Wednesday, October 31 | 8:25 – 8:50 am ET

ASO Design, Discovery and Research innovation in ASO technology

PRESENTER

Konstantina Skourti-Stathaki, PhD

Director, ASO Design and Discovery

Nano-rare Patient Colloquium 2024

Hosted by: Biogen

n-lorem FOUNDATION

Integrated Processes & Building a Cohesive Team

Providing an optimal ASOs to nano-rare patients is the product of high-quality processes and years of expertise and experience

Sarah Glass

Molecular Geneticist
Clinical trial expertise
Operational Management

Konstantina (Nadina) Skourti-Stathaki

RNA Expert
ASO discovery and design

Julie Douville

Toxicologist
ASO expertise
Non-clinical development

Laurence (Laury) Mignon

Neuroscientist
ASO expertise
Clinical development

Amy Williford

Communications
Educator
Fundraising expertise

Establishing Systems Processes Creating Unified Cohesive Team | Creating Optimal ASOs ATTC - RMC | Preclinical to Regulatory RMC - IND | Clinical & Safety Patient Mgmt & Treatment STAR - DSMB | Supporting Patient Journey Communication /Education

n-Lorem Benefits From More Than 35 Years of ASO Expertise and Builds on continuous Research Innovation to Assure Each Patient is Treated With the Best ASO Possible

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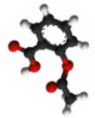
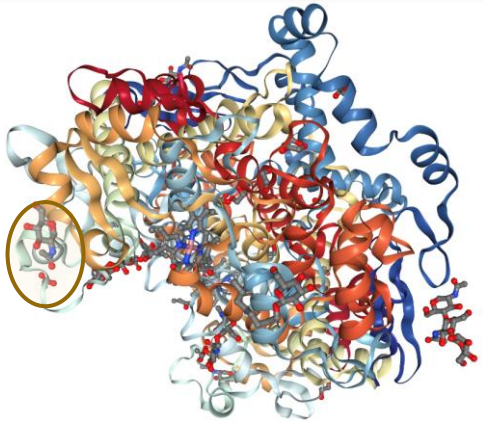
Drugs and Their Targets

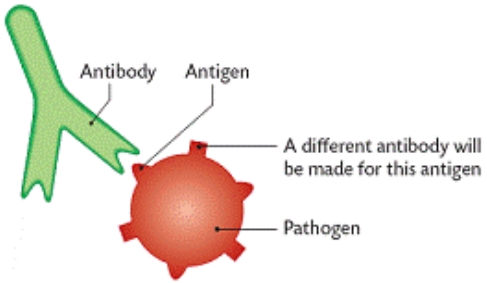
Small Molecule drug (aspirin)





Protein target (cyclooxygenase-2 or COX2) with aspirin

Monoclonal Antibody bound to target antigen



ASO binding to target RNA

ASO Technology Makes it Possible to Do What We Do at n-Lorem

Attribute	Small Molecule	Monoclonal Antibodies	RNA-Targeted (ASO)
Information Content	- - - -	++	+++++
Rules of Engagement	Still largely unknown	Partially understood	Well understood, easy to use
Learning from Previous Drugs	- - - - ”change a methyl change the drug”	Limited transferability	Broad transferability
Cost of Drug Discovery	Extremely expensive	Less expensive, but costly	Modest
Rate of Drug Discovery	Slow – Decades	Years	Months
Selectivity for Target	- - - -	++	++++
Versatility	++++	+	++++
Cost of Goods	++++	- - -	++++
Advancing Technology	No	Minimal	Extensive, rapid advancement

What Do We Do in the n-Lorem Lab and How Do We Do it?

Nano-rare Patient Colloquium 2024

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The n-Lorem Lab Does ASO Discovery, Directed and Basic Research

- ASO Discovery
- Directed research to better understand targets and mutations
- Basic research to advance ASO technology

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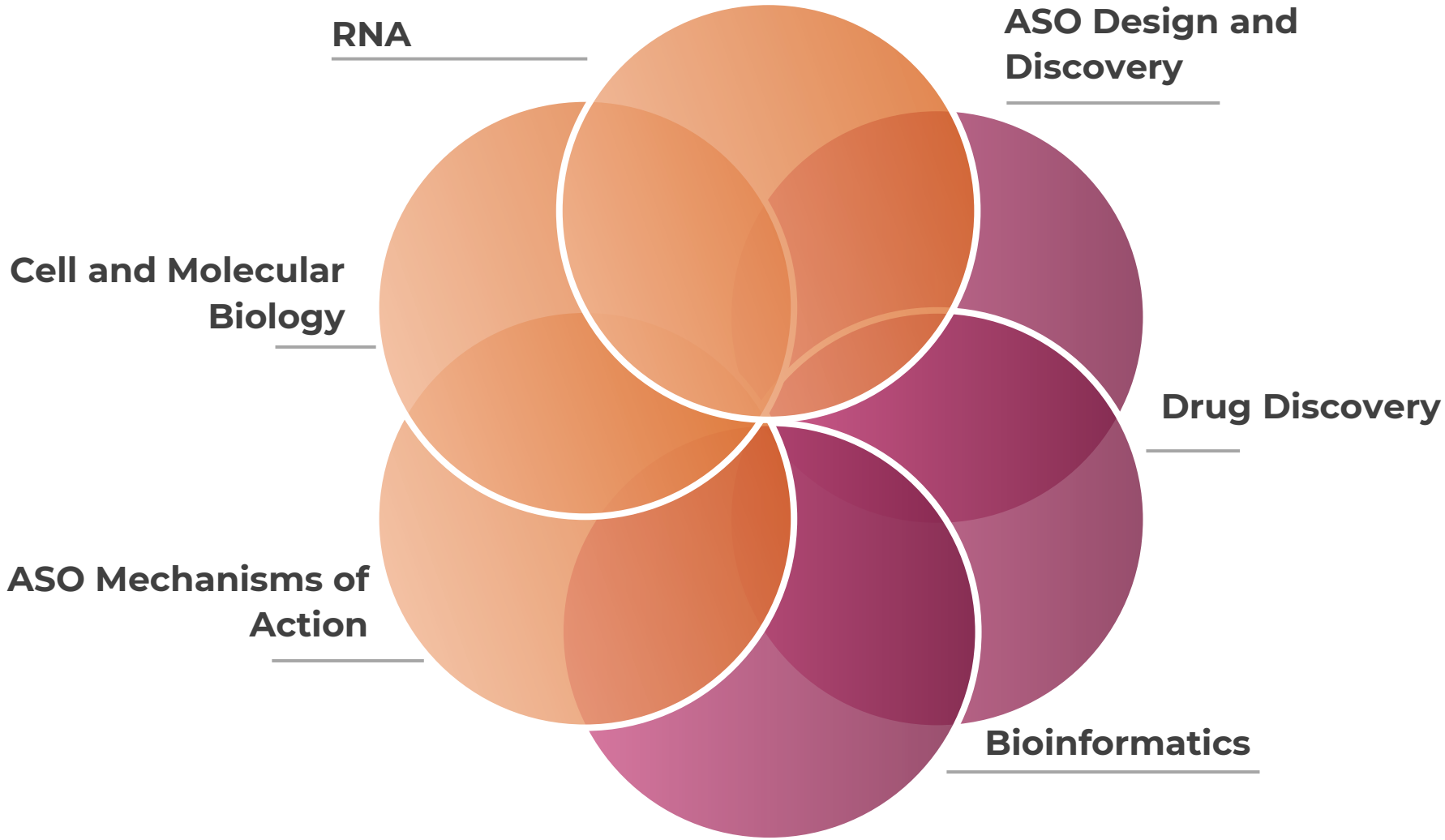
- ASO Discovery
- Directed research to better understand targets and mutations
- Basic research to advance ASO technology

We purposefully created a lab and a team with the right skills, right experience to meet the demand in the highest quality possible

The n-Lorem Lab is a Scientific Team with Complementary Skills and Experience

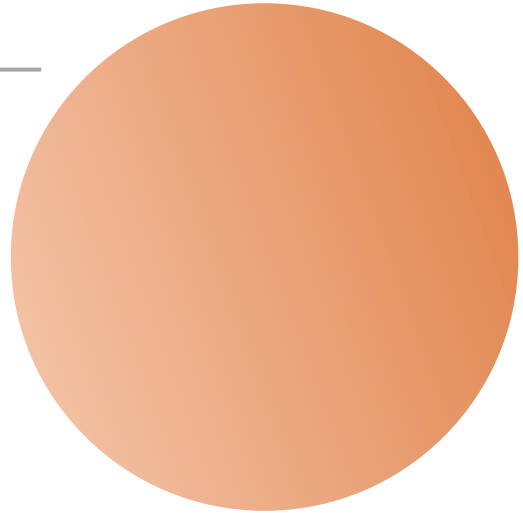


The n-Lorem Lab is a Scientific Team with Complementary Skills and Experience

RNA

RNA is our target, our medicine and the molecule that drives health and disease

Konstantina Skourti-Stathaki,
Anthony Vu,
Ria Thomas,
Craig McIntosh
Stan Crooke



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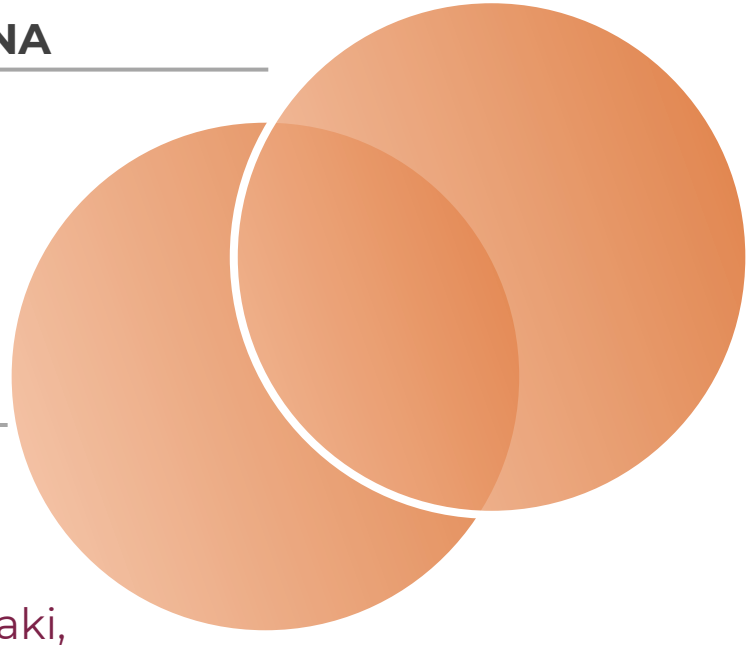

Cell and Molecular Biology

Understand the phenotype and evaluate if ASO can rescue

Konstantina Skourti-Stathaki,
Wei Zhang,
Craig McIntosh,
Julia Pytte,
Ana Foroughishafiei,
Swappi Mittal
Stan Crooke

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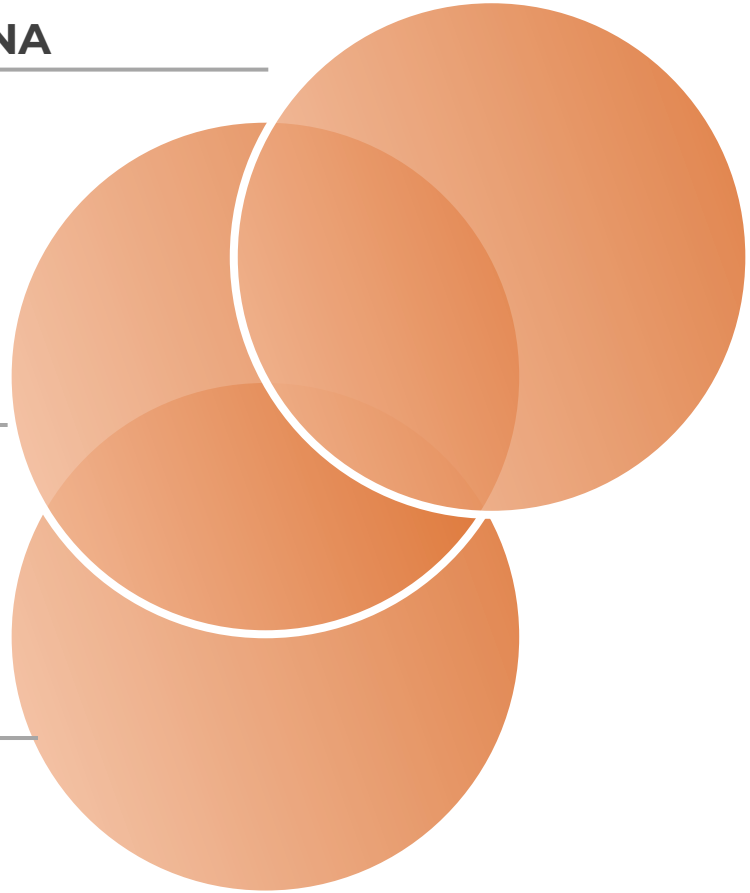
ASO Mechanisms of Action

Understand what we need to do and how we can improve it even more



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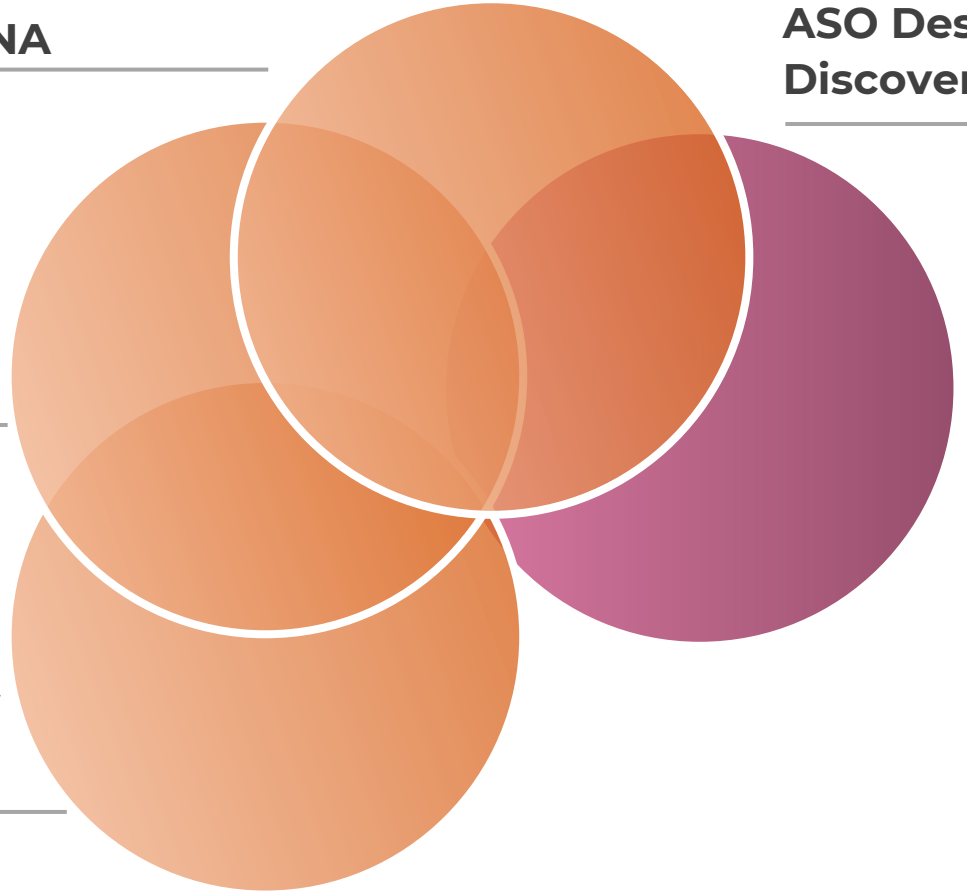

ASO Design and Discovery

This is the core of what we need to do to identify the optimal ASO

Konstantina Skourti-Stathaki,
Emily Miyoshi,
Anthony Vu,
Julia Pytte,
Lesley Saldana,
Elena Gonzalez,
Swappi Mittal, Ana Foroughishafiei,
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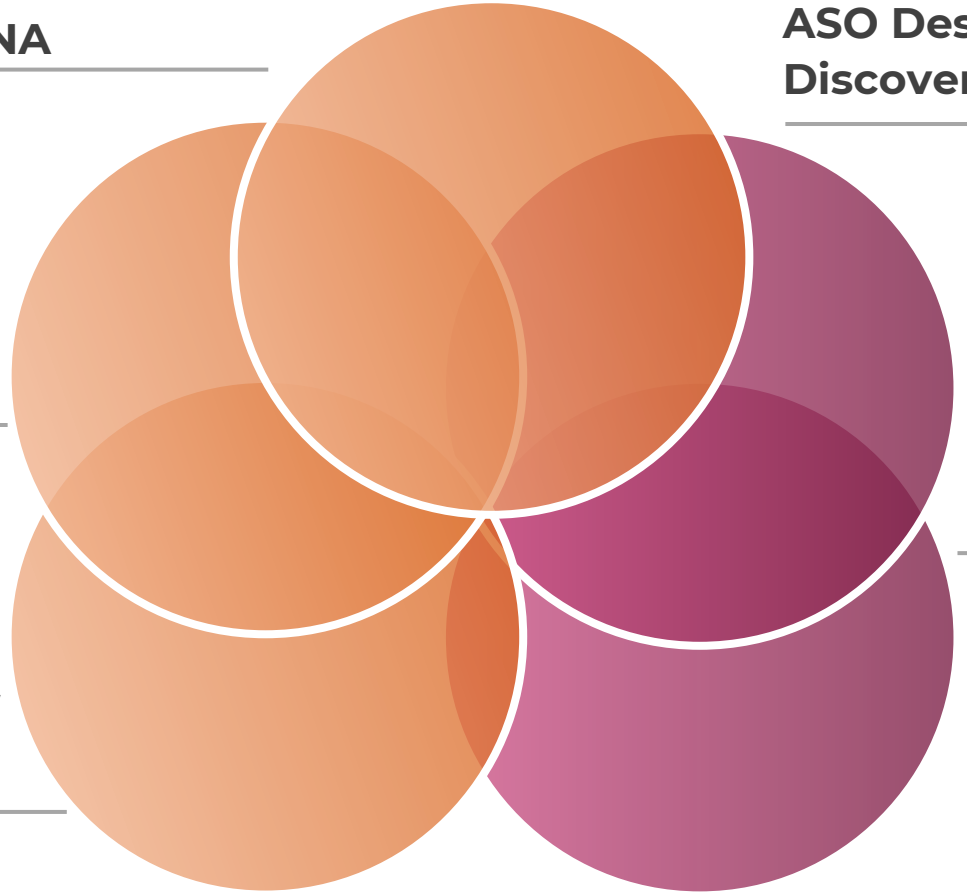

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ASO Design and Discovery

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Drug Discovery

Specific skillset needed

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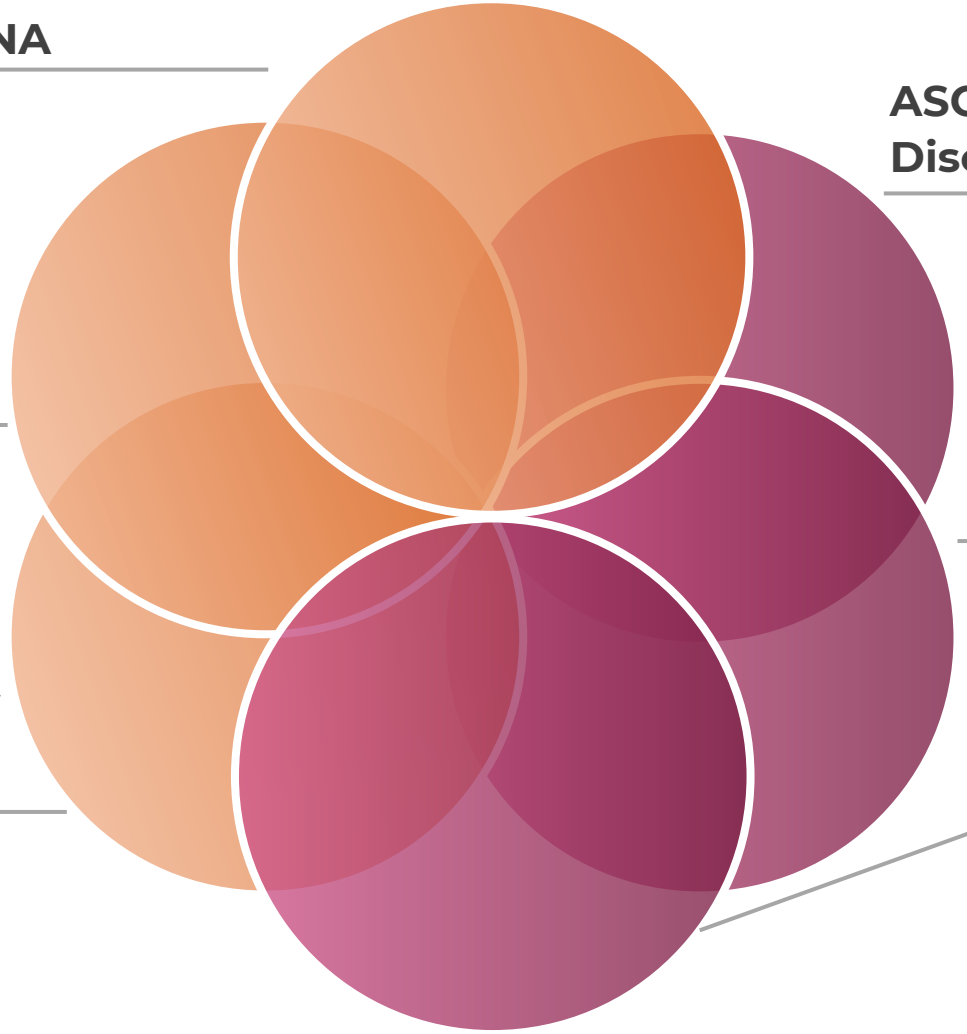


ASO Design and Discovery

This is the core of what we need to do to identify the optimal ASO

Drug Discovery

Specific skillset needed

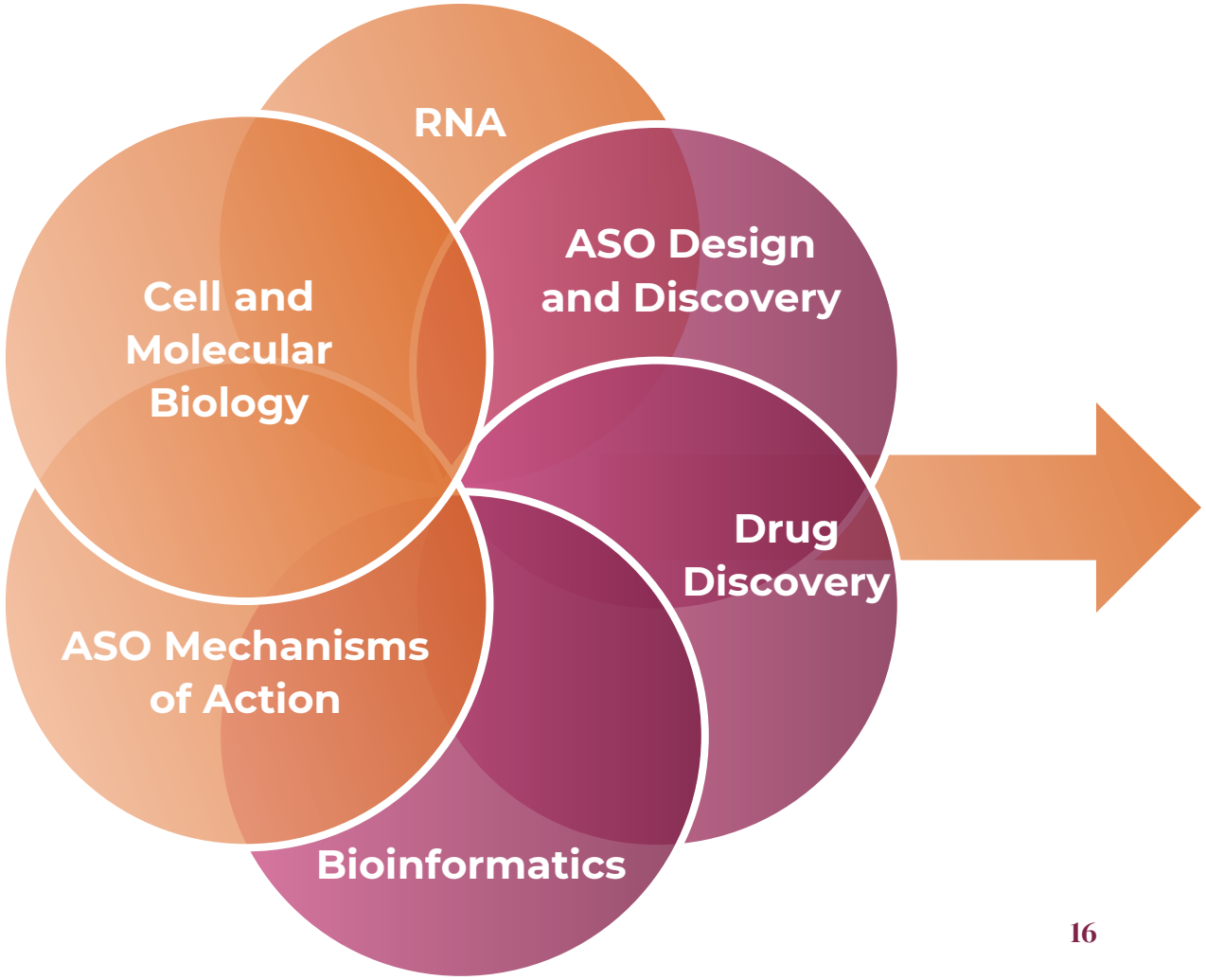
Bioinformatics

AI-driven ASO design and discovery

Emily Miyoshi
Anthony VU
Konstantina Skourti-Stathaki
Stan Crooke

The n-Lorem Lab is a Scientific Team with Complementary Skills and Experience

These skills and experience mean that we provide the best possible decisions for our patients



The n-Lorem Lab

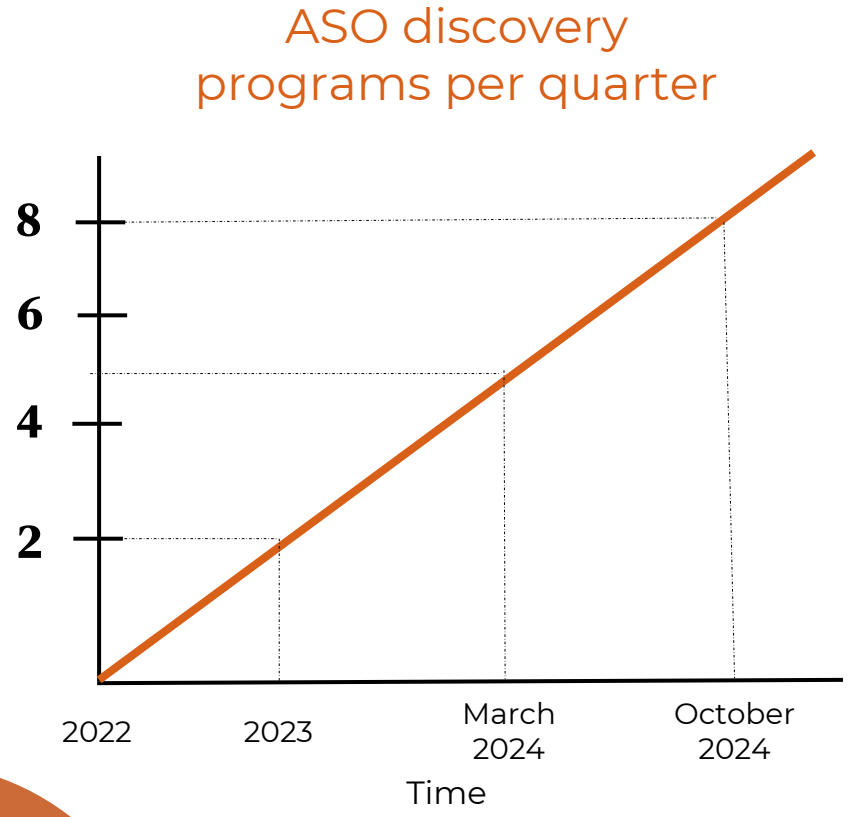
We started as a very small lab that we paid for at Ionis but given the demand, we needed a more robust capacity

Rapid Progress with a Newly Established Lab

We started as a very small lab that we paid for at Ionis but given the demand, we needed a more robust capacity

Here is what we have accomplished!

Rapid Progress with a Newly Established Lab



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Conclusions

- In less than 2 years, we now have ~27 programs that have completed discovery and are progressing into pre-clinical development
- We are at a rate of completing ~8 programs per quarter and continuing to grow
- We have the capacity to double or triple next year, if we raise the funds

ASO Design and Discovery Process

Checkpoints of ASO Design and Discovery Process

Review of a case
ASO amenability
ASO Design Strategy

Step 1

ASO Design

Discovery

Did we find optimal ASOs?
- Move to tolerability
- Start over
- Discontinue the case

Present at RMC
Lead selection
Challenging case / discontinuation

Can ASO Technology Meet the Needs of the Patient?

- Our task is to determine if an ASO strategy may be devised:
  - Is the mutation amenable to an ASO?
    - Impact: Are we confident that we know the nature of the mutation.
  - What ASO design is optimal? Do we know/ understand enough about the proximal pathological molecular mechanisms?
    - Impact: Will the ASO be able to correct these pathological mechanisms?
    - By understanding the cellular phenotype, we can ask if we can correct relevant phenotype
    - We often add new insights into the pathology of the disease.
  - What is the ASO design strategy?
    - Impact: Do we need an allele-selective ASO, a non-allele selective ASO, or a steric-blocking ASO?
  - What mutations can we not fix? True null mutations

Can ASO Technology Meet the Needs of the Patient?

Areas of consideration for ATTC

- **Clinical Genetics :** Favorable Benefit risk ratio
- **ASO Design & Discovery:** Amenable to H1 ASO; allele-selectivity required for now but if additional experiments are done, we could consider non-AS approach
- **Clinical Development:** Straightforward Treatment Goals
- **Clinical Operations:** Logistically straightforward with clear support from institution.

Checkpoints of ASO Design and Discovery Process

Review of a case
ASO amenability
ASO Design strategy

ASO Design

Step 2

Discovery

Did we find optimal ASOs?
- Move to tolerability
- Start over
- Discontinue the case

Present at RMC
Lead selection
Challenging case / discontinuation

Different ASOs for Different Strategies

Gapmer (5-10-5; RNA-DNA-RNA)

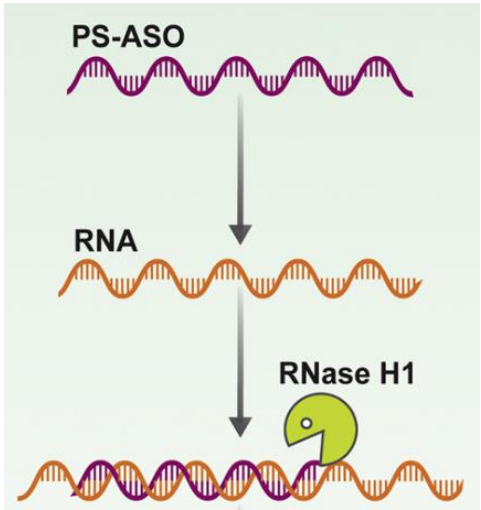
DNA base

2' MOE modified RNA base

Phosphodiester Linkage (PO)

Phosphorothioate Linkage (PS)

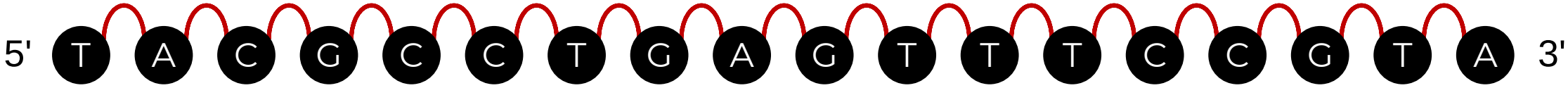
RNase H1 activity

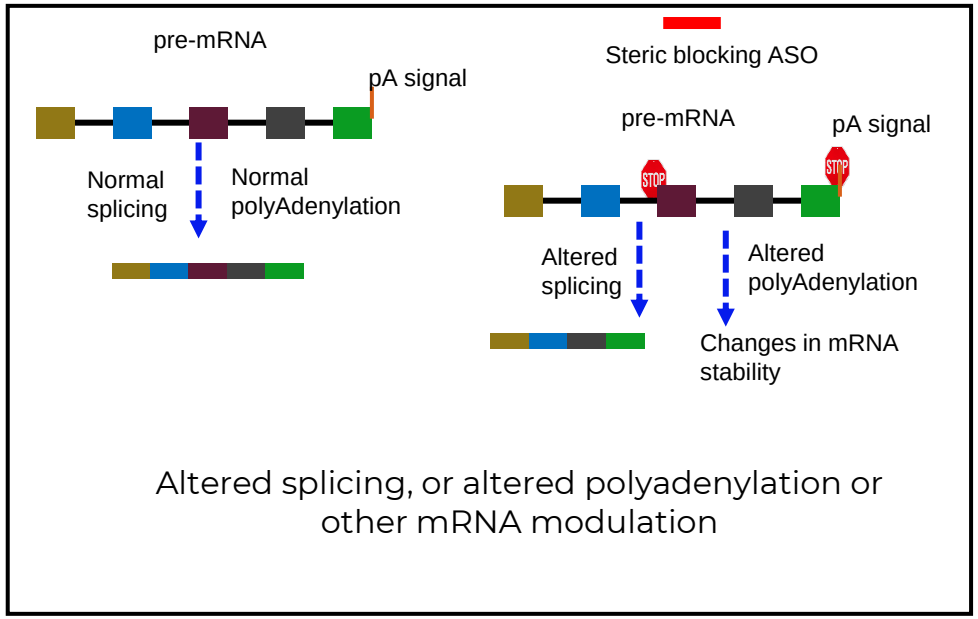


target RNA degraded

Different ASOs for Different Strategies

Splicing/ steric blocking ASO (18mer, Full MOE)



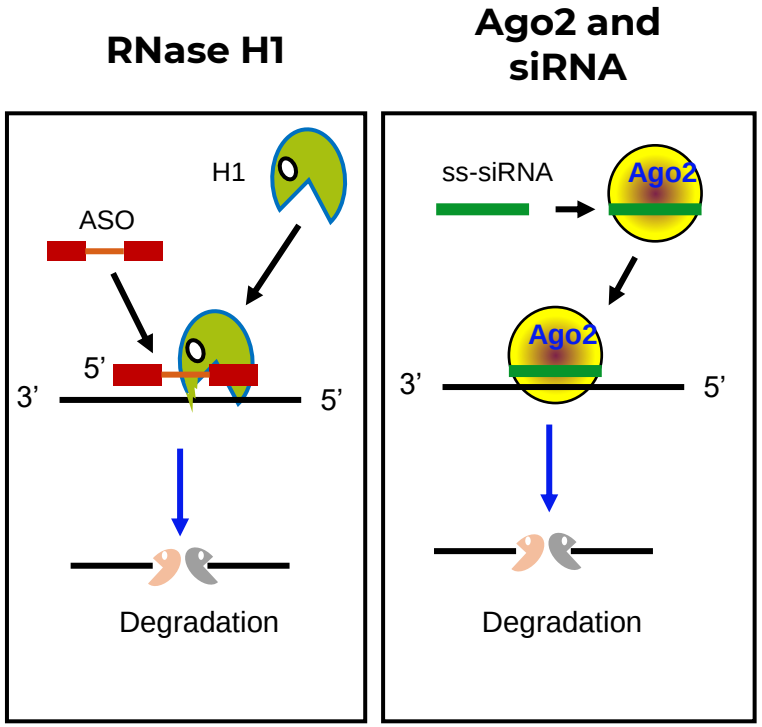


2' MOE modified RNA base

Phosphorothioate Linkage (PS)

2 ASO Mechanisms: RNA Degradation

- We have led the way in understanding all kinds of mechanisms to be used with ASOs



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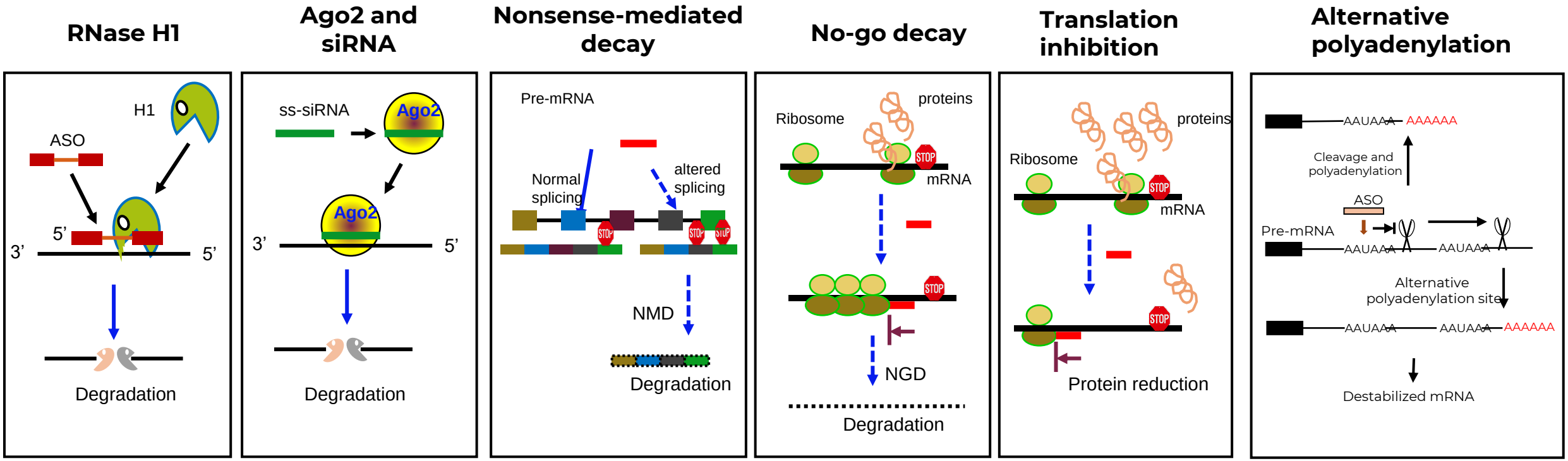
2 ASO Mechanisms: RNA Degradation and mRNA Modulation

- We have led the way in understanding all kinds of mechanisms to be used with ASOs



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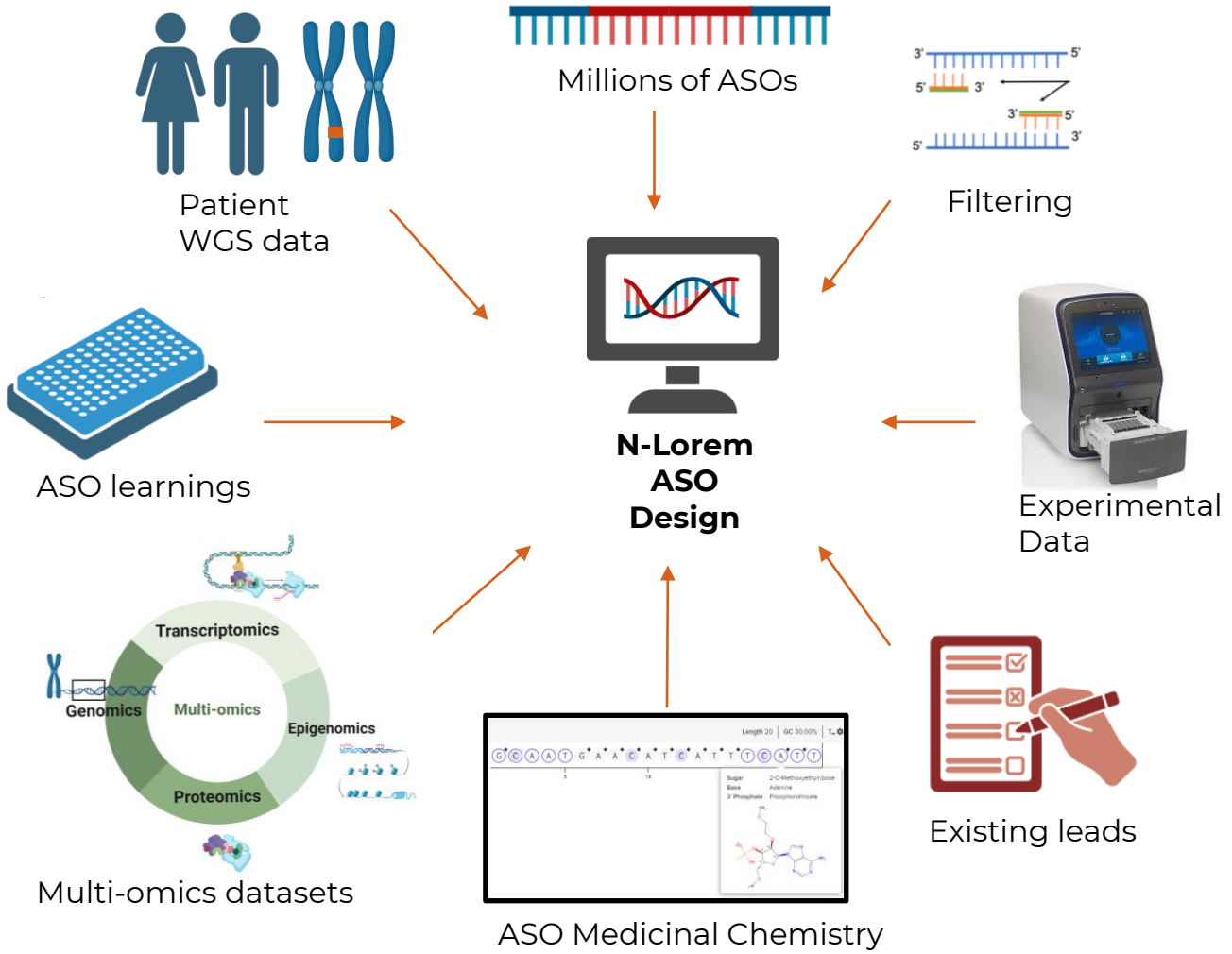
AI-informed ASO Design Makes ASO Discovery Highly Efficient

- 35 years of ASO expertise
- Millions of ASOs
- All relevant cell and animal models
- Hundreds of thousands of patients
- Continuous learnings from WGS data
- Design ASOs to reach more patients
- State-of-the-art integration of multi-omics data



Checkpoints of ASO Design and Discovery Process

Review of a case
ASO amenability
ASO Design strategy

ASO Design

Discovery

Step 3

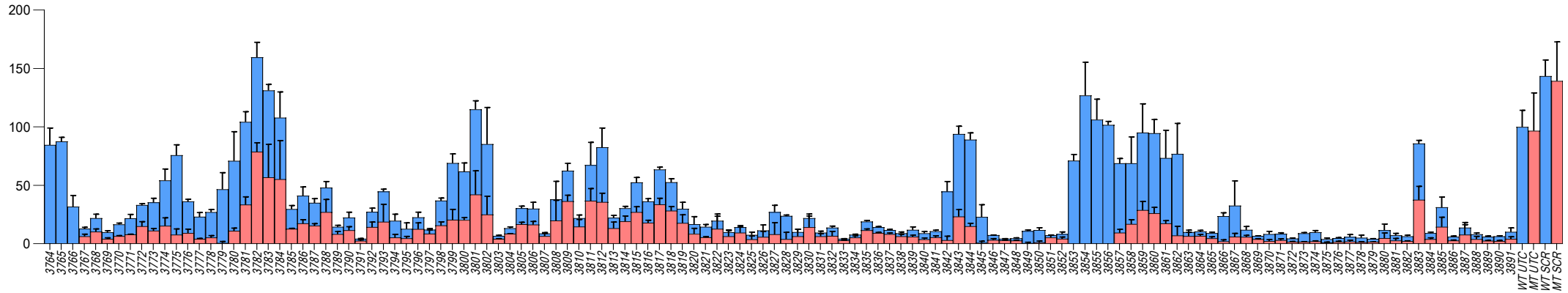
Did we find optimal ASOs?
- Move to tolerability
- Start over
- Discontinue the case

Present at RMC
Lead selection
Challenging case / discontinuation

A Rigorous, High-throughput, High-quality ASO Discovery Process: Discovery of an Allele-selective ASO

Screening Step	Purpose	Approximate Minimum Numbers of ASOs Typically Evaluated	Minimum Criteria
Primary ASO screen	To identify optimal sites in target RNA for ASO and H-1 binding	~500	>80% target reduction

Single dose screening



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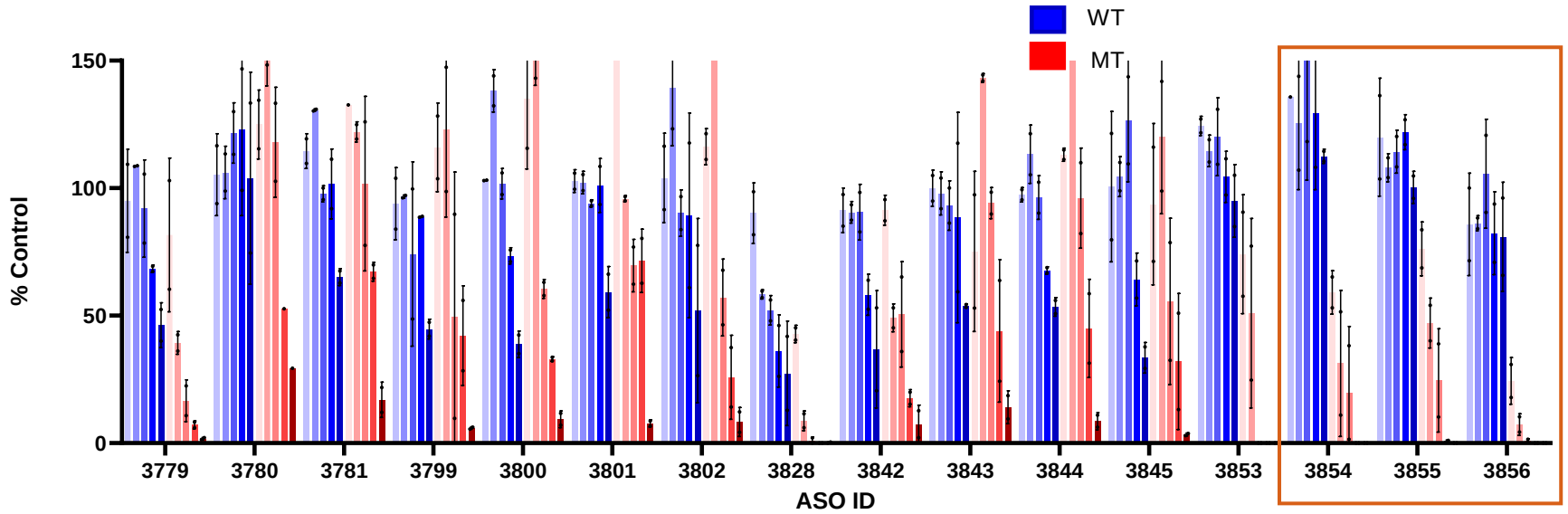
Dose response evaluation of multiple ASOs	To select at least 20 ASOs for in vivo tolerability screening	~50-75	IC50<1 >80% reduction Selectivity with >10 fold



Dose response screening

A Rigorous, High-throughput, High-quality ASO Discovery Process

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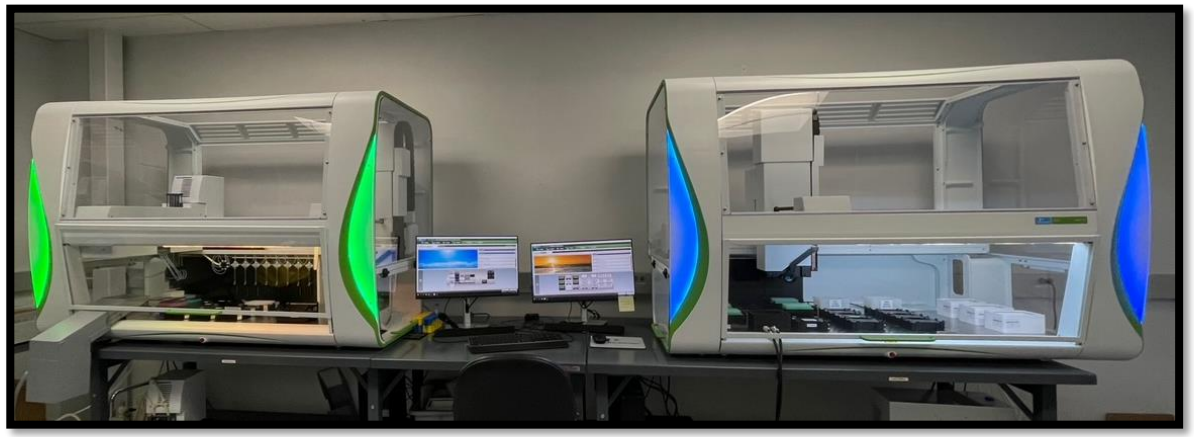
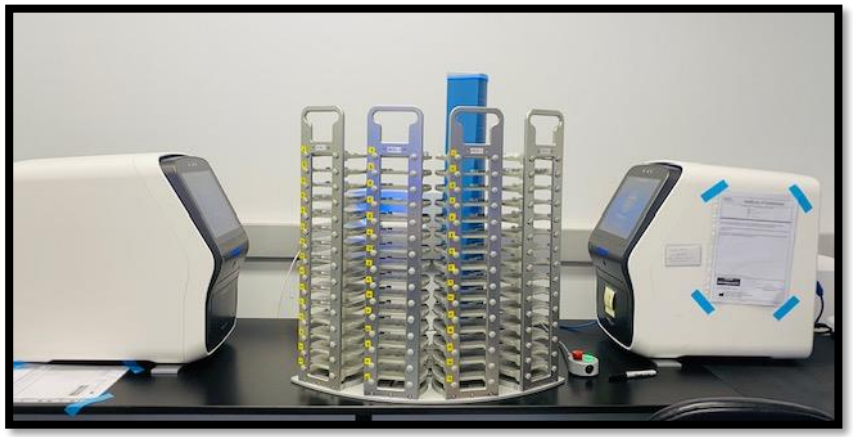
Dose response evaluation of multiple ASOs	To select at least 20 ASOs for in vivo tolerability screening	~50-75	IC50<1 >80% reduction Selectivity with >10 fold
In vitro off-target analysis	To confirm selectivity of ASO for target RNA vs. any worrisome off-target	As many as necessary	~10-fold difference in IC50s for target RNA vs. off target
BJAB Assay	To exclude activators of innate immunity	~50-75	Less than 2-fold increase in TNF-alpha at high ASO concentrations





Critical Decisions and Challenges We Address

Decisions:

- Discontinue a case
- Do we have the best ASO possible, or do we need to redesign?

Challenges:

- Encounter differences with published literature/ data
- We don't know enough about the nature of the mutation
- Highly structured RNA
- Small number of SNPs for allele-selective ASOs

We Do Everything We Can Before We Discontinue a Case

- Are the ASOs we have sufficient, given the function of the gene?
- If we do not know, we go ahead and perform research experiments
  - How does the cell tolerate the specific ASO?
  - Does it still do its normal function?
  - Can the ASO rescue the pathological phenotypes?
- If allele-selectivity is not sufficient, we bring the case to RMC and recommend to discontinue

A Minor Error in Sequencing Proved to be a Major Problem for ASO Discovery

- Discrepancy in indel sequence was resolved enabling the treatment of all four patients with the same ASO

Genetic Report		Long-read WGS	
Indel Variant	**Sequencing Method**	**Variants**	**Sequencing Method**
c.2053-3358_2053-3350 delinsTGTTTTTTACATGACAGGT	Sanger seq	c.2053-3358_2053-3350 delinsTGTTTTTTACATTACAGGT	Long-read WGS
c.2053-3358_2053-3350 delinsTGTTTTTTACATTACAGGT	NGS (likely short read)	c.2053-3358_2053-3350 delinsTGTTTTTTACATTACAGGT	Long-read WGS
c.2053-3358_2053-3350 delins (sequence not specified)	Sanger/capillary seq	c.2053-3358_2053-3350 delinsTGTTTTTTACATTACAGGT	Long-read WGS
c.2053-3358_2053-3350 delinsTGTTTTTTACATGACAGGT	Sanger seq and NGS (likely short read)	c.2053-3358_2053-3350 delinsTGTTTTTTACATTACAGGT	Long-read WGS

Directed Research to Better Understand Targets and Mutations

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What is the Nature of JIP3 R578C Mutation and is it ASO Amenable?

Open questions that needed answering before moving to ASO discovery

- Is JIP3 R578C a toxic gain of function mutation? ?
- What are the proximal molecular pathological mechanisms? ?
- How much reduction of wild type JIP3 is tolerated? ?
- Does PS ASO reduction of mutant JIP3 reverse all pathologic phenotypes? ?

What is the Nature of JIP3 R578C Mutation and is it ASO Amenable?

We answered these questions to design and discover the most optimal ASOs

Is JIP3 R578C a toxic gain of function mutation?	YES	Yes, confirmed that this mutation is toxic gain of function
What are the proximal molecular pathological mechanisms?	Determined	It affects multiple cellular functions of JIP3 including apoptosis, endosome mobility, dopamine signaling
How much reduction of wild type JIP3 is tolerated?	Determined	Moderate reduction is tolerated
Does PS ASO reduction of mutant JIP3 reverse all pathologic phenotypes?	YES	Yes, allele-selective ASOs rescued all proximal, molecular pathological phenotypes

Basic Research to Advance ASO Technology and Reach More Patients

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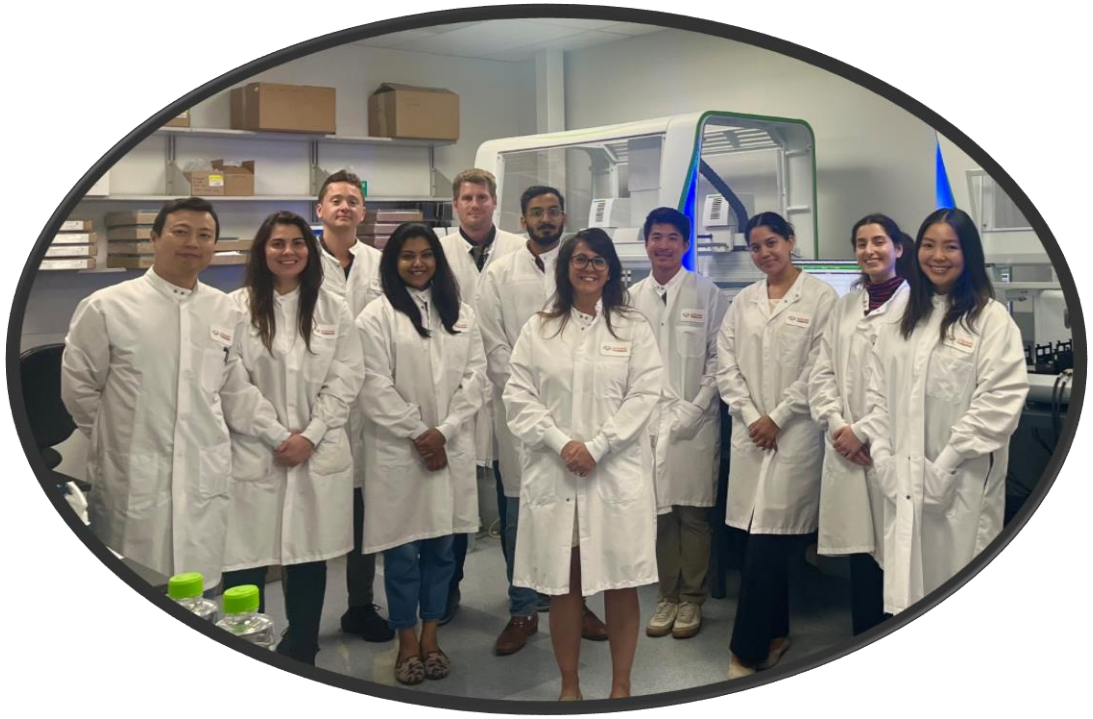
How Can We Expand to Reach More Patients?

- Mechanisms to avoid adverse events and immune activation
  - Why? We could make even better ASOs and be even more efficient
- Enhance allele-selectivity
  - Why? ~50% of our programs are allele-selective. We can make better ASOs and reach our patients faster and help even more patients
- Upregulation strategies with ASOs
  - Why? ~40% of our declined cases require an ASO-mediated protein upregulation strategy. We want to be able to accept and treat these patients

We have the knowledge and expertise to address these issues today

Conclusions

- We have an outstanding team in place to discover optimal ASOs
- We have the capabilities to do this rapidly, in a cost-effective and high-throughput manner



Conclusions

- We have an outstanding team in place to discover optimal ASOs
- We have the capabilities to do this rapidly, in a cost-effective and high-throughput manner
- We apply and share our learnings for each patient to maximize our opportunities
- 100% commitment to the patient: The most important decision that we are making in every step of the way is –

Is the ASO sufficient and optimal for this patient?

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Join Us As We Make The World A Better Place, One Patient, One Family at a Time