Antisense Oligonucleotide (ASO) Treatment in Epilepsy

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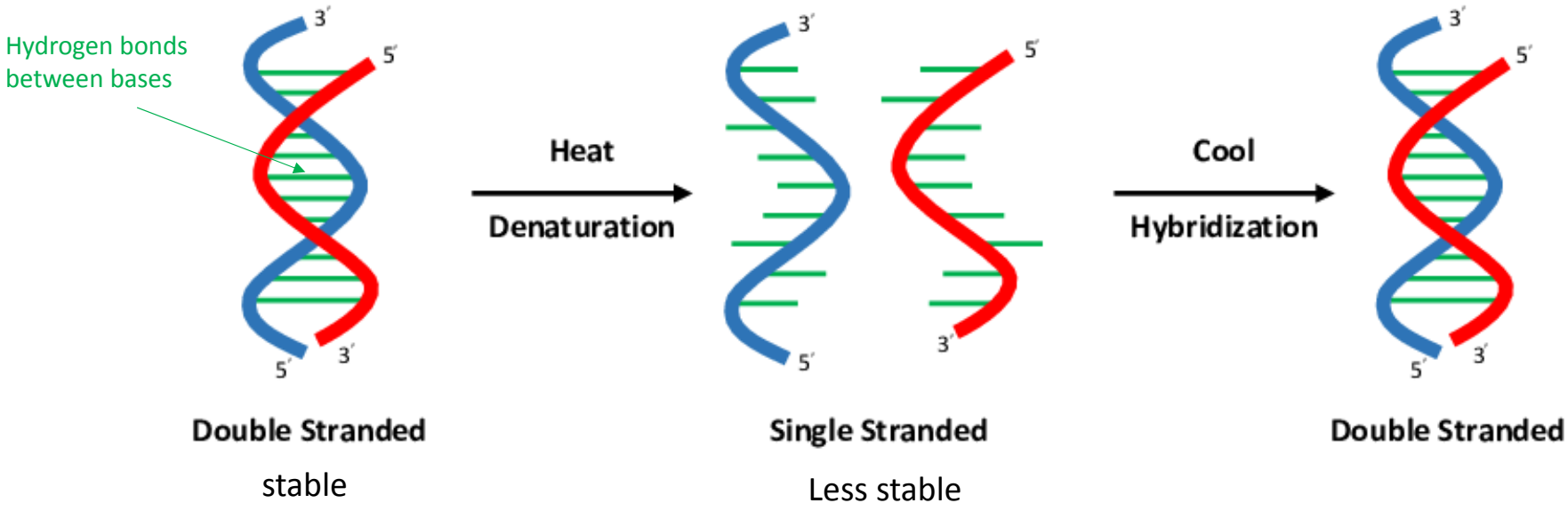
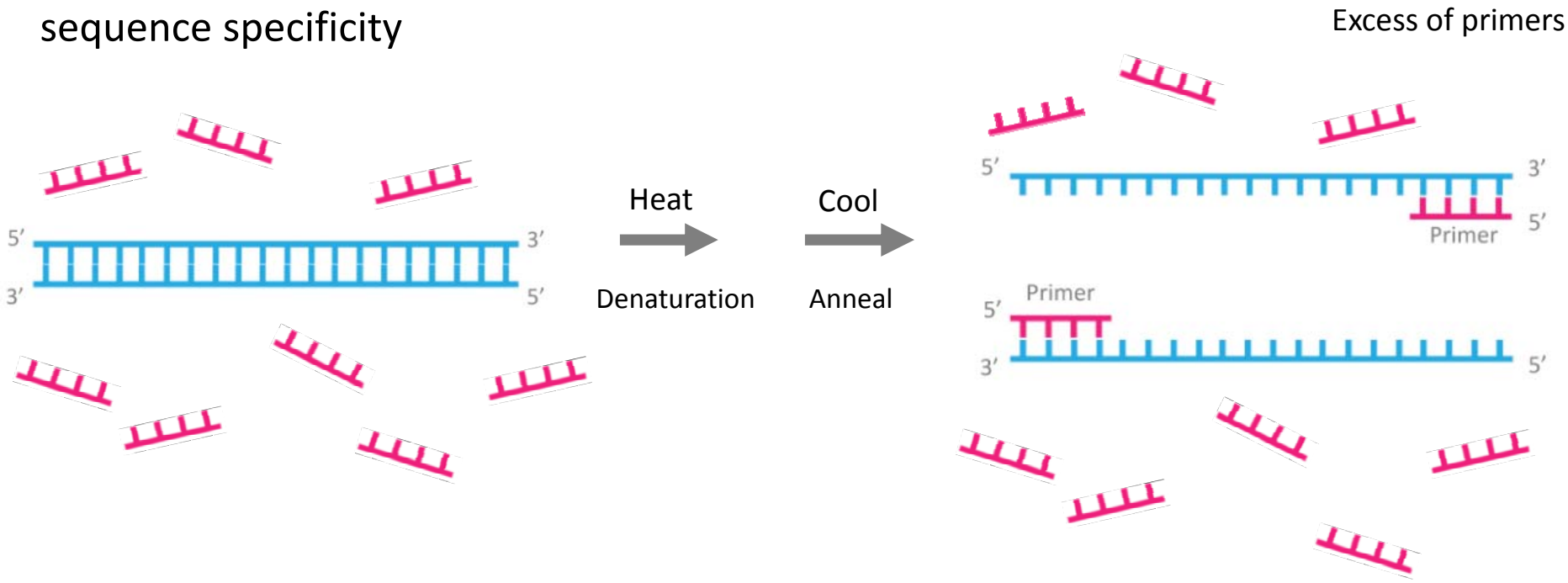
Double-stranded DNA is thermodynamically stable



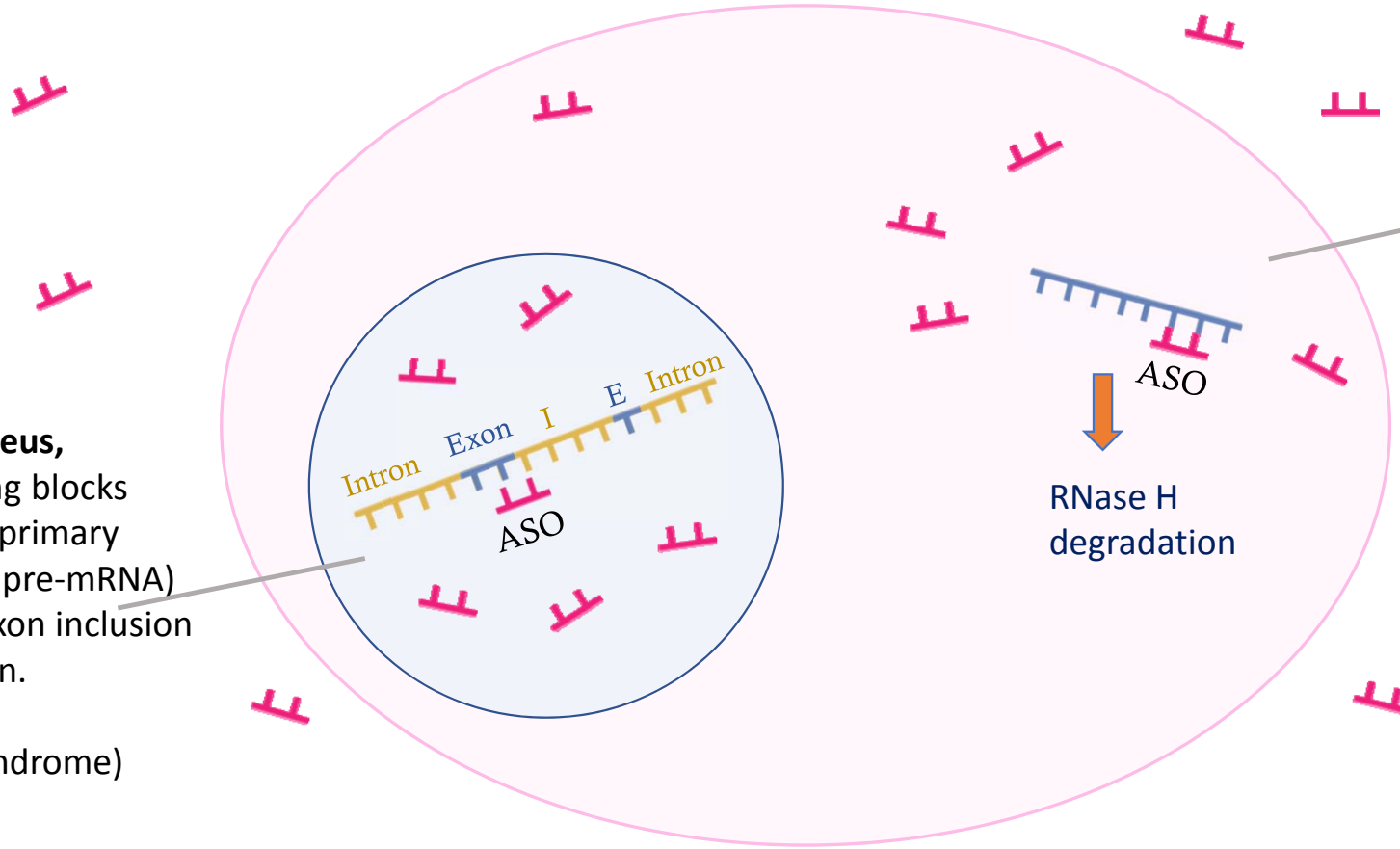
https://www.researchgate.net/figure/Figure-A1-Principles-of-DNA-denaturation-and-hybridization_fig5_279969077

Even short oligos (~20 bp) can form stable double-stranded hybrids

Example: PCR primers;
sequence specificity



Antisense oligos (ASOs) are taken up by neurons from the CSF and hybridize with single-stranded RNA in the nucleus or cytoplasm



In the nucleus,
ASO binding blocks splicing of primary transcript (pre-mRNA) to direct exon inclusion or exclusion.

(Dravet Syndrome)

In the cytoplasm,
ASO binding to mature mRNA results in degradation of RNA/DNA hybrid by RNaseH.

(*SCN8A encephalopathy*)

Need for an “ASO-walk” to identify effective ASOs

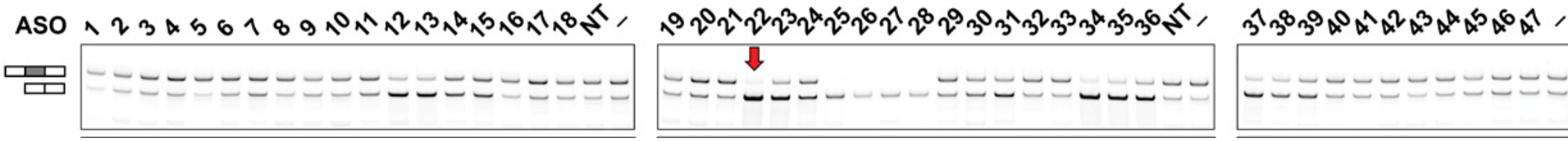
It is necessary to empirically test many ASOs to identify accessible targets.

The single-stranded RNA targets in cells adopt secondary structures (stem-loops, ds regions) that prevent ASO binding

Intronic targets in a pre-mRNA may be excised early during splicing.

(There is no “heating step” to denature the target.)

In this example, ASO # 22 was most effective in targeting the grey exon.



Han et al, 2020

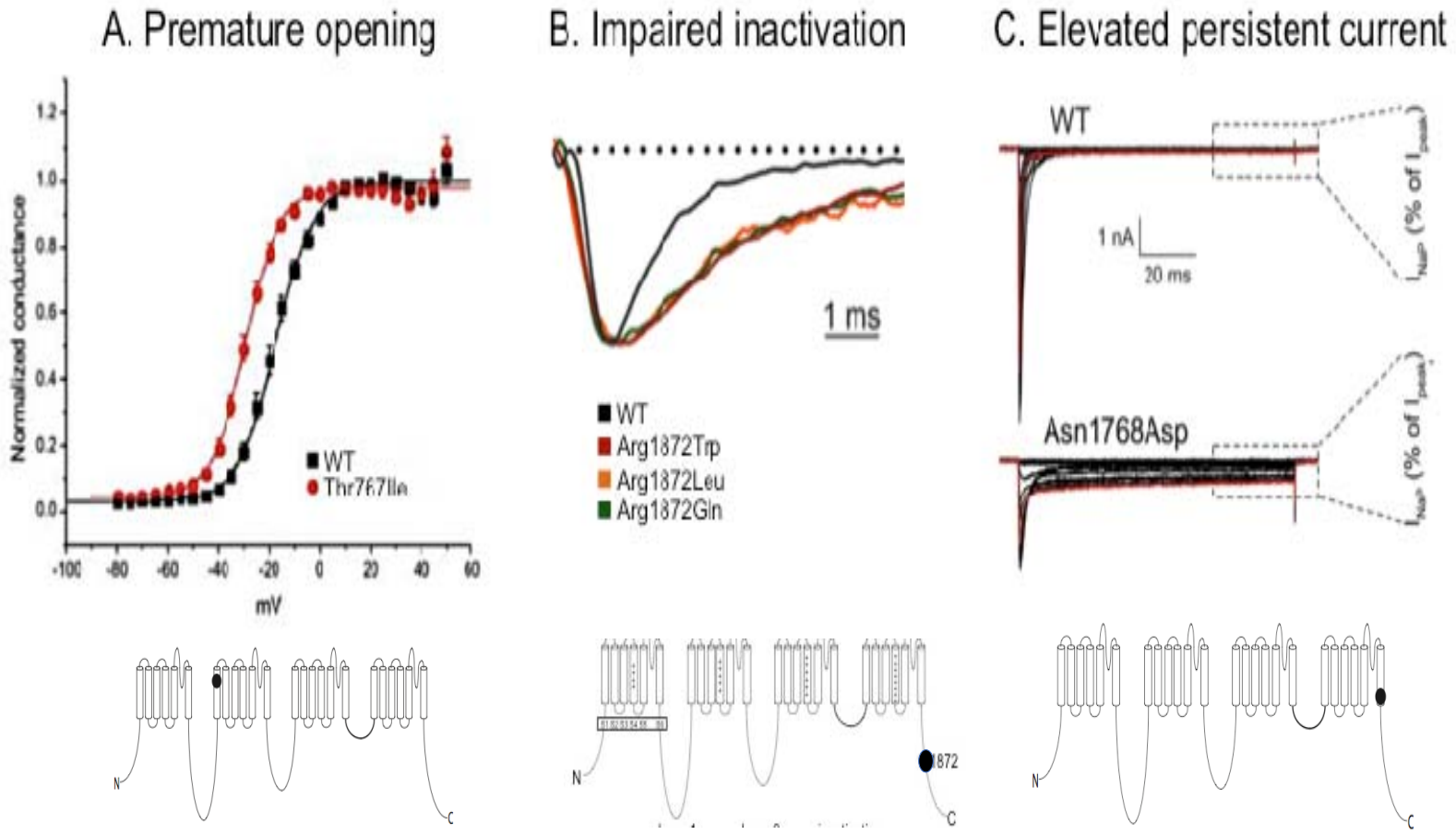
Therapy I. **Decreasing** gene expression with an ASO:

SCN8A developmental and epileptic encephalopathy (DEE)

SCN8A encodes $Na_v1.6$, a major sodium channel in excitatory neurons in the CNS
- early onset (average 4 months)
- multiple seizure types
- cognitive impairment, developmental delay
- 50% nonambulatory

Predominant Molecular Mechanism:
- *de novo* mutations
- missense (amino acid substitutions)
- GOF: gain of function: protein present with altered biophysical properties

Mechanisms of gain-of-function mutations of *SCN8A*



T767I R1872W N1768D

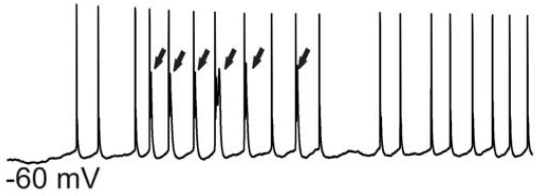
Meisler et al, Epilepsia 2016

Neuronal hyperexcitability: GOF mutations of SCN8A result in spontaneous firing in mice models of patient mutations

Wildtype +/+

N1768D

CA1



hippocampus

CA3

(10 sec slice recordings)

Lopez-Santiago, Yuan et al PNAS 2017

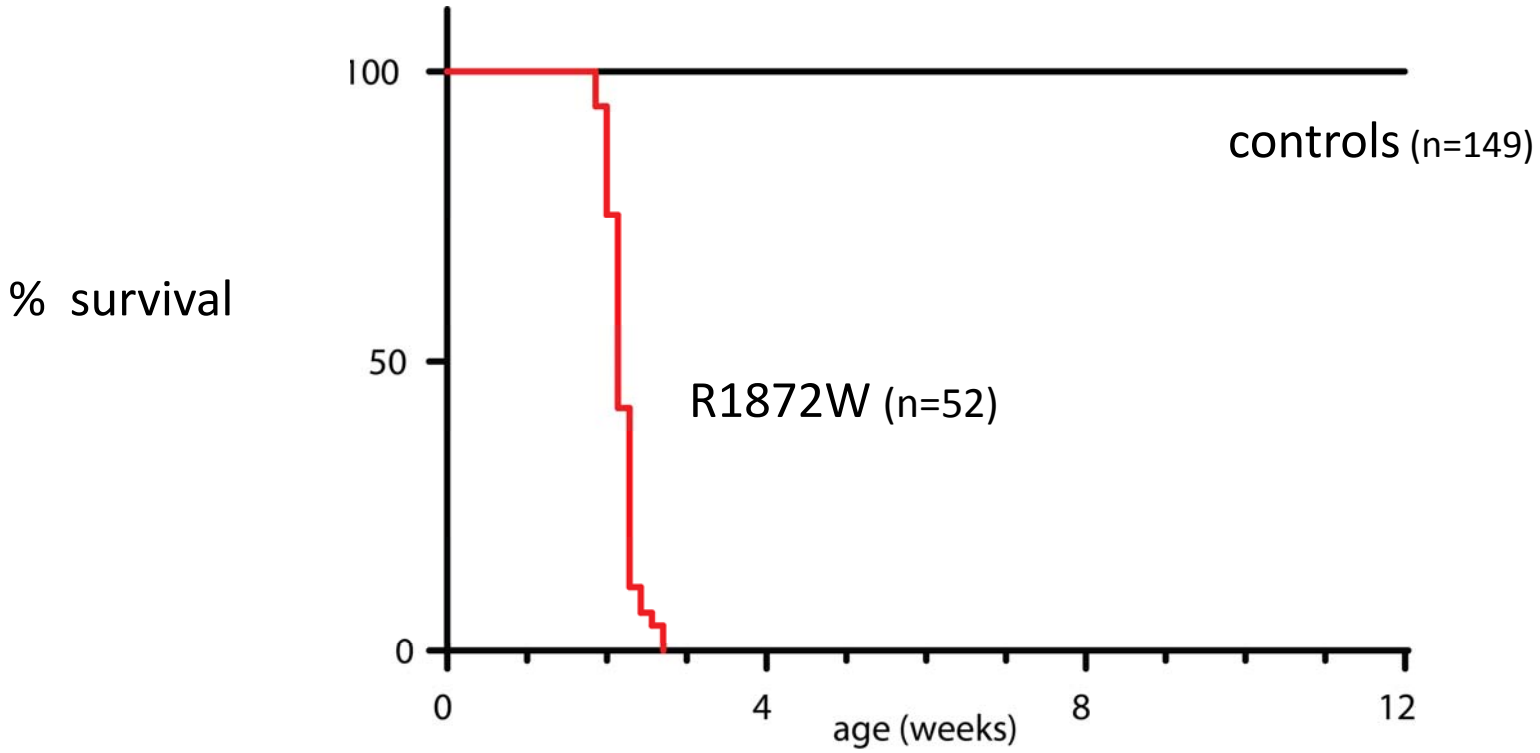
Scn8a Antisense Oligonucleotide Is Protective in Mouse Models of *SCN8A* Encephalopathy and Dravet Syndrome

Guy M. Lenk, PhD,[1] Paymaan Jafar-Nejad, MD,[2] Sophie F. Hill, BS,[1,3] Lucas D. Huffman, MS,[3,4] Corrine E. Smolen, MS,[1] Jacy L. Wagnon, PhD,[1] Hayley Petit, BS,[1] Wenxi Yu, PhD,[1] Julie Ziobro, MD,[5] Kritika Bhatia, BS,[5] Jack Parent, MD,[5] Roman J. Giger, PhD,[3,4,5] Frank Rigo, PhD,[2] and Miriam H. Meisler, PhD[1,3,5]

University of Michigan and IONIS Pharmaceuticals

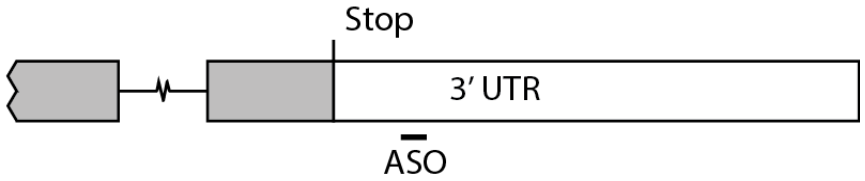
ANNALS of NEUROLOGY FEB 2020

Early onset epilepsy and sudden death in mice expressing *Scn8a*-R1872W



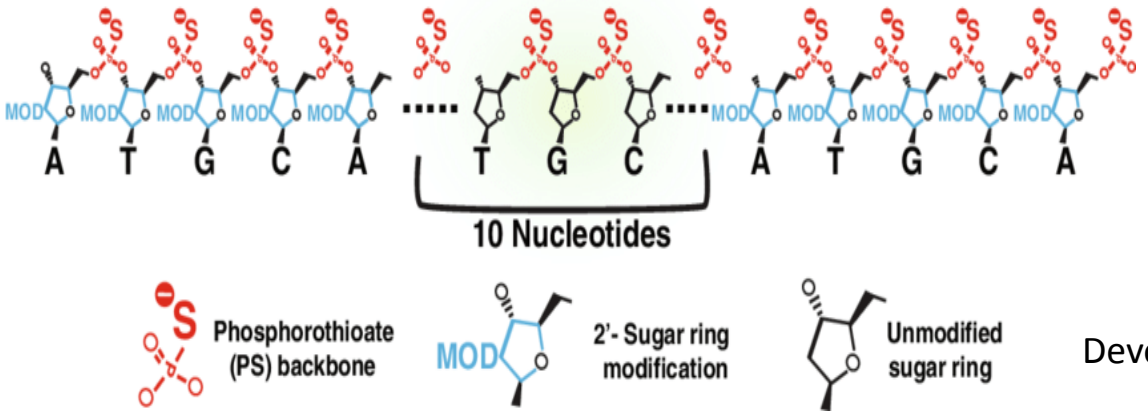
Bunton-Stashyshn et al, Brain 2019

Can we treat *SCN8A* encephalopathy by reducing expression of $Na_v1.6$ using an ASO to activate mRNA degradation?



5′-GACGA TTAGT GACAT AGGCT - 3′ **20mer**

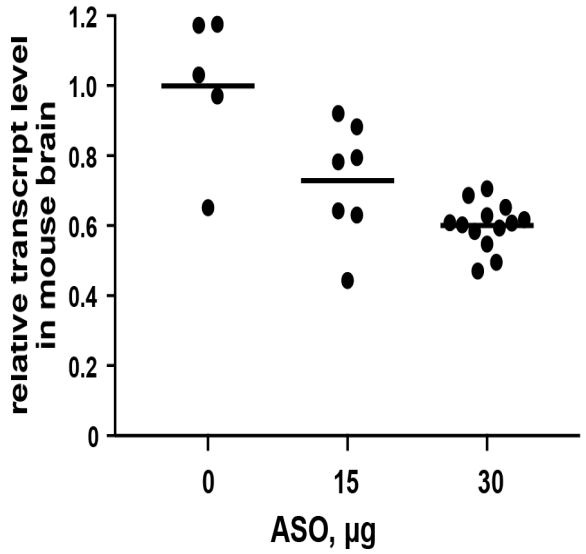
Chemical modification for *in vivo* stability: "Gapmer"



Devos & Miller (2013)

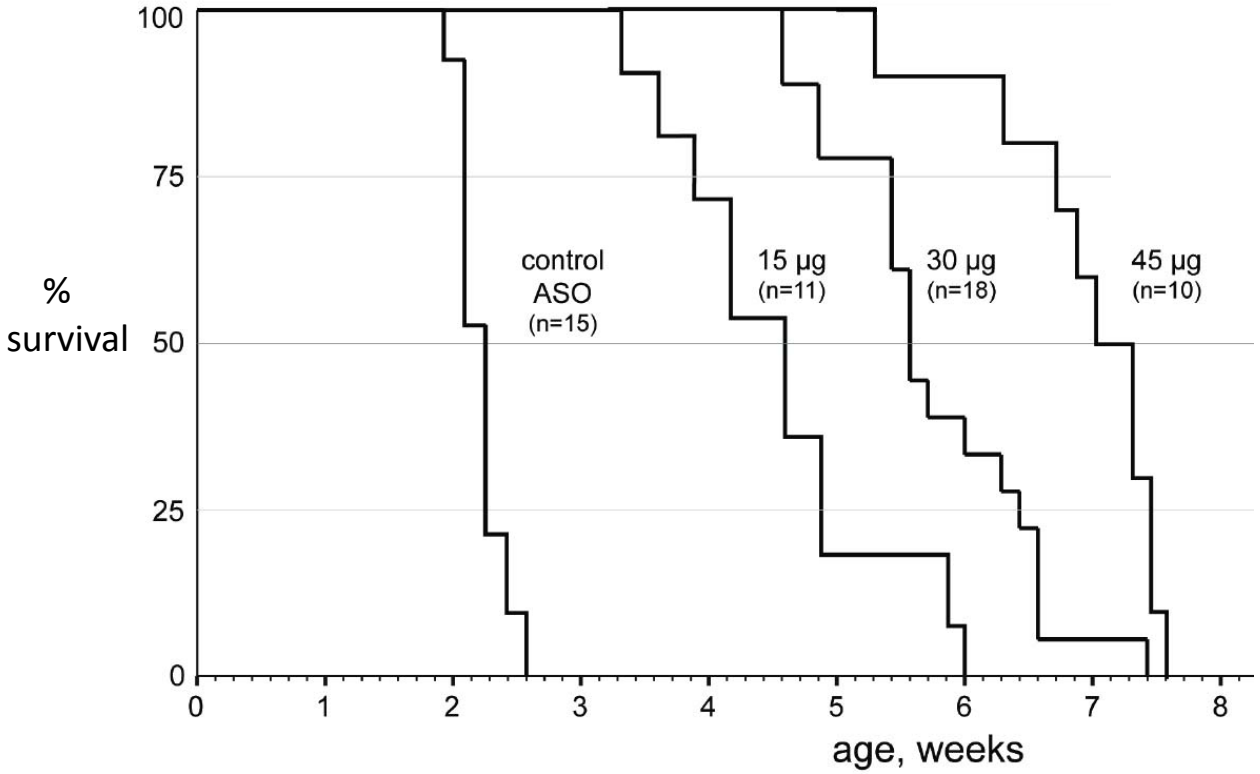
ASO reduces *SCN8A* transcript in brain of WT mice

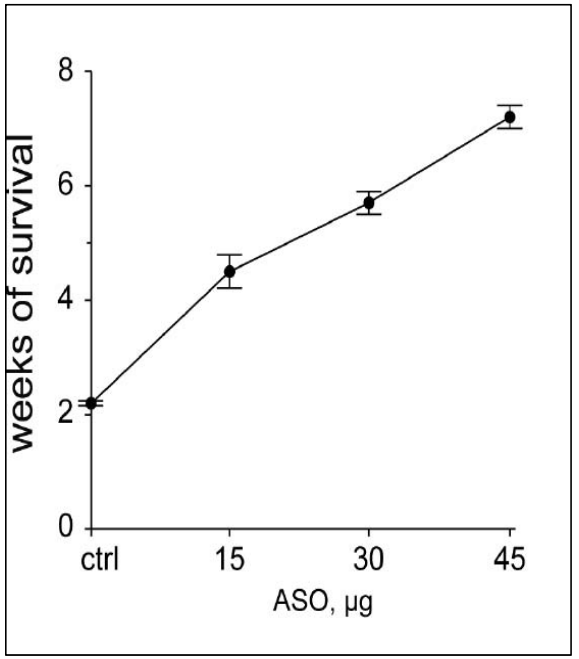
ASO administered by **intracerebroventricular injection at postnatal day 2**



Lenk et al, Ann Neurol 2020

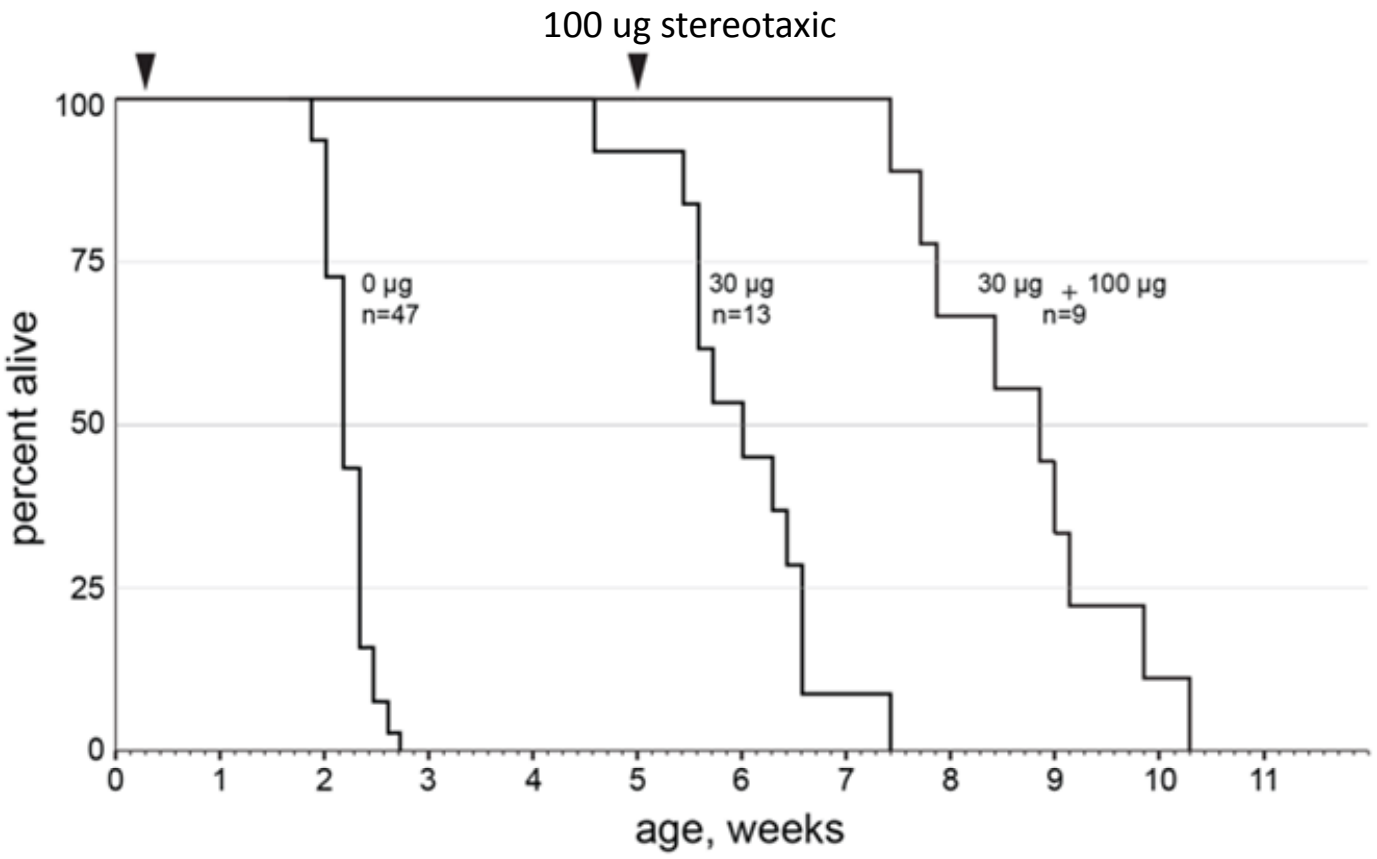
Scn8a-ASO treatment at P2 extends survival of R1872W mice





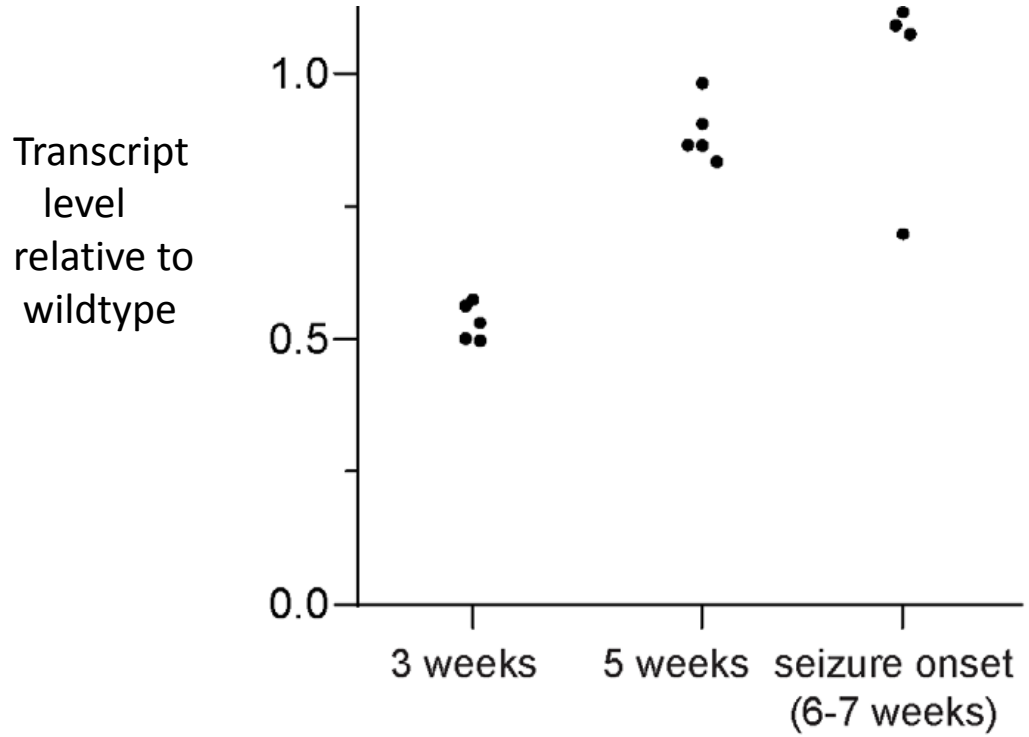
Lenk et al, Ann Neurol 2020

Repeat administration of ASO further extends seizure-free survival, from 2w to 9w



Lenk et al, Ann Neurol 2020

Seizures begin after mRNA returns to wildtype level



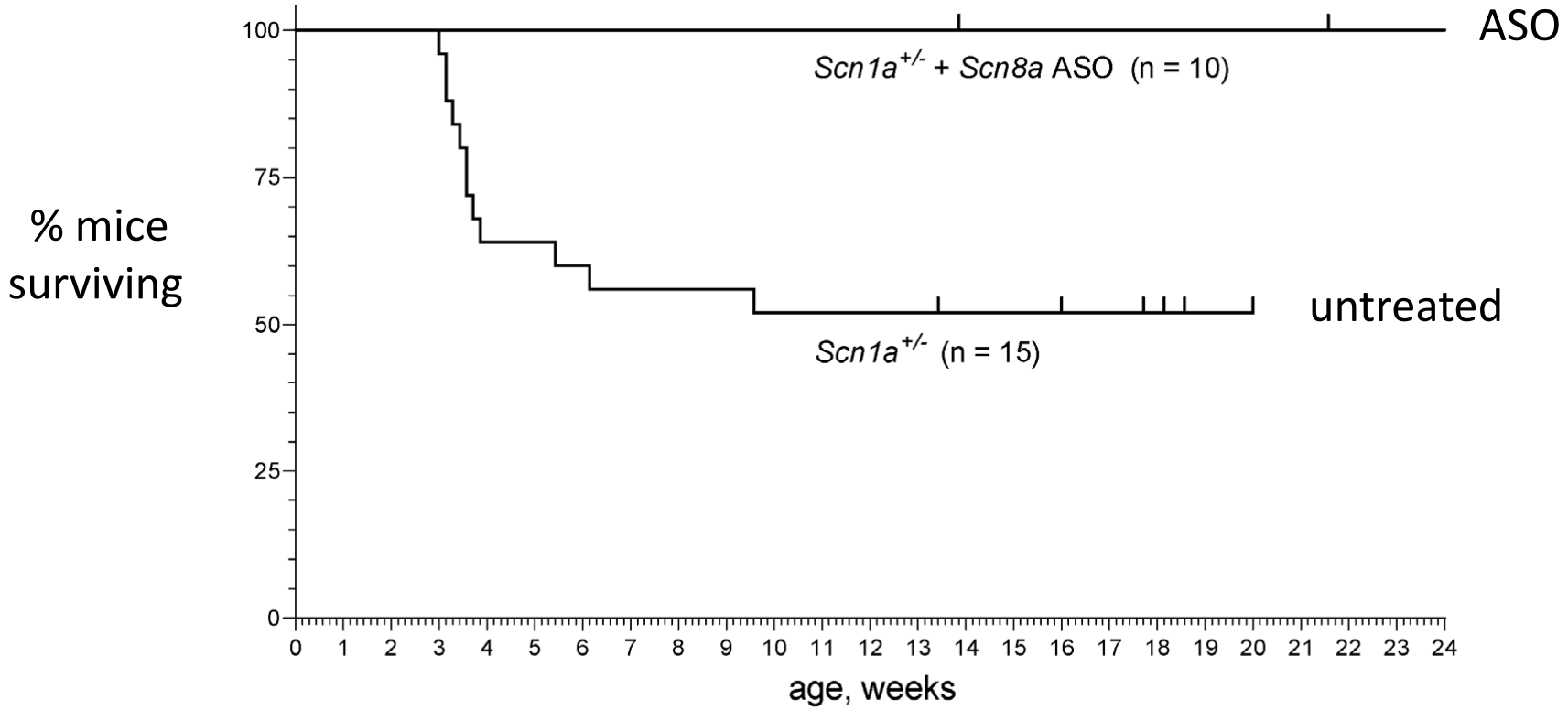
Lenk et al, Ann Neurol 2020

Could reduction of *SCN8A* expression be therapeutic

for other types of genetic epilepsy,

by reducing neuronal excitability regardless of cause?

ASO to *SCN8A* rescues Dravet Syndrome mice (*SCN1A*$^{+/-}$)



Lenk et al, Ann Neurol 2020

Summary 1

SCN8A ASO to 3' UTR reduces mRNA *in vivo*

Reduced *SCN8A* expression rescues seizures
in mouse model of *SCN8A* DEE
and in mouse model of Dravet mice

Therapy II: **Increasing** gene expression with an ASO

Haploinsufficient *SCN1A*$^{+/-}$ in Dravet Syndrome

SCN1A encodes $Na_V1.1$, a major CNS sodium channel in inhibitory neurons
- onset during first year of life
- febrile seizures, multiple seizure types
- cognitive impairment, developmental delay
- less severe than *SCN8A* encephalopathy

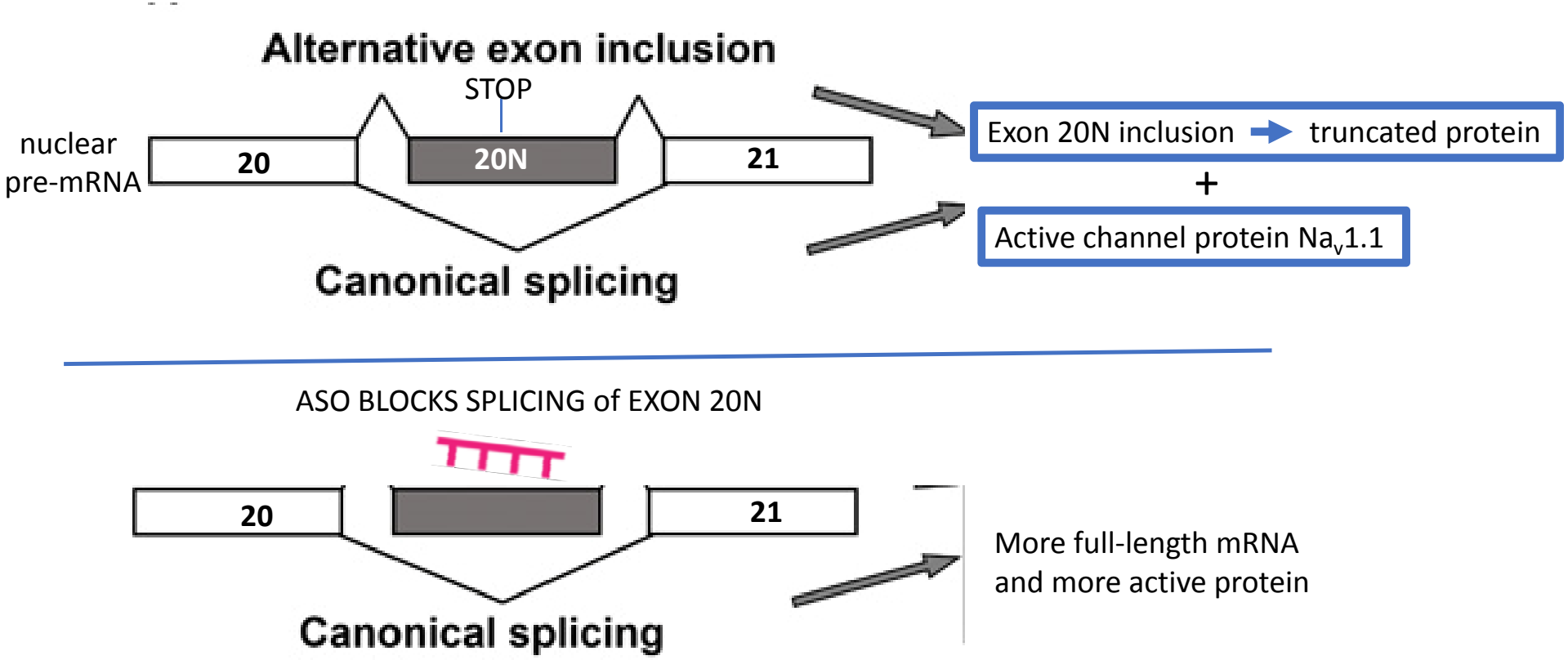
Predominant molecular mechanism:
- *de novo* mutations
- LOF: Loss of function: 50% protein truncation, 50% missense

ASO treatment of Dravet Syndrome

- *SCN1A* contains a highly expressed, alternatively spliced ‘poison exon’ (exon 20N) that introduces an in-frame stop codon in the mRNA resulting in protein truncation and loss-of-function. (significant % of mRNA)

- Blocking inclusion of the poison exon with an ASO to the pre-mRNA increases the amount of correctly-spliced mRNA and rescues haploinsufficient mice

ASO BLOCKS splicing of "Poison exon" 20N in *SCN1A*



Carvill et al, Am. J. Hum. Genet. 2018

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE AUG 2020

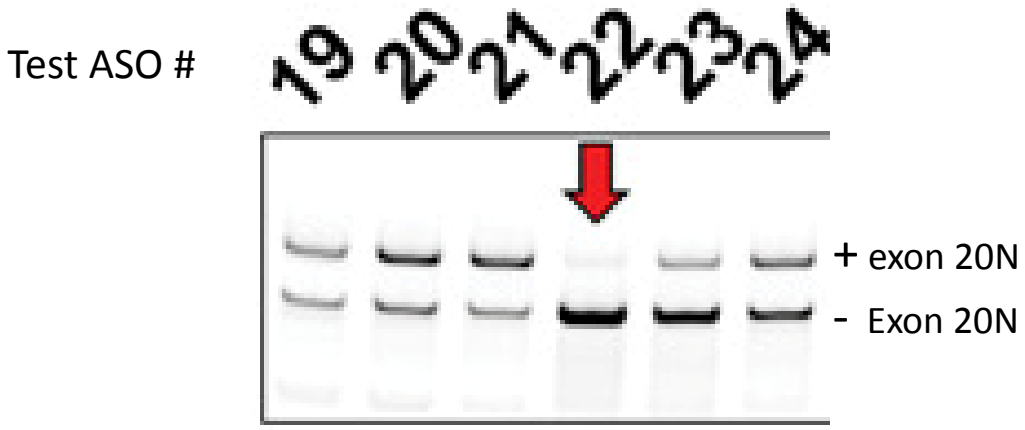
EPILEPSY

Antisense oligonucleotides increase *Scn1a* expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome

Zhou Han[1], Chunling Chen[2], Anne Christiansen[1], Sophina Ji[1], Qian Lin[1], Charles Anumonwo[2], Chante Liu[2], Steven C. Leiser[3], Meena[1], Isabel Aznarez[1], Gene Liau[1], Lori L. Isom[2]*

University of Michigan and STOKE Therapeutics

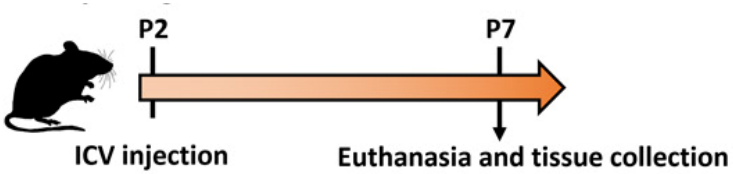
RT-PCR of RNA from ReN cells treated with test ASOs;
assay result with RTPCR primers in exon 20 and exon 21

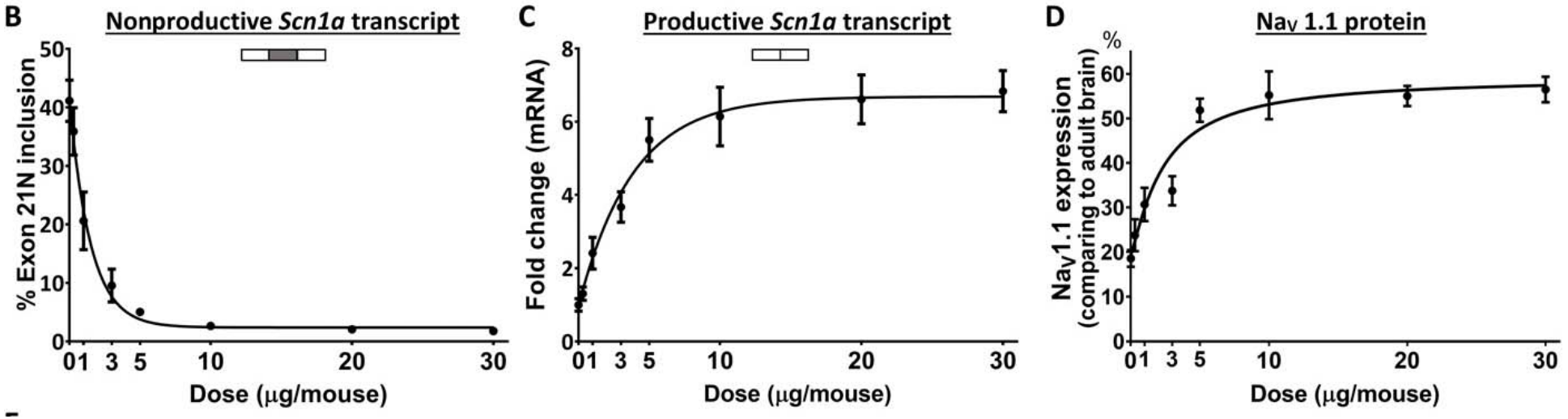


ASO # 22 blocks inclusion of exon 21N from the Scn1a mRNA

Han et al., Sci Transl Med 2020

In vivo administration of ASO increases Nav1.1 expression in mouse brain

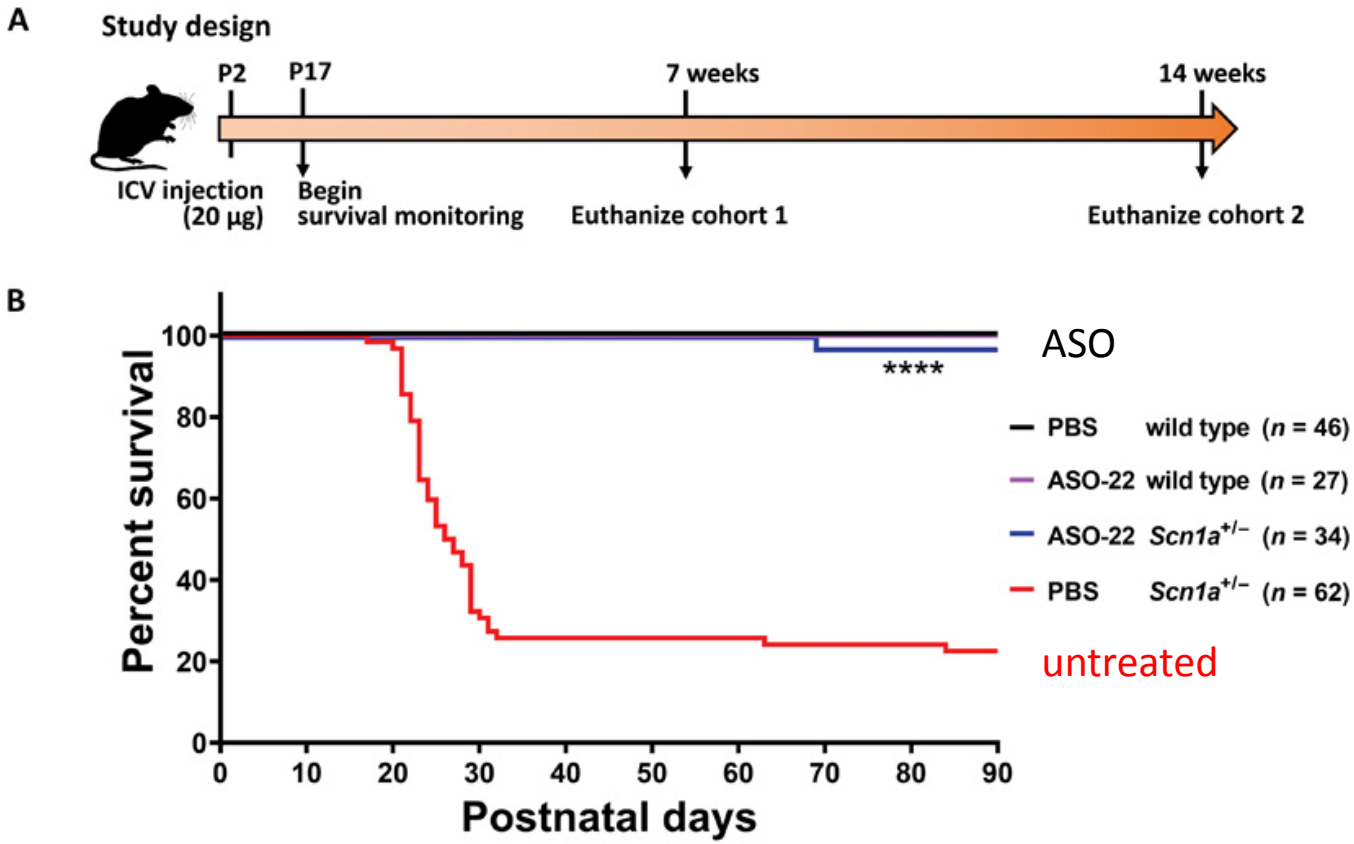




Han et al., Sci Transl Med 2020

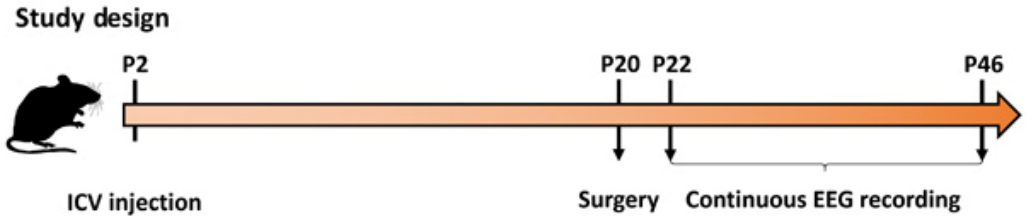
Dose dependence

ASO to Scn1a-exon 20N rescues survival of Dravet Syndrome Mice

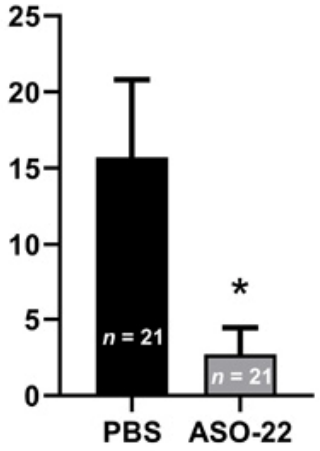


Han et al., Sci Transl Med 2020

ASO to Scn1a-exon 20N reduces seizures in Dravet Syndrome Mice



Total # seizures
between P22 and P46



Han et al., Sci Transl Med 2020

Summary: Therapeutic potential of ASOs for genetic epilepsies

Positive features

- Specificity is confered by the sequence of the ASO
- ASOs are inexpensive to produce
- Broad applicability to GOF and LOF mutations

but....

- Intra-thecal administration; ASOs do not cross blood-brain barrier
- Limited *in vivo* stability: repeat after 3 to 6 months
- Important to know the mechanism of the patient mutation.
  (e.g some *SCN8A* mutations result in LOF, some Dravet is GOF

Meisler Laboratory

current

Guy Lenk
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Wenxi Yu
Young Park
Xu Cao
Aparna Sumanth
Pooja Varanasi
Sydney Musser

recent

Jacy Wagnon
Rosie Bunton-Stasyshyn
Corrine Smolen
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Yukun Yuan
Chad Frasier

Jack Parent
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Roman Giger
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