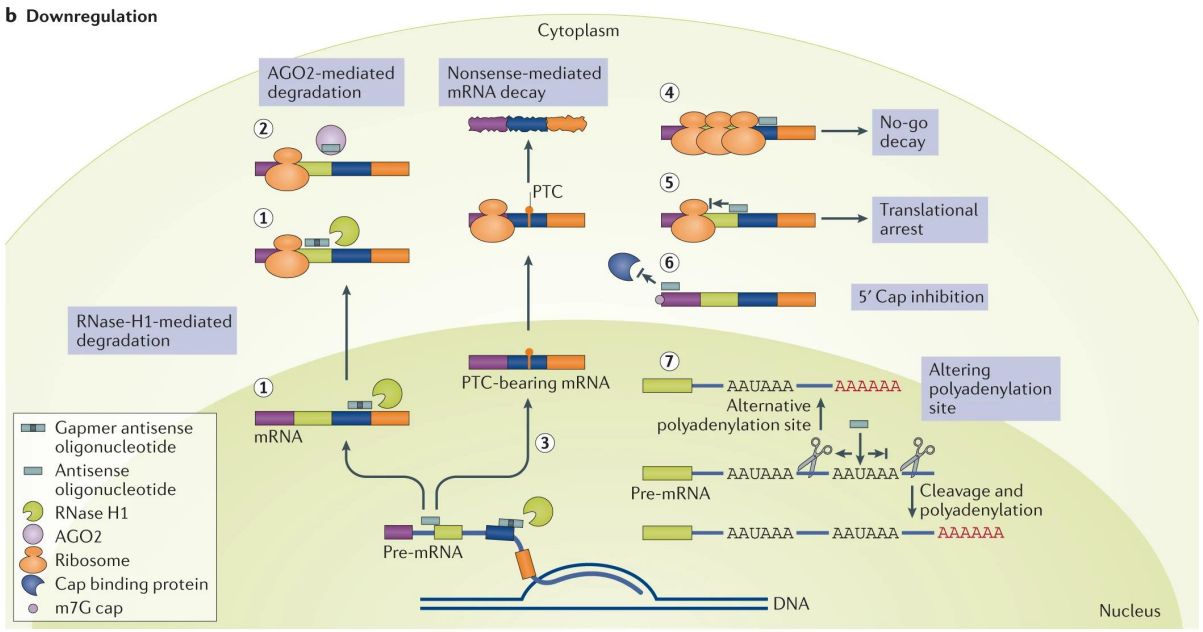
- Steric hindrance ASOs
  - trigger alternative splicing of pre-mRNAs, leading to mRNAs without PTCs, thereby increasing the stability and levels of mRNAs induce cleavage of RNA by RNase H1
  - inhibit miRNA function can increase expression of the miRNA target genes
  - enhance translation by inhibiting translation suppression elements, such as upstream open reading frames (uORFs) and translation inhibitory elements (TIEs) within the 5′ untranslated region (UTR)
- Gapmer ASOs
  - inhibit NMD
  - target promoter regions to enhance transcription

ASOs: Downregulation



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- Gapmer ASOs
  - induce cleavage of RNA by RNase H1
    - cytoplasm = reduces mRNA level
    - nucleus = terminate transcription
  - induce AGO2-mediated RNA degradation, similar to siRNAs
  - cleave 5’-cap and 3’-polyA tails
- Steric hindrance ASOs
  - modulate splicing, generating mRNAs with premature termination codons, leading to nonsense-mediated decay
  - block ribosome scanning and arrest translation
  - bind to the 5’-end of a mRNA inhibiting the binding of translation initiation factors