

Antisense-Mediated Correction of Aberrant Splicing in a Model Genetic Disease of Children

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Spinal Muscular Atrophy (SMA)

Autosomal recessive disease of children and infants

A disease of lower motor neuron degeneration

Second leading genetic cause of infant mortality



Type I
12 years



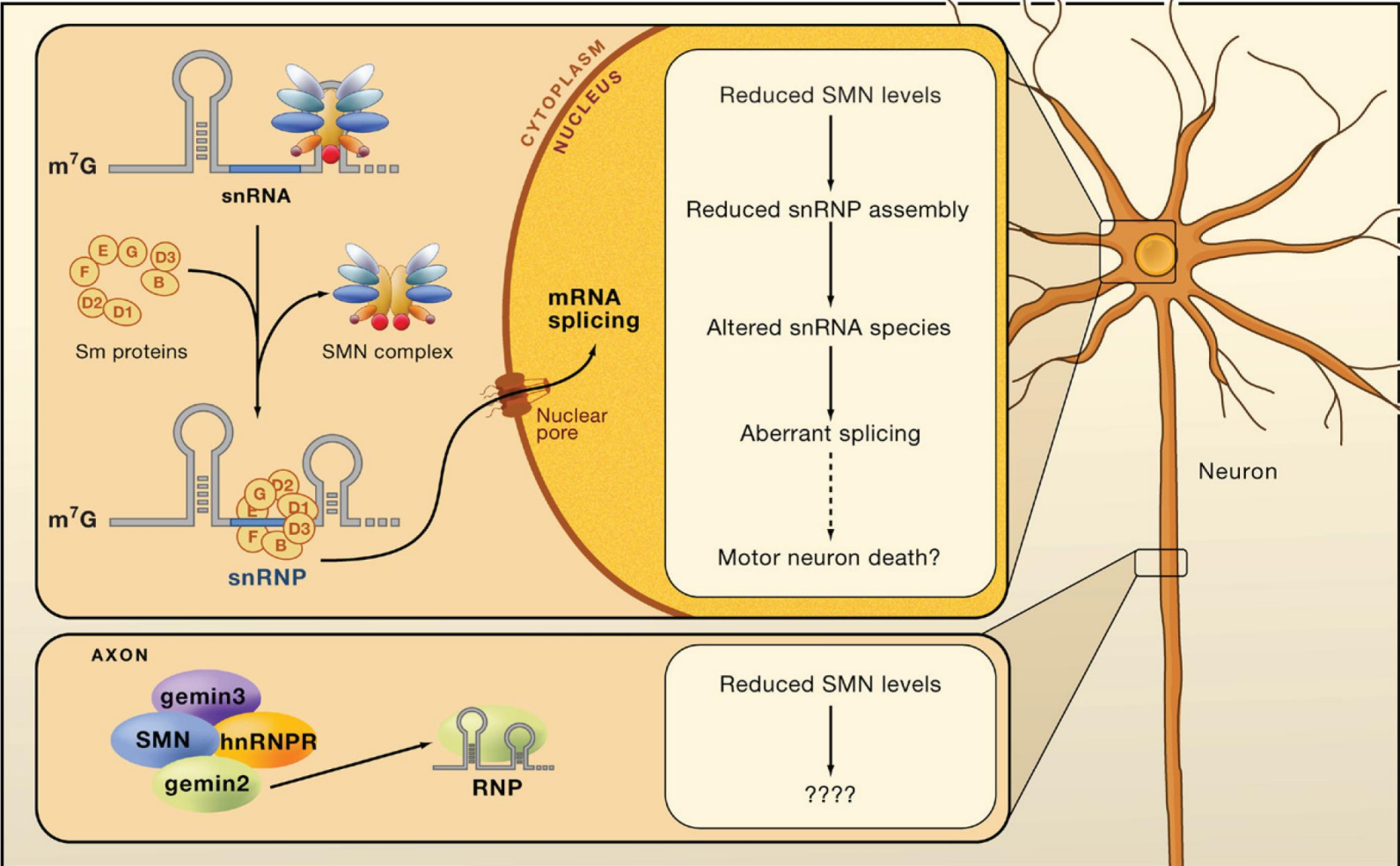
Type II
7 Years



Type III
3 Years

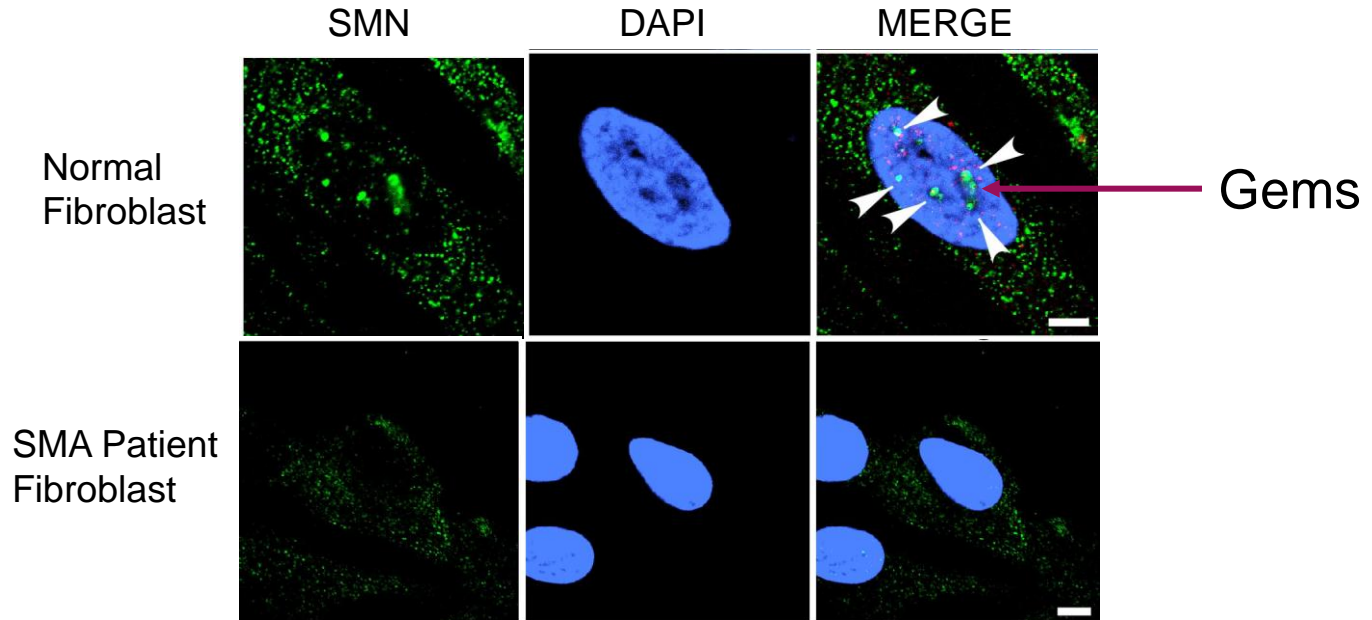
Courtesy: Families of SMA (FSMA.ORG)

SMA is a disease of low levels of Survival Motor Neuron (SMN)



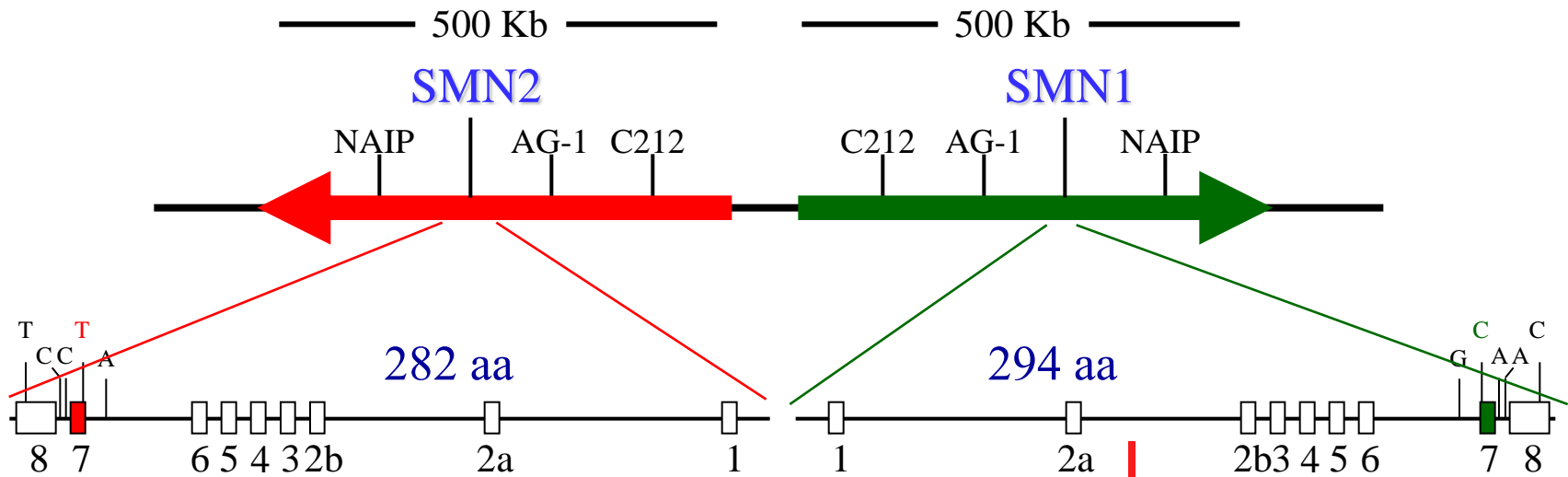
Hallmarks of disease

Low levels of SMN bodies (Gems) in nucleus of SMA fibroblasts



Why do SMA patients have low levels of SMN?

Two *SMN* Genes as inverted repeats on 5q11-q13



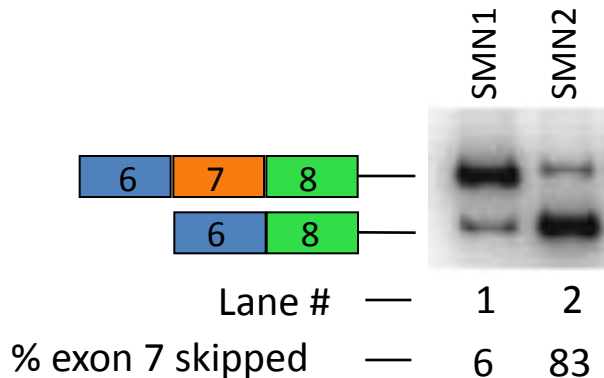
All types of SMA
carry copies of *SMN2*

Δ SMN1

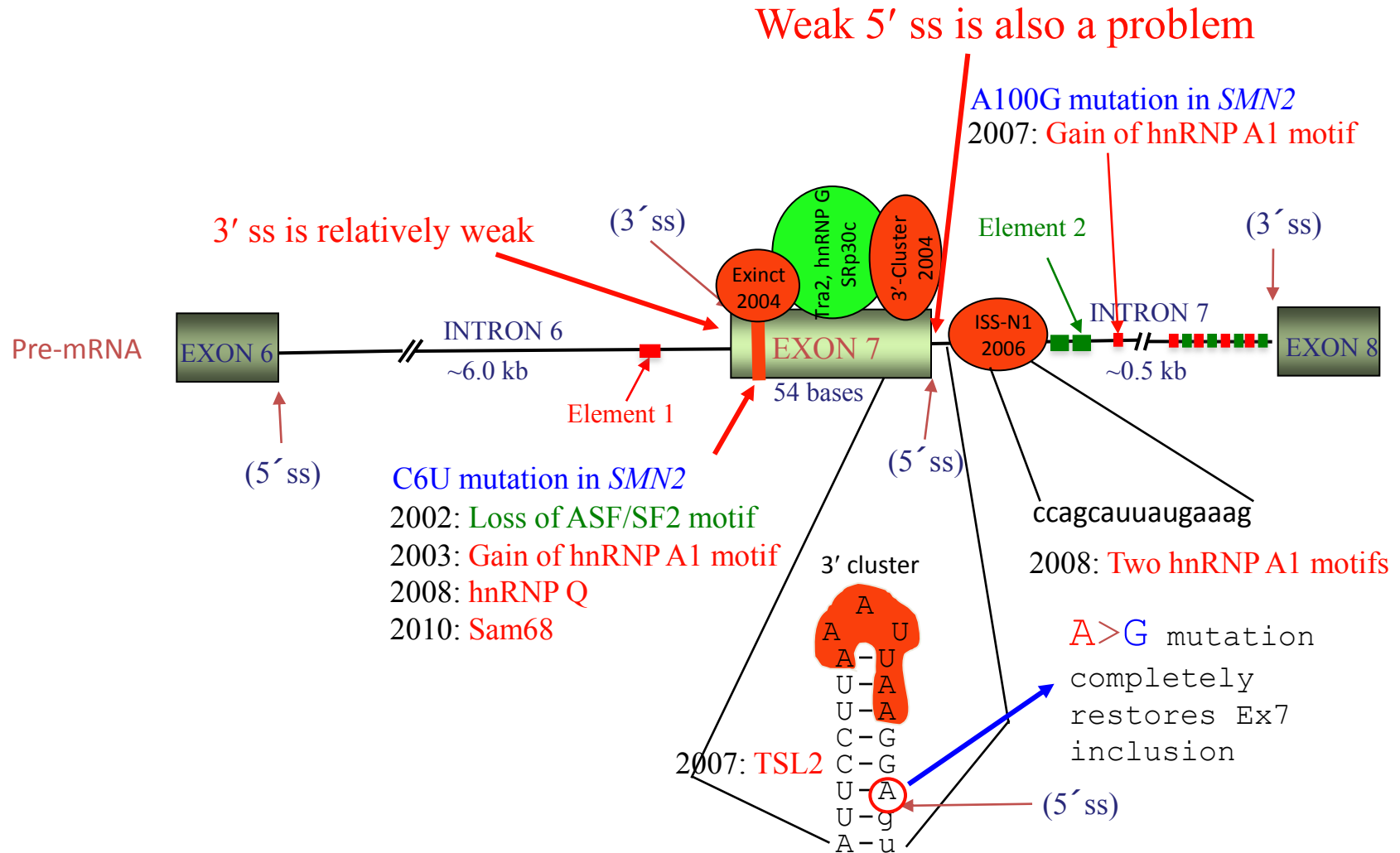
Spinal Muscular Atrophy (SMA)

Affects 1 in ~6,000 babies
~1 in 40 people are carriers

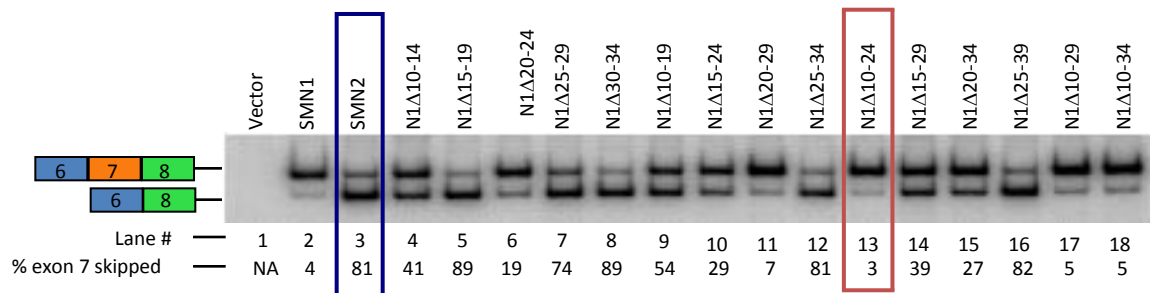
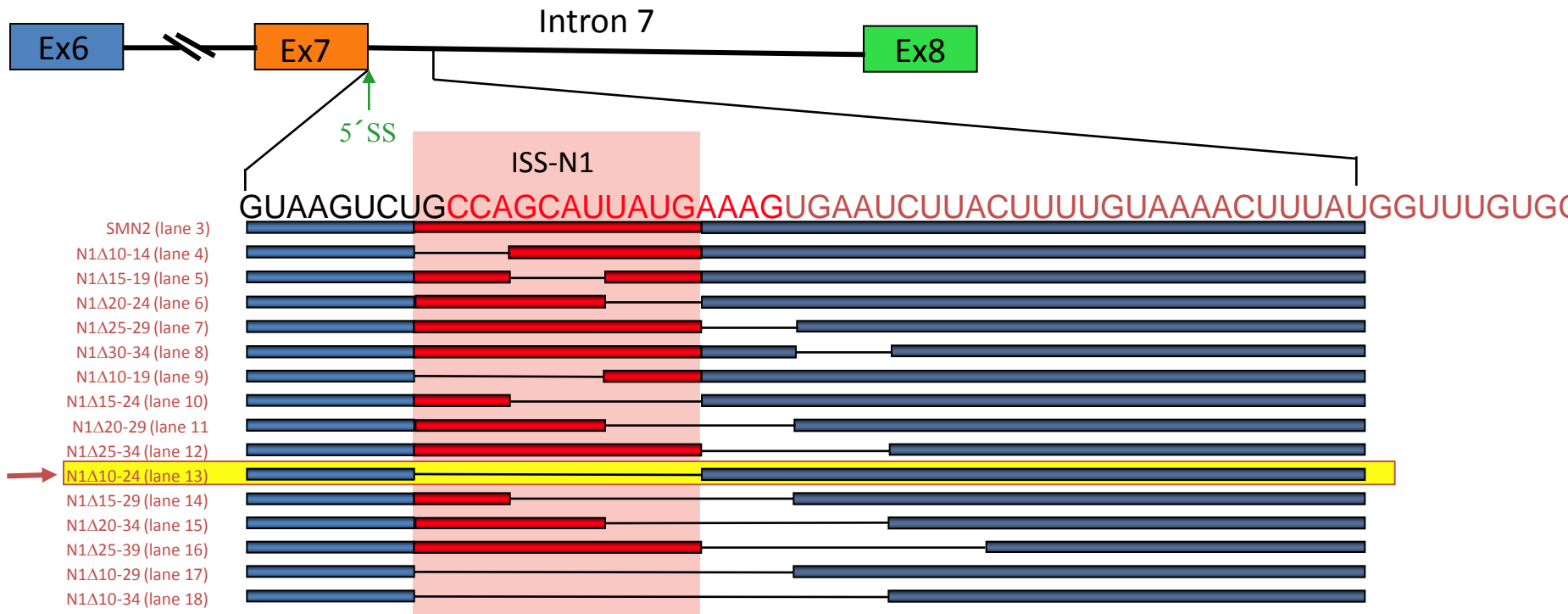
Three types of SMA
Type I, II and III



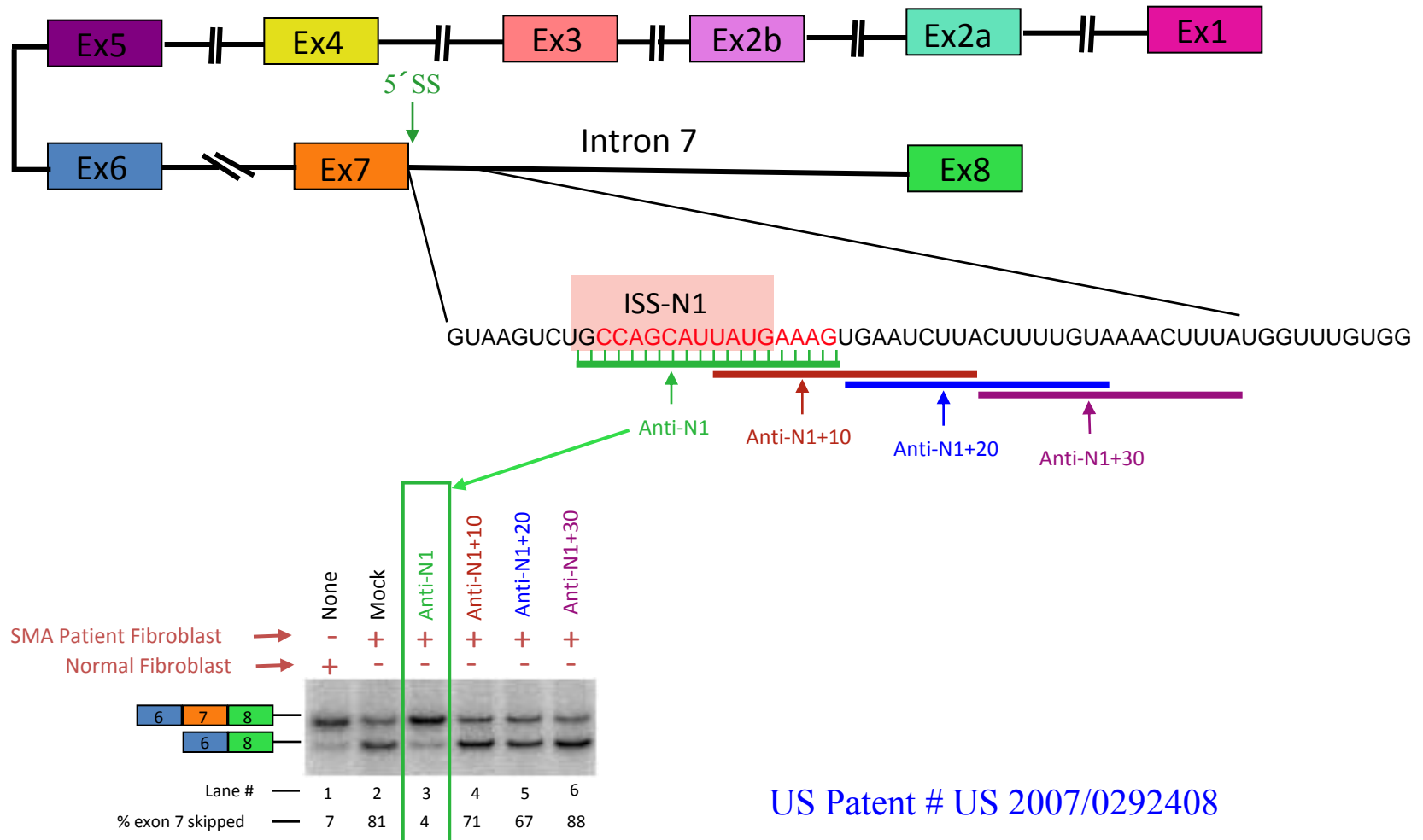
What causes skipping of SMN2 exon 7 ?



ISS-N1: A novel intronic splicing silencer: deletion of ISS-N1 corrects splicing



Effect of ISS-N1-targeting ASOs on *SMN2* splicing in SMA Type I patient cells (GM cells)



US Patent # US 2007/0292408

Singh et al (2006) Mol. Cell. Biol. 26, 1333-1346

Antisense Masking of an hnRNP A1/A2 Intronic Splicing Silencer Corrects *SMN2* Splicing in Transgenic Mice

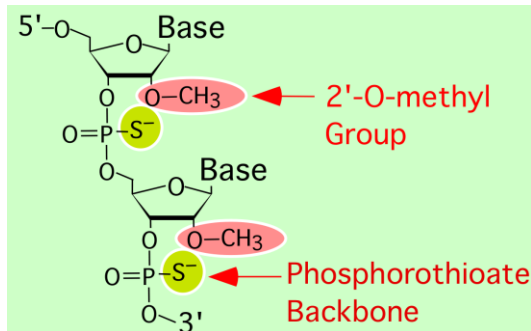
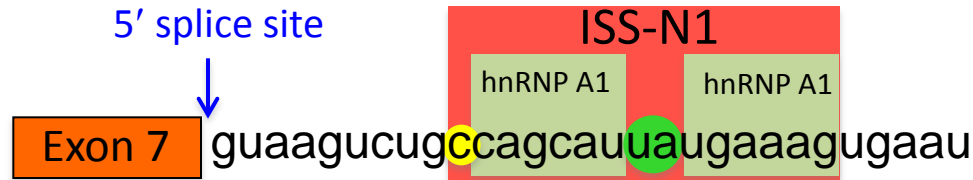
Yimin Hua,¹ Timothy A. Vickers,² Hazeem L. Okunola,¹ C. Frank Bennett,² and Adrian R. Krainer^{1,*}

¹Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, NY 11724, USA; ²Isis Pharmaceuticals, 1896 Rutherford Road, Carlsbad, CA 92008, USA

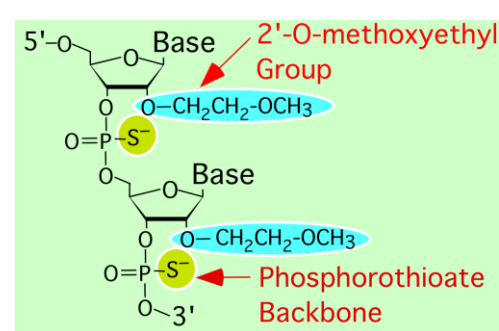
*Correspondence: krainer@cshl.edu

DOI 10.1016/j.ajhg.2008.01.014. ©2008 by The American Society of Human Genetics. All rights reserved.

834 The American Journal of Human Genetics 82, 834–848, April 2008



Singh et al., 2006



Hua et al., 2008/ ISIS Pharmaceuticals

In vivo experiments on ISS-N1 as a target

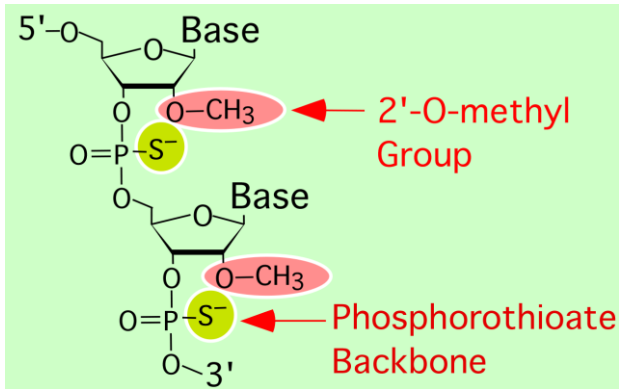
Oligonucleotide-Mediated Survival of Motor Neuron Protein Expression in CNS Improves Phenotype in a Mouse Model of Spinal Muscular Atrophy

Jason H. Williams, Rebecca C. Schray, Carlyn A. Patterson, Semira O. Ayitey, Melanie K. Tallent, and Gordon J. Lutz
Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102

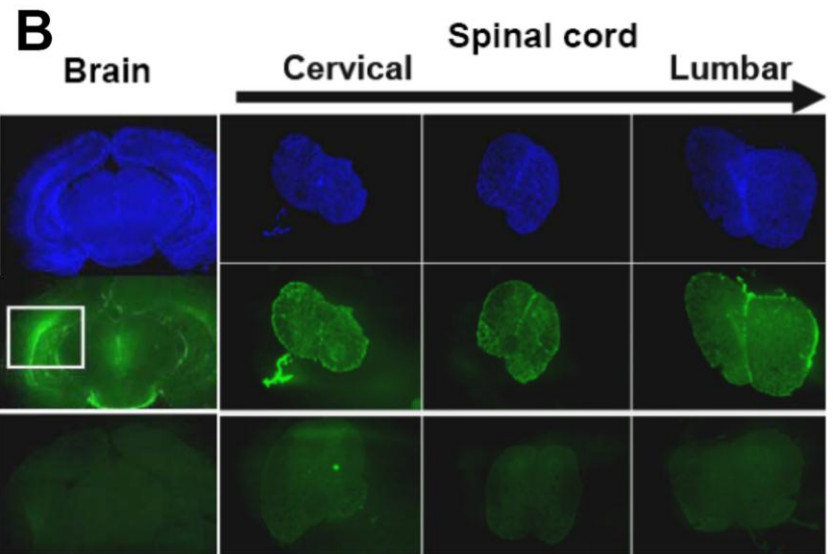
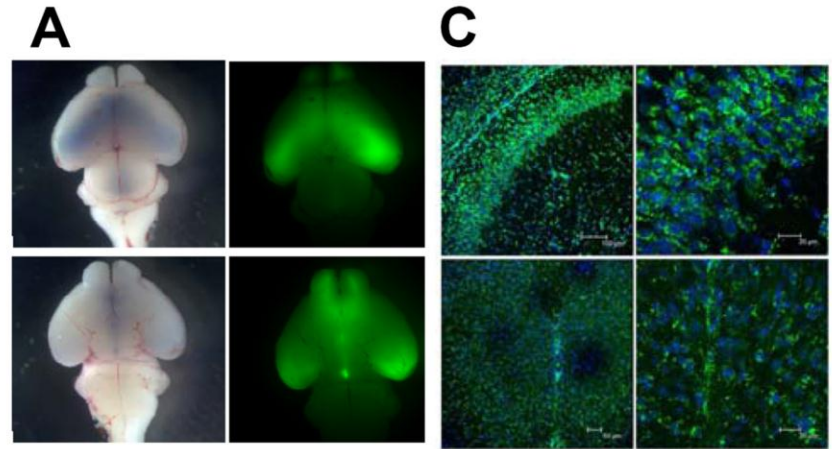
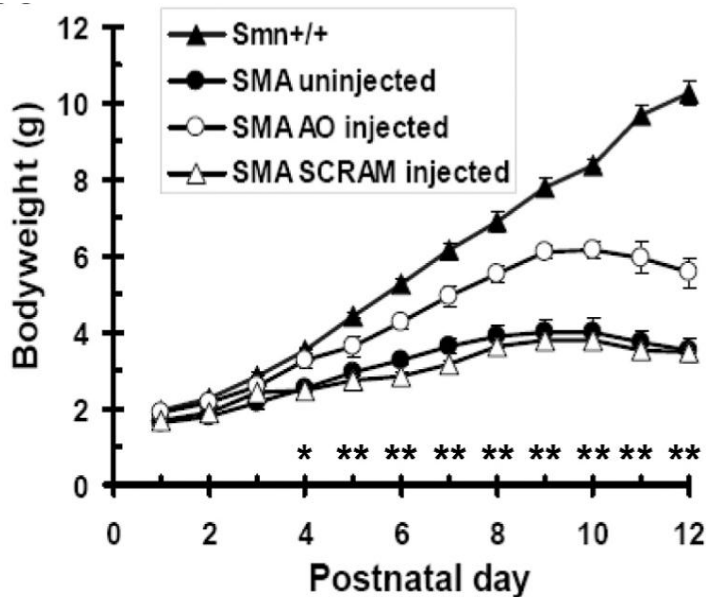
The Journal of Neuroscience, June 17, 2009 • 29(24):7633–7638 • **7633**

In vivo experiments on ISS-N1 as a target

ICV delivery of antisense oligonucleotides



Singh et al (2006) Mol. Cell. Biol. 26, 1333



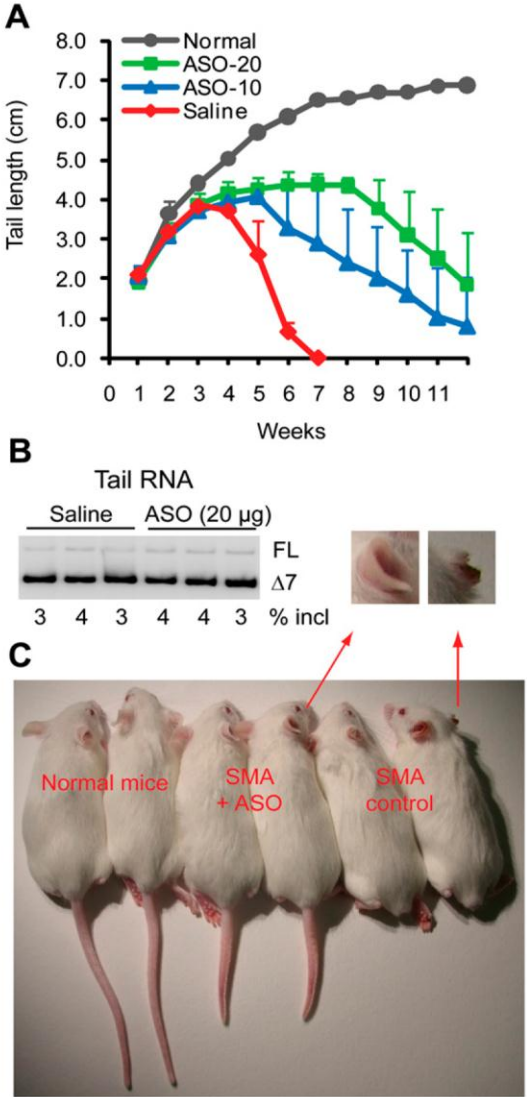
In vivo experiments on ISS-N1 as a target

Antisense correction of SMN2 splicing in the CNS rescues necrosis in a type III SMA mouse model

Yimin Hua,¹ Kentaro Sahashi,¹ Gene Hung,² Frank Rigo,² Marco A. Passini,³ C. Frank Bennett,² and Adrian R. Krainer^{1,4}

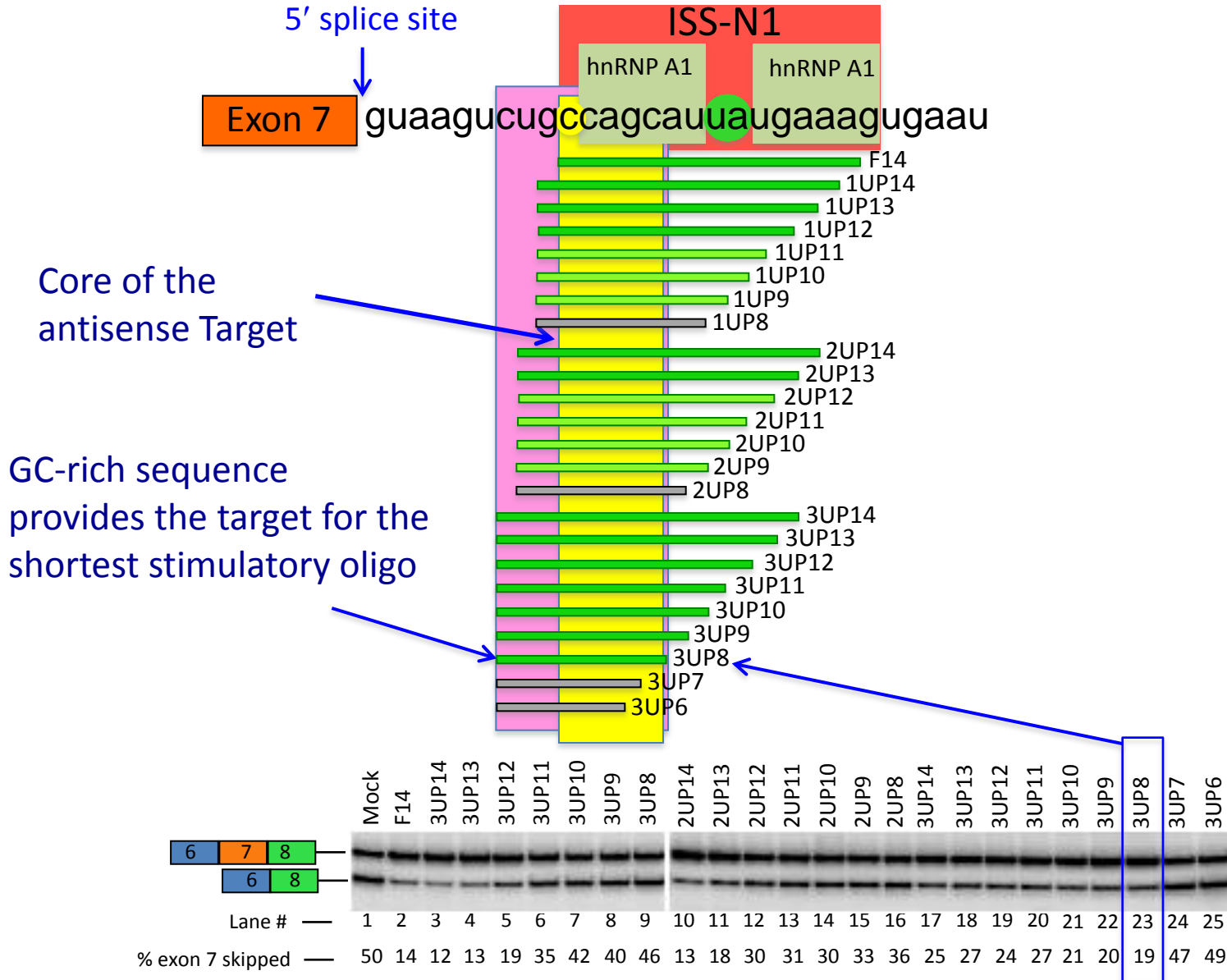
¹Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA; ²Isis Pharmaceuticals, Carlsbad, California 92008, USA; ³Genzyme Corporation, Framingham, Massachusetts 01701, USA

In vivo experiments on ISS-N1 as a target

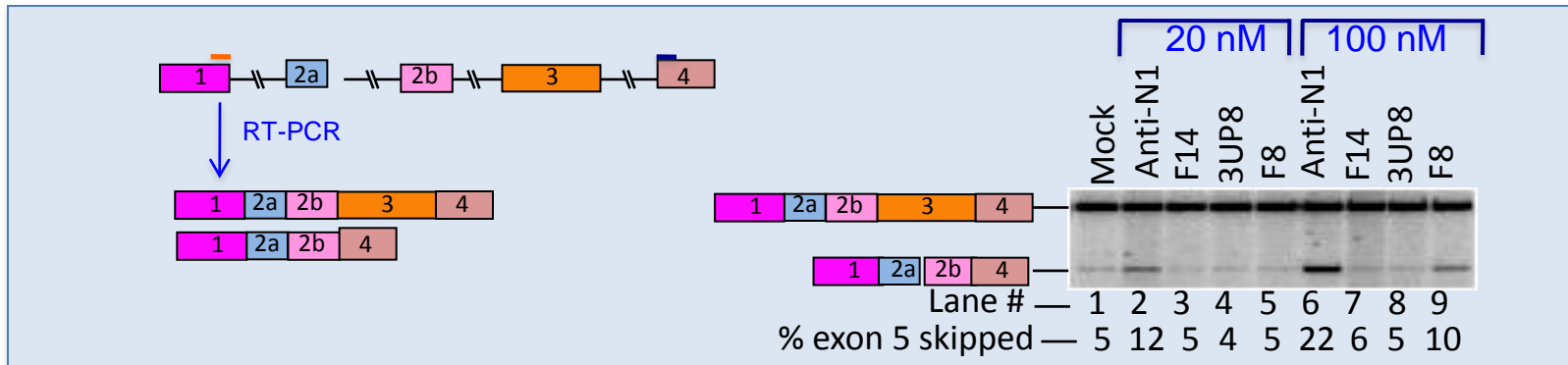
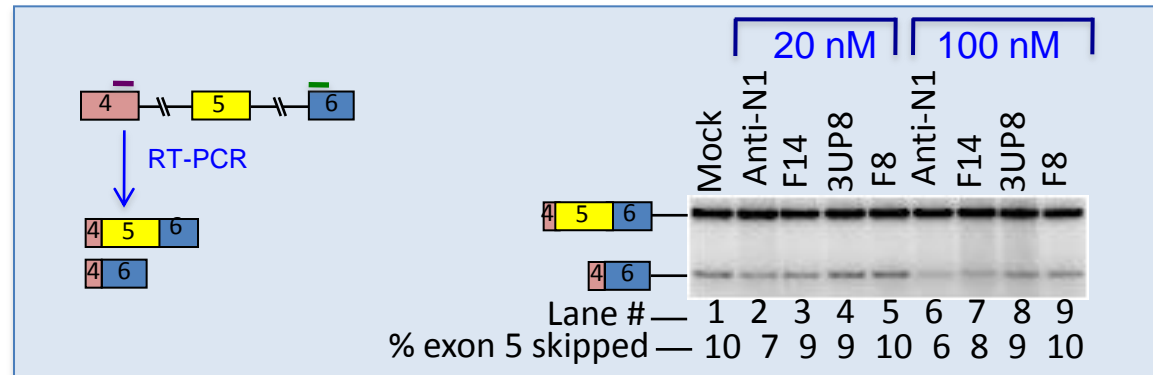
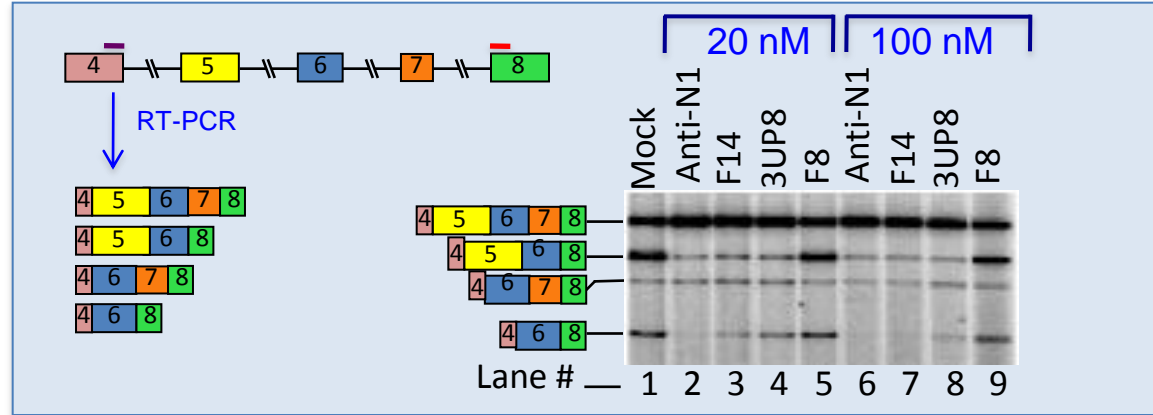
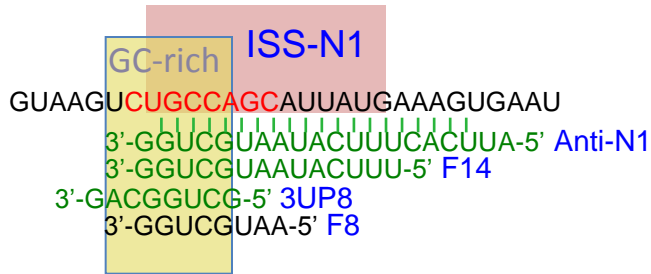


How short an oligo can be for
splicing correction?

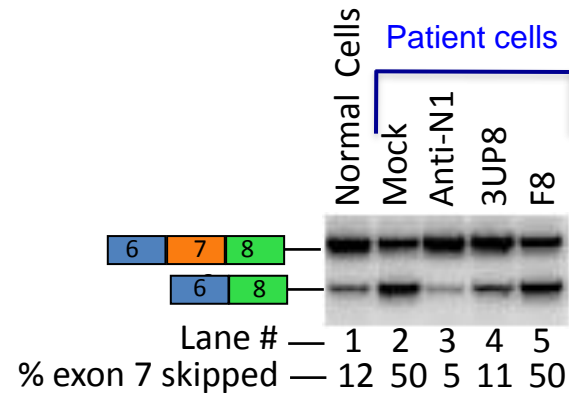
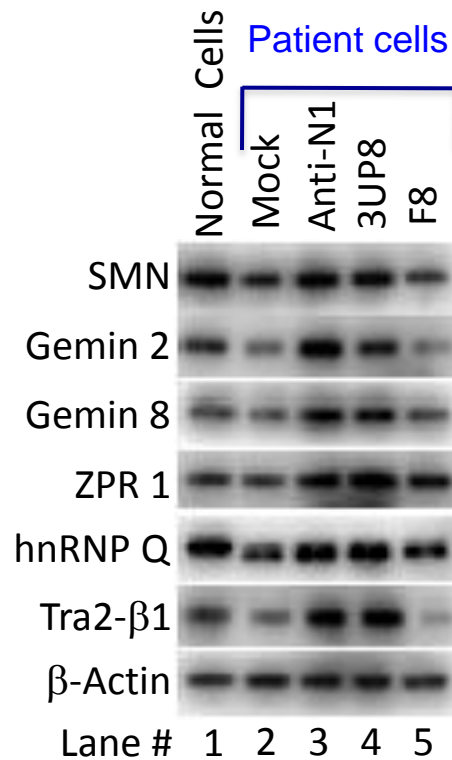
Shortest ASO to correct splicing in a patient cell line



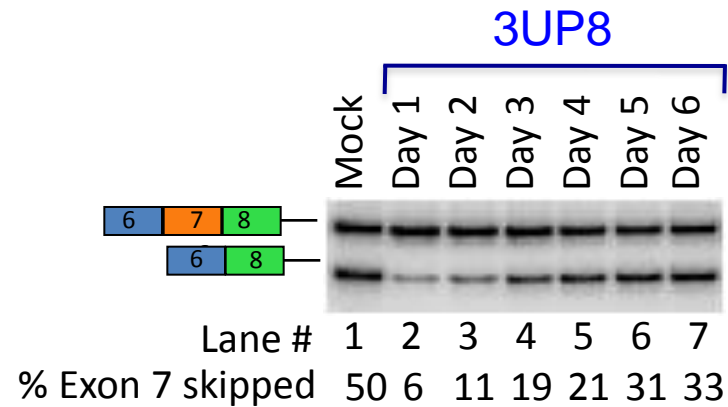
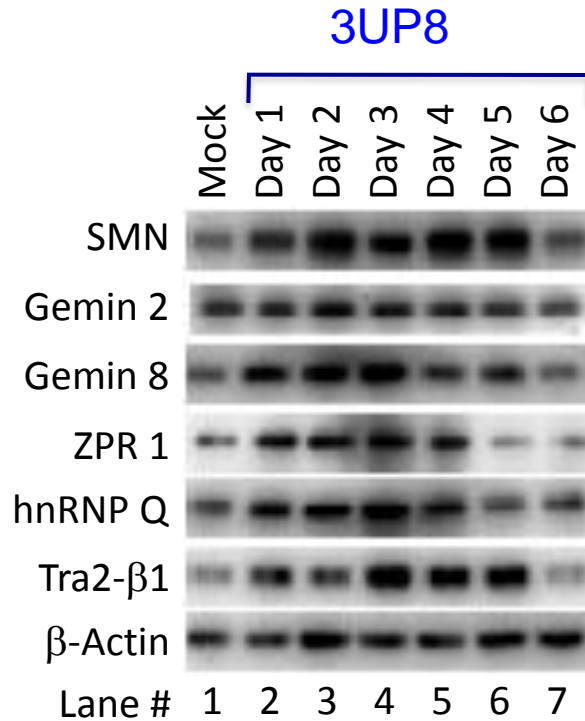
Off-target effect of an ASO is the matter of size



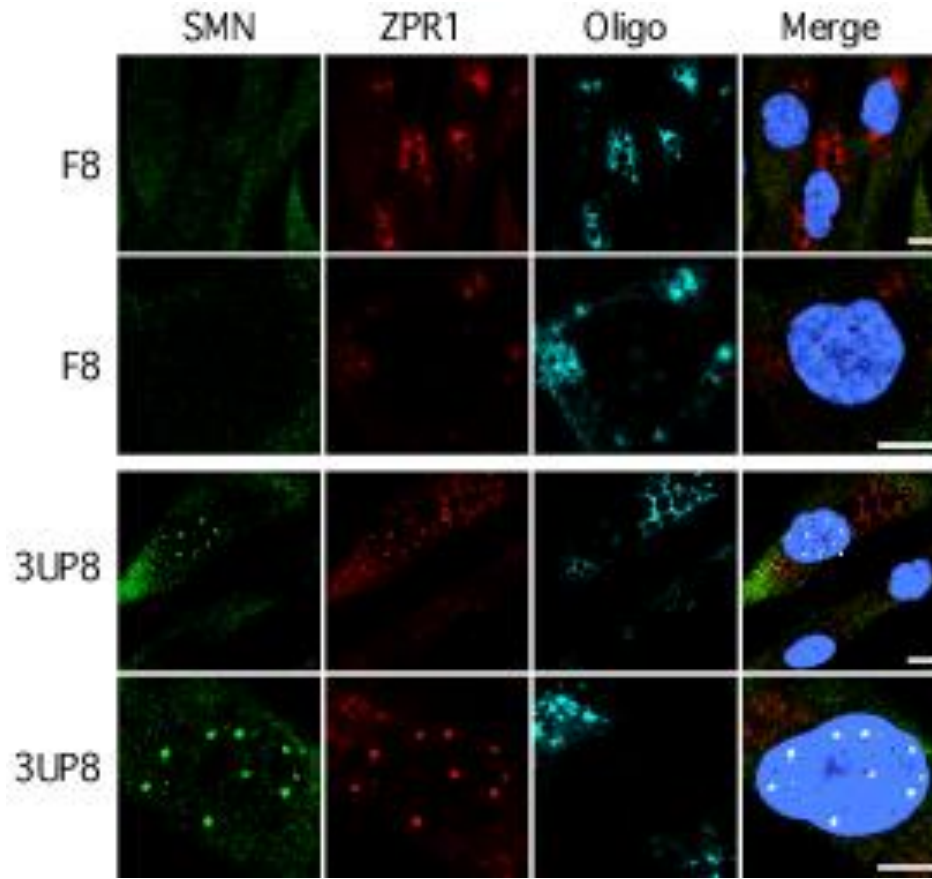
The 8-mer ASO restores high levels of SMN and other proteins in SMA patient cells



The stimulatory effect of a single low dose of the 8-mer ASO lasts for five days



The 8-mer ASO triggers subcellular reorganization characteristic of the healthy cells



Acknowledgments

Iowa State University

Natalia Singh, Ph.D.

Maria Shishimorova, Ph.D.

Dhruva Bhattachrya, Ph.D.

Meirong Zhou, Ph.D.

Joonbae Seo

Katrin Hollinger

Eric Ottesen

Mariah Lawler

Daya Upreti

UMASS MED School

LuCheng Cao, Ph.D.

Elliot Androphy, M.D.

Junning Wang, Ph.D.

Yan Wang, M.S.

COLLABORATORS/CONSULTANTS

Dr. Robin Reed (Harvard Medical School)

Dr. Ryszard Kole (North Carolina)

Dr. Tariq Rana (Burnham Institute for Biomedical Research)

Dr. Stefan Stamm (University of Kentucky)

Dr. Kum Kum Khanna (Australia)

Dr. Shuowei Cai (Dartmouth)

CURRENT SUPPORT

NIH/NINDS

PECASE

Salsbury Endowment, ISU

PAST SUPPORT

American Parkinson's Disease association

Families of SMA, USA

Muscular Dystrophy Association, USA

Iowa Center for Advanced Neurotoxicology