n-lorem FOUNDATION

2023 Nano-rare Patient Colloquium

ASO Experience and Scientific Excellence Driving the n-Lorem Discovery and Development Platform

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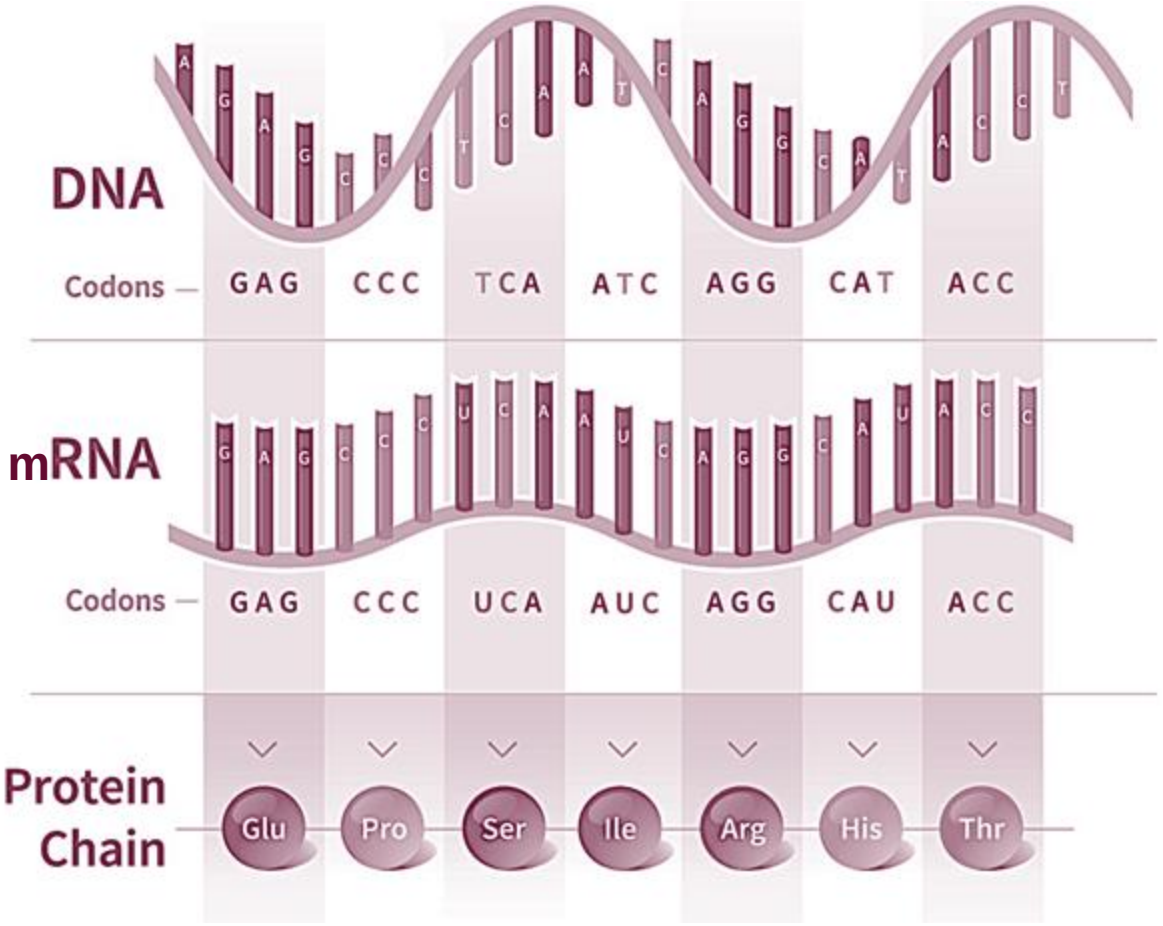
Hosted by Biogen

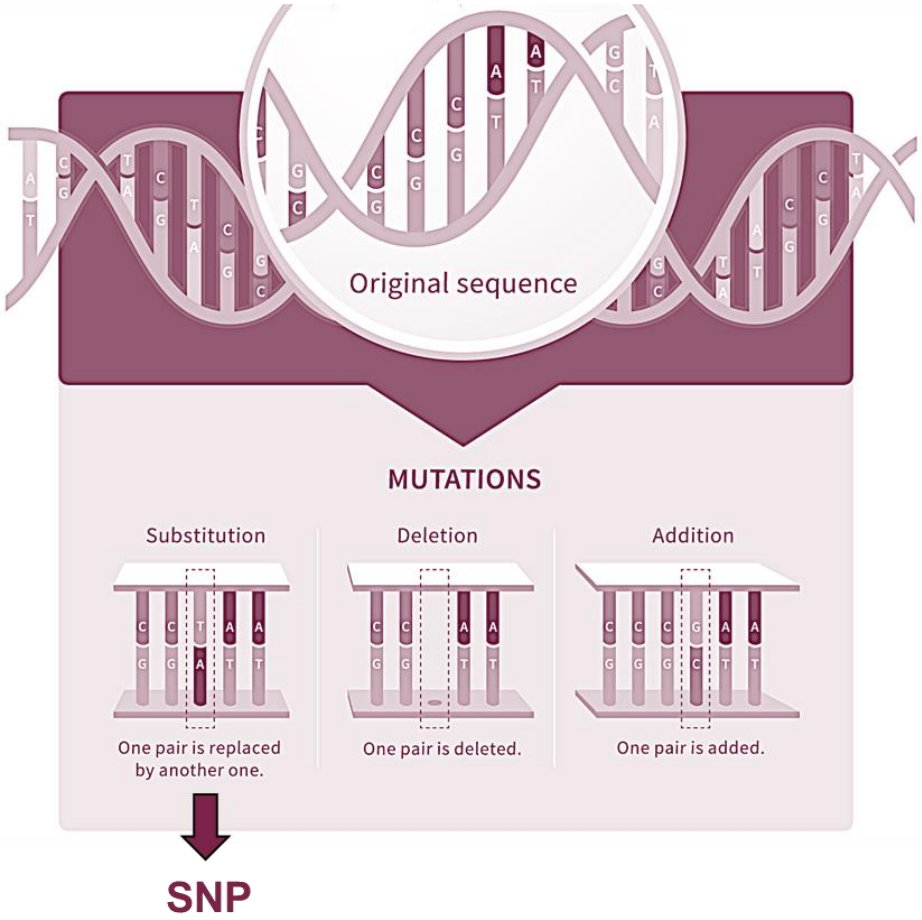
From Genes to Proteins

- Genes are made of DNA
- Building blocks of DNA are made of 4 bases – A, T, C, G - organized in a specific sequence encoding information
- Information from DNA is transcribed into messenger RNA molecule which bears instructions
- mRNA instructions are read by the machinery of the cell to make proteins
- Proteins are important to the structure, function, and regulation of the body

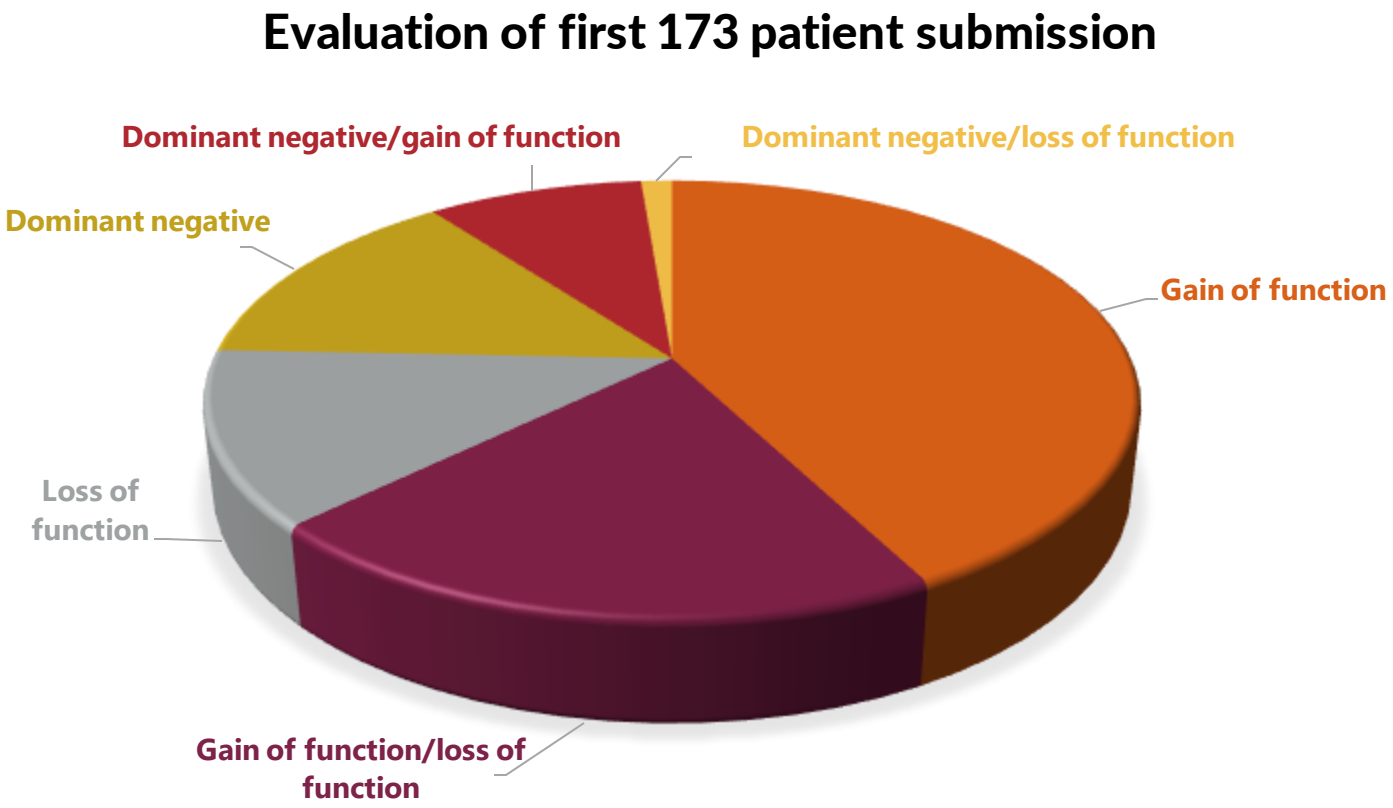


What is a Mutation?

- A mutation is a change in the normal sequence of nucleotides
- Most of the times, mutations are corrected, benign or silent
- Sometimes, mutations can change a critical part of the instructions to make a protein

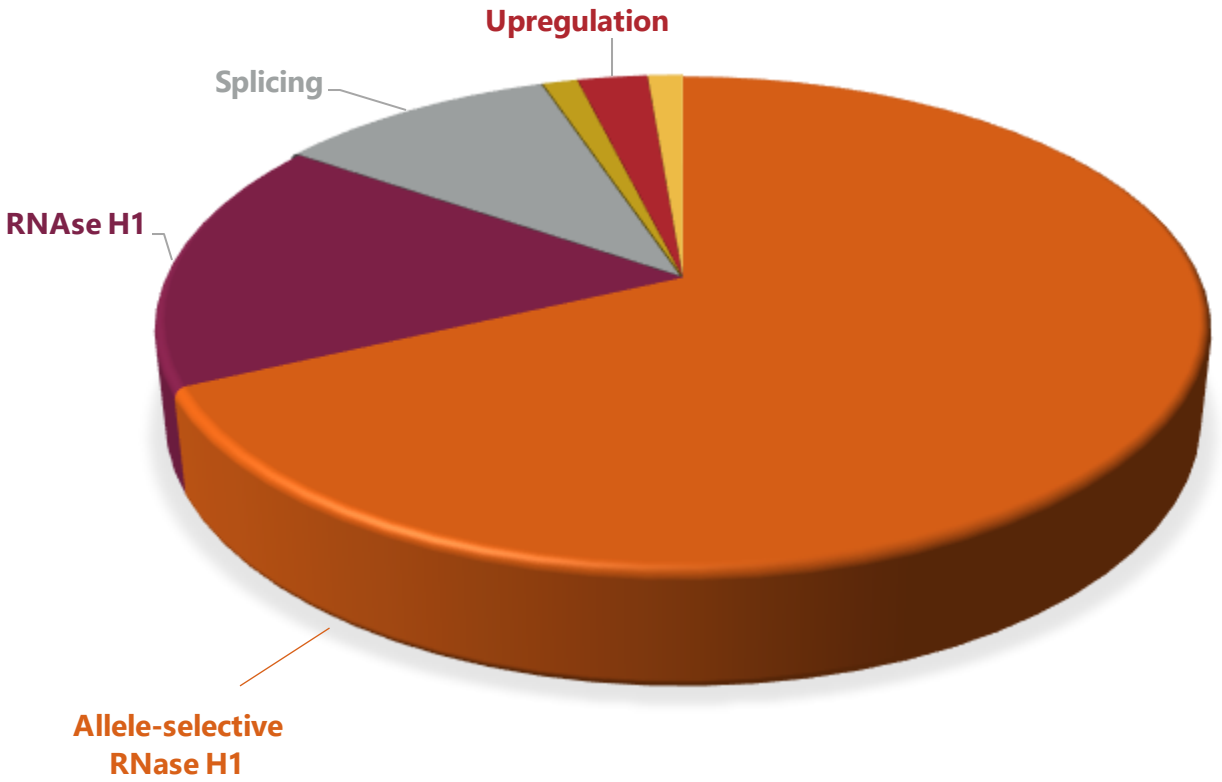


Consequences of Mutations for Nano-Rare



- Different mutations have different consequences
  - Protein becomes inactive or degraded
  - Protein acquires a new function
- Each individual patient submission is thoroughly evaluated to identify an adequate ASO strategy

Predominance of Allele-Specific RNase H1 Programs in the Current Portfolio



- Gain of Function mutations can be addressed by RNase H1 ASOs
- Many n-Lorem patients require allele-selective strategy to preserve function of the target gene
- Allele-selective strategies require greater skill, expertise and screening in order to identify an optimized ASO for that patient

n-Lorem Platform is Based on ASO Technology and FDA Guidelines

- Antisense technology is
  - Rapid
  - Versatile
  - Validated
  - Cost effective
  - Scalable
- Supported by the FDA
  - 4 guidelines issued in 2021 to specifically address the development of individualized ASOs

Some Recent Reviews on RNA-Targeted Drug Discovery

The Interaction of Phosphorothioate-Containing RNA Targeted Drugs with Proteins Is a Critical Determinant of the Therapeutic Effects of These Agents

Stanley T. Crooke,* Punit P. Seth, Timothy A. Vickers, and Xue-hai Liang

SURVEY AND SUMMARY

Phosphorothioate modified oligonucleotide–protein interactions

RNA-Targeted Therapeutics

Stanley T. Crooke, Joseph L. Witztum, C. Frank Bennett, and Brenda F. Baker

Towards next generation antisense oligonucleotides: mesylphosphoramidate modification improves therapeutic index and duration of effect of gapmer antisense oligonucleotides

Brooke A. Anderson, Graeme C. Freestone, Audrey Low, Cheryl L. De-Hoyos, William J. Drury III, Michael E. Østergaard, Michael T. Migawa, Michael Fazio, W. Brad Wan, Andres Berdeja, Eli Scandalis, Sebastien A. Burel, Timothy A. Vickers, Stanley T. Crooke, Eric E. Swayze, Xuehai Liang and Punit P. Seth

JBC REVIEWS

Antisense technology: A review

Stanley T. Crooke, Xue-Hai Liang, Brenda F. Baker, and Rosanne M. Crooke

REVIEWS

Antisense technology: an overview and prospectus

Stanley T. Crooke, Brenda F. Baker, Rosanne M. Crooke and Xue-hai Liang

ARTICLE IN PRESS

Biochemical Pharmacology

Antisense drug discovery and development technology considered in a pharmacological context

Stanley T. Crooke, Xue-hai Liang, Rosanne M. Crooke, Brenda F. Baker, Richard S. Geary

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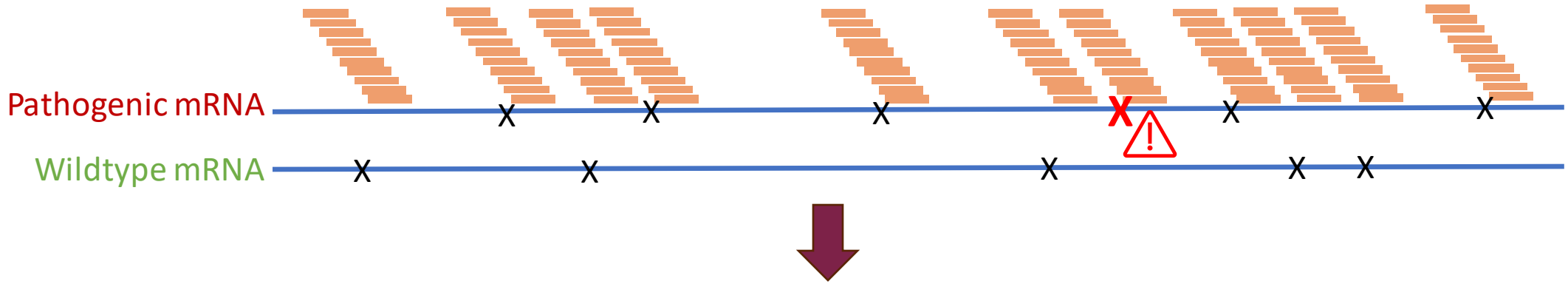
Current Process To Discover and Develop Optimal RNase H1 ASOs for CNS Disease

Screening Step	Purpose	Approximate Minimum Numbers of ASOs Evaluated	Minimum Criteria
ASO design including in silico off-target assessment	Exclude motifs associated with ASO structure, repeat sequences, cytotoxicity, pro-inflammatory effects and off targets Include attractive motifs	Scan entire pre-mRNA Apply algorithms	All important attractive motifs included, unattractive excluded
Primary ASO screen	To identify optimal sites in target RNA for ASO and H-1 binding	~500	>80% target reduction
Dose response evaluation of multiple ASOs	To select at least 20 ASOs for in vivo tolerability screening	~50-75	IC50 1umol (free uptake)
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
Single dose tolerability screening in rodents at high dose including histopathology of CNS	To identify optimally tolerated lead ASOs	20	Exclude poorly tolerated candidate and include ASO with an optimal therapeutic index General Histology / AIF1 Microglia / GFAP Astrocytes
Repeat dose GLP 3-month rodent toxicity	To identify NOAEL and cell-types at risk	1-3	An attractive therapeutic index with an acceptable NOAEL
GMP Manufacturing	Quality ASO drug substance	1	Pure, stable lyophilized ASO
Sterile Fill and Finish	Quality, stable and sterile ASO drug product	1	Sterile vials for administration

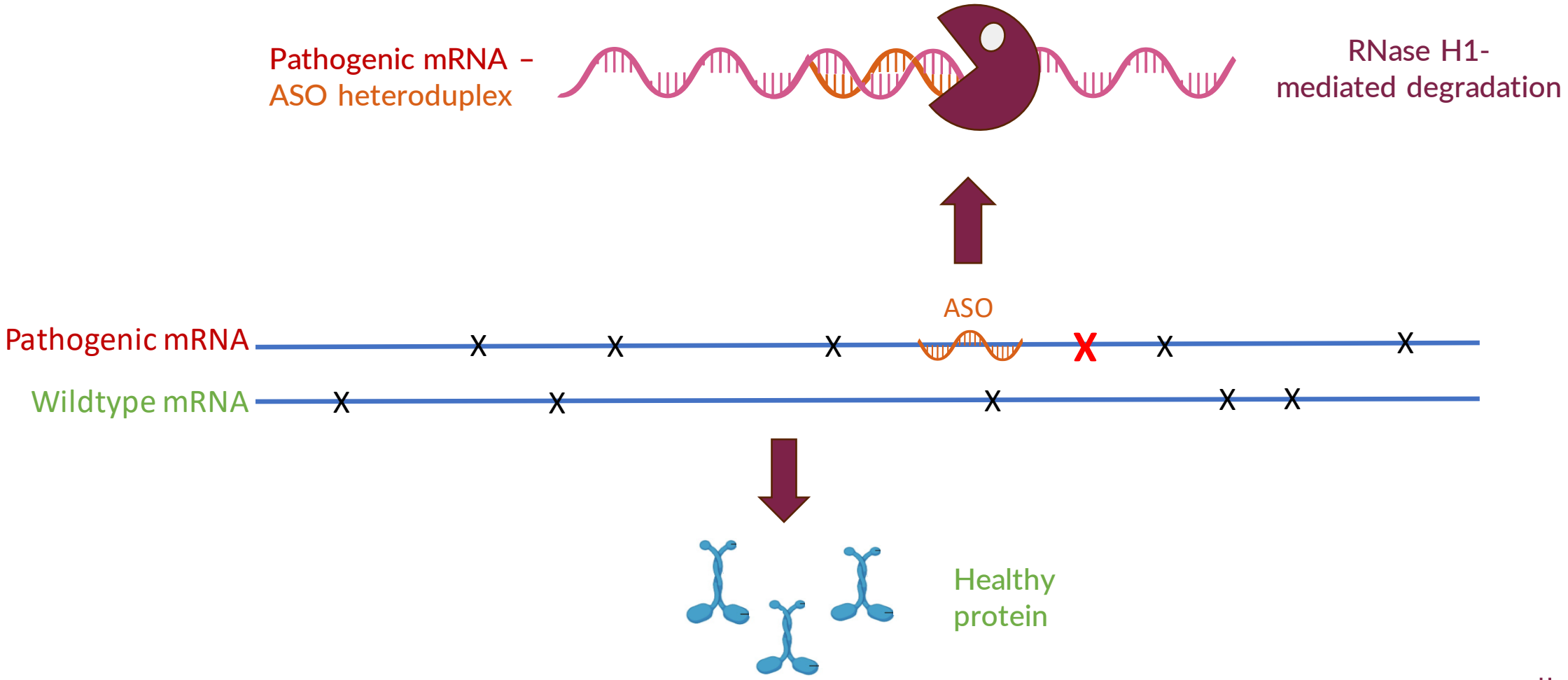
Extensive Screening to Identify the Best ASO

STEP 1: Identifying SNPs to design allele-specific ASOs

- 2 copies of each gene; pathogenic mutation can happen on either allele
- Benign changes in bases – SNPs – can also be present on either allele
- SNPs can be used to design ASOs targeting **only** the pathogenic mRNA
- Each SNP site allows the design of 20 different ASOs



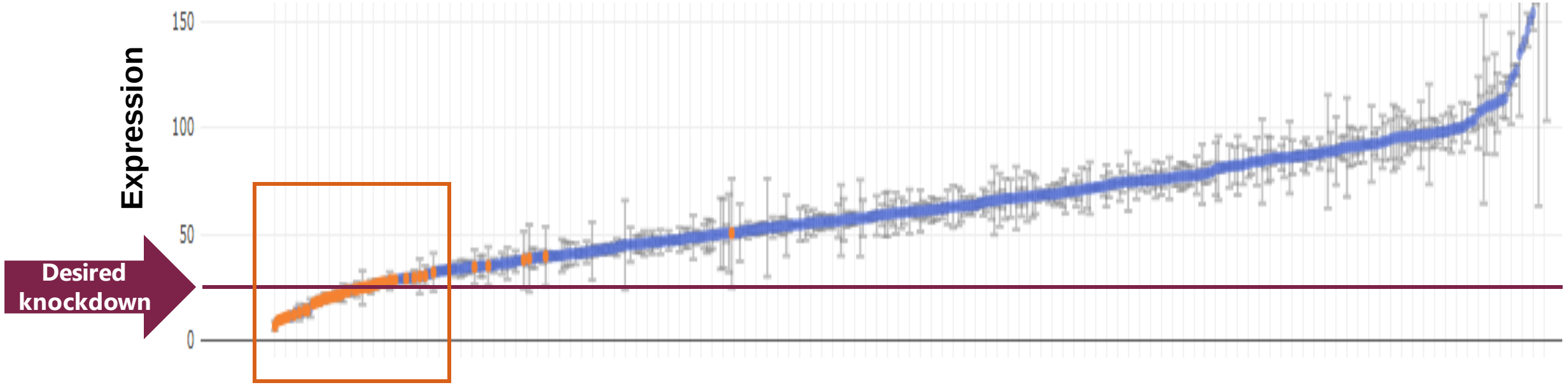
Allele-Specific RNase H1 ASO Triggers Degradation of Mutant mRNA while Preserving Wildtype mRNA



Extensive Screening to Identify the Best ASO

STEP 2: Single Dose Assay

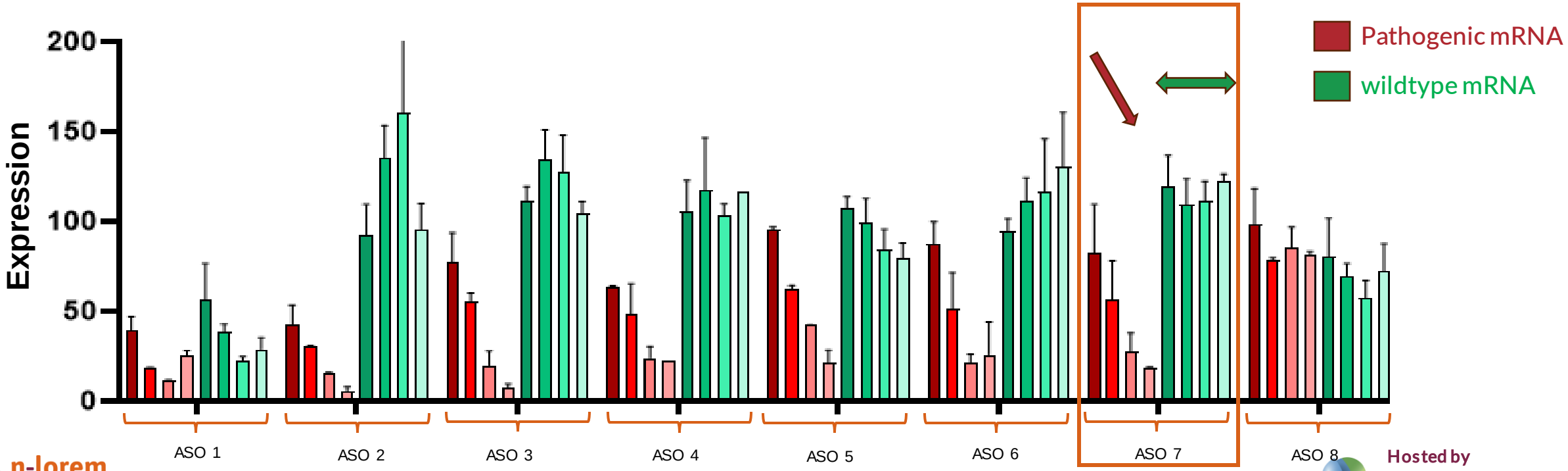
- ASOs are tested at a single concentration to evaluate selectivity and potency
- ASOs showing ~80% of reduction of pathogenic mRNA will move forward



Extensive Screening to Identify the Best ASO

STEP 3: Dose Response Assay

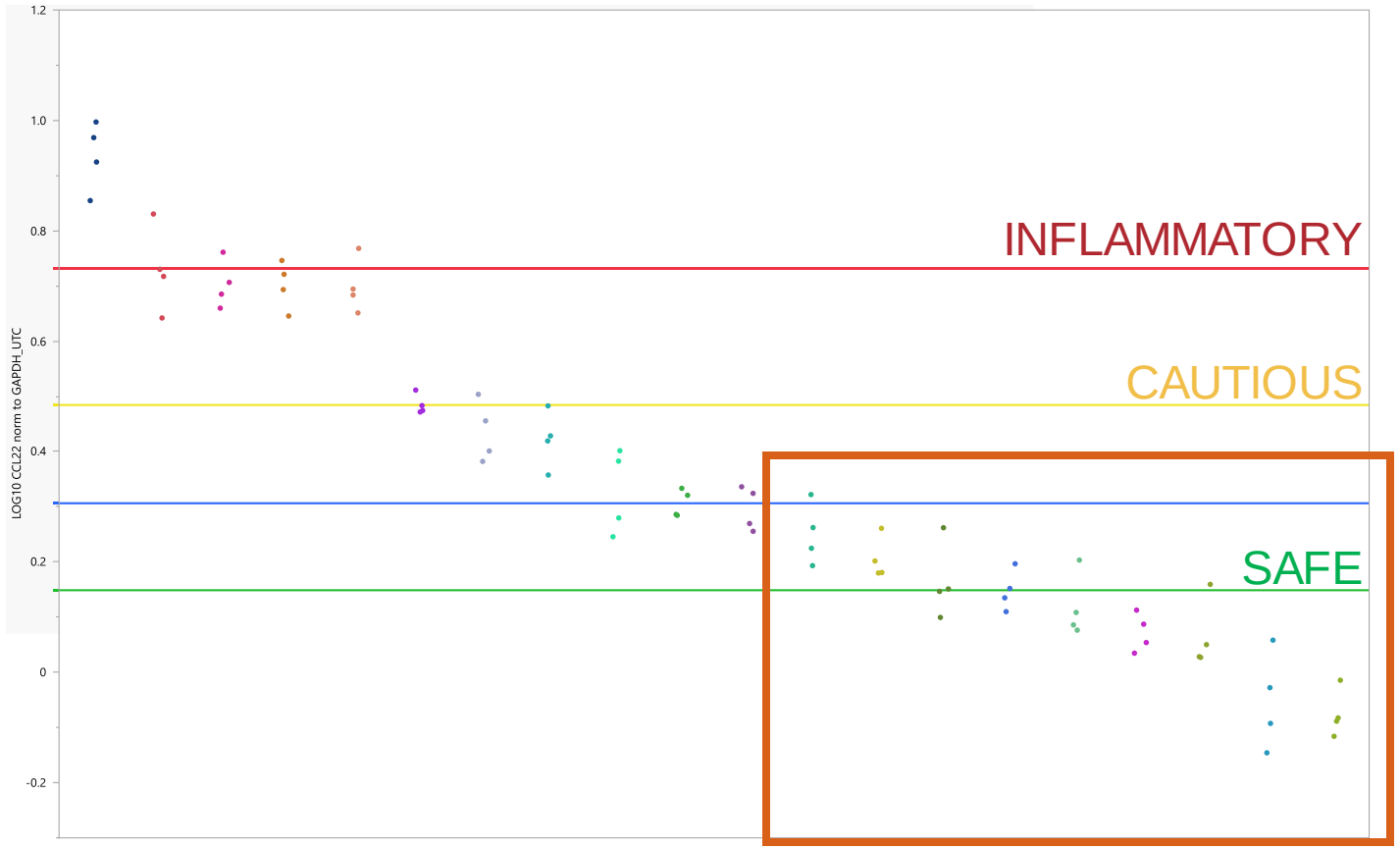
- Assay evaluates potency of ASOs for pathogenic and wildtype mRNA
- ASOs reducing pathogenic mRNA in a dose responsive manner while sparing wildtype expression will move forward



Extensive Screening to Identify the Best ASO

STEP 4: Inflammation Assay (BJAB)

- Assay designed to measure innate immune activation
- Peripheral cell line is used (BJAB)
- All ASO candidates are benchmarked against known controls to rank their pro-inflammatory potential
- ASOs identified as safe will move forward



Extensive Screening to Identify the Best ASO

STEP 5: Identify Potential Off Target Effects (in silico and in vitro)

- Analysis designed to identify nonspecific binding of ASOs to mRNA from other genes
- Nonspecific binding of ASOs could reduce expression of critical genes
- 2 step process:
  - In silico-based analysis highlighting genes with partial match to ASO sequence
  - In vitro assay evaluating actual effect of ASOs on expression of the off target genes
- Having a thorough understanding of the patient condition is essential to evaluate the true liability of any confirmed off target effects

Extensive Screening to Identify the Best ASO

STEP 6: Small Scale Synthesis

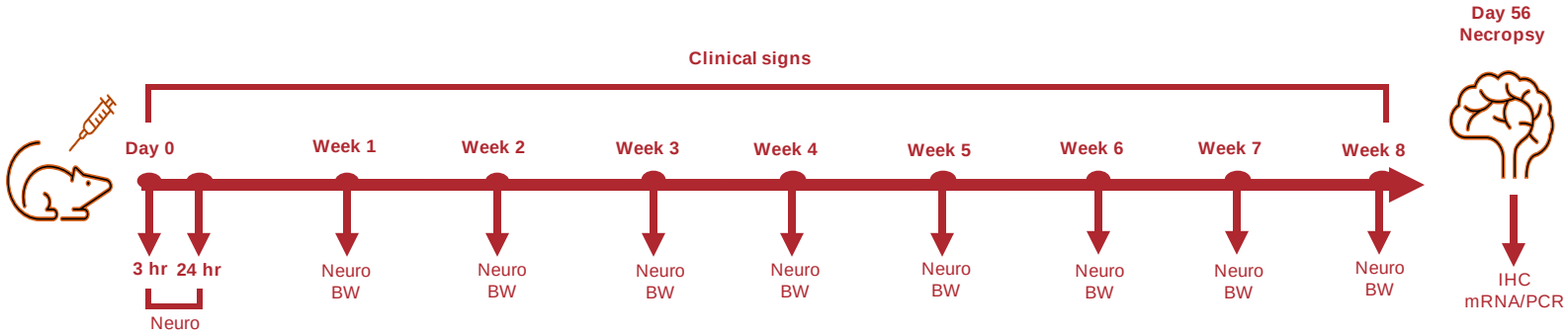
- Small scale synthesis of **~20 ASO** candidates is required for use in animal studies to further evaluate the different candidates
- Strict criteria are used for purity at this stage, ensuring that animals are exposed to a batch of material representative of the clinical batch

Extensive Screening to Identify the Best ASO

STEP 7: in vivo Tolerability Study

- Study designed to identify poorly tolerated ASOs
- Route of administration mimics clinical route
- Rodents receive a single injection at high dose of **top ~20** ASOs identified from in vitro screen and observed for 8 weeks

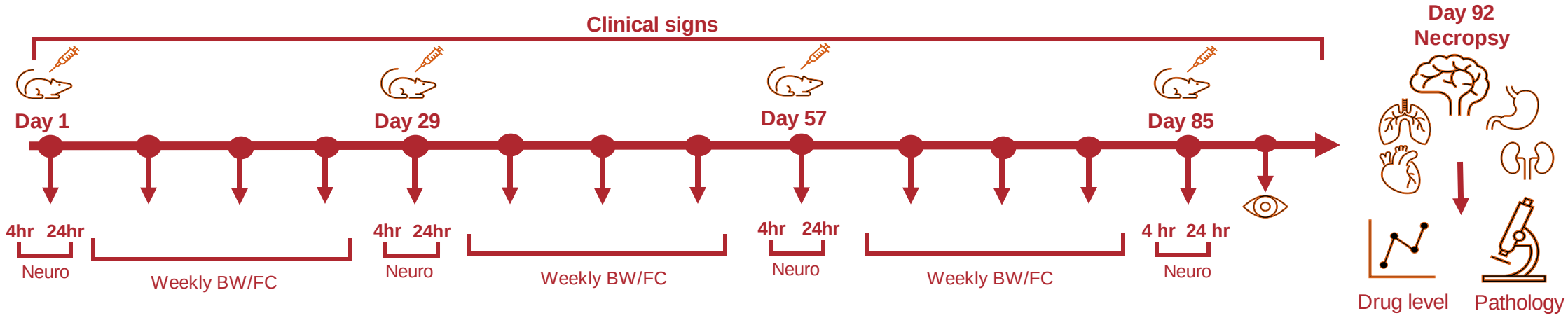
- Animals evaluated for:
  - clinical signs
  - neurobehavioral parameters
  - body weights
  - toxicity and inflammation markers in spinal cord and brain; histology as required



Extensive Screening to Identify the Best ASO

STEP 8: GLP Toxicology Study

- Study designed to identify potential toxicity of lead ASO after repeated dosing over 3 months, considered representative of chronic dosing in the clinic
- Specific design will change based on targeted organ and ASO strategy
- Stricly regulated study: all parameters are controlled to yield robust data
- Animals are injected once a month and thoroughly evaluated for a battery of parameters (general and organ-specific)



Extensive Screening to Identify the Best ASO

STEP 9: GMP Manufacturing of the Clinical ASO

- Similar to GLP toxicology, GMP manufacturing is regulated and operates by a set of rules ensuring quality and reproducibility at every step
- The lead ASO is synthesized according to strict criteria for purity, salt content, bioburden, etc to ensure adequacy for human administration
- A single batch typically yields sufficient material to treat a patient for 10 years or more
- Depending on each institution's pharmacy capabilities, ASO may require formulation into a liquid, sterile form, ready for administration following manufacturing

Importance of Rigor in Nonclinical Data

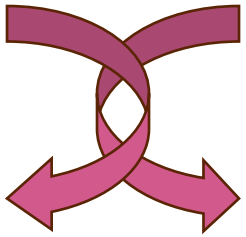
- FDA individualized guidelines outline a lean nonclinical pathway to filing
- Nonclinical data represent a significant part of the IND
- Therefore, rigor and quality are of the utmost importance at every step

In vitro and In vivo Data	In vitro screening data In silico analysis Animal Tolerability and Toxicology Studies
Manufacturing Information	Details of composition, manufacturer, stability, and controls for manufacturing the drug substance and drug product
Clinical Protocol and Investigator Information	Detailed protocol Qualifications of investigator Informed consent document

n-Lorem Discovery and Development Platform Delivers Optimized ASOs

n-Lorem has developed a scalable model for mutation-driven ASO discovery and development

Every program is tailored to the needs of each patient



In depth ASO expertise and experience

Agility and flexibility in decision making process

n-Lorem process is informed by millions of ASOs studied over decades

Integrating Decisions:
from ASO Design to Patient Treatment

- At every step, all available data are evaluated in the context of the patient
- Exact number of ASOs and criteria at each step may vary slightly from program to program depending on the mutation, target gene, ASO strategy
- ASO candidates moving to the next steps are always carefully selected
- Benefit/risk assessed in real-time as the ASO program and patient phenotype progress
- Each step is difficult, but the integration is even harder

Integrating Decisions:
from ASO Design to Patient Treatment

The most important question:
Is this ASO adequate for this patient?

THANK YOU

More importantly, our patients and families thank you!

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