> …CRISPR–Cas has opened up new avenues in our understanding of how cells repair DNA damage, in our ability to engineer cells and in the possibility of developing new, RNA-dependent therapies for previously intractable genetic diseases

DNA template is available the cell will attempt to copy genetic information from it during repair, thereby inserting new genetic information into the genome, as demonstrated by Mali et al. and Cong et al.

From its origins as a bacterial immune system, CRISPR–Cas has been developed into an all-purpose tool for tailored engineering of genomes in a range of species. In the span of less than a decade, CRISPR–Cas has opened up new avenues in our understanding of how cells repair DNA damage, in our ability to engineer cells and in the possibility of developing new, RNA-dependent therapies for previously intractable genetic diseases.

Ross Cloney,
Senior Editor, *Nature Communications*

MILESTONE STUDIES Jinek, M. et al. Programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816–821 (2012) | Gasiunas, G. et al. Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc. Natl. Acad. Sci. USA* **109**, E2579–E2586 (2012) | Jinek, M. et al. RNA-programmed genome editing in human cells. *eLife* **2**, e00471 (2013) | Mali, P. et al. RNA-guided human genome engineering via Cas9. *Science* **339**, 823–826 (2013) | Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).
FURTHER READING Pickar-Oliver, A. & Gersbach, C. A. The next generation of CRISPR–Cas technologies and applications. *Nat. Rev. Mol. Cell Biol.* **20**, 490–507 (2019).

> This seminal work established siRNA–GalNAc as a promising therapeutic delivery approach to treat diseases involving liver-expressed genes

on the RNA sense strand resulted in a higher-affinity binding to ASGPR on liver cells, and a more robust silencing in vivo.

This seminal work established siRNA–GalNAc as a promising therapeutic delivery approach to treat diseases involving liver-expressed genes. Despite halting of the development of the first siRNA–GalNAc-based drug (Revusiran) during clinical trials in 2016, the impressive silencing efficiency, good safety profile and encouraging results from more recent clinical trials of drugs for acute hepatic porphyria (Givosiran) and cardiovascular disease with elevated LDL cholesterol (Inclisiran), established GalNAc conjugation as a promising solution for therapeutic siRNA delivery to the liver.

Alfredo Sansone,
Senior Editor, *Nature Communications*

MILESTONE STUDIES Nair, J. K. et al. Multivalent *N*-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* **136**, 16958–16961 (2014) | Matsuda, S. et al. siRNA conjugates carrying sequentially assembled trivalent *N*-acetylgalactosamine linked through nucleosides elicit robust gene silencing in vivo in hepatocytes. *ACS Chem. Biol.* **10**, 1181–1187 (2015).
FURTHER READING Sardh, E. et al. Phase 1 trial of an RNA interference therapy for acute intermittent porphyria. *N. Engl. J. Med.* **380**, 549–558 (2019) | Ray, K. K. et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N. Engl. J. Med.* **376**, 1430–1440 (2017).

MILESTONE 14

An antisense oligonucleotide splicing modulator to treat spinal muscular atrophy

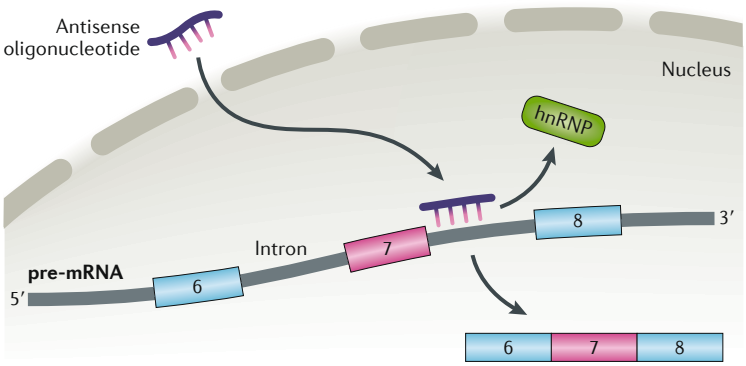


Figure adapted with permission from Rigo, F. et al. *J. Cell Biol.* **199**, 21–25 (2012)

On 23 December 2016, the United States Food and Drug Administration (FDA) approved the antisense oligonucleotide (ASO) drug nusinersen (Spinraza) to treat spinal muscular atrophy (SMA), a fatal genetic disease that can affect children and adults. The approval was the culmination of a successful collaboration between researchers in academia and industry, with support and assistance from patient advocacy groups and regulatory agencies.

SMA is a devastating neuromuscular disease that affects 1 in 10,000 people and is caused by mutations in the gene survival of motor neuron 1 (*SMN1*). Without functional SMN protein, the motor neurons in the spinal cord and brain stem degenerate, resulting in muscle weakness and atrophy. Of the infants born with SMA, 60% show symptoms before six months of age, with median life expectancy of less than two years. A paralog of *SMN1* in the human genome, *SMN2*, encodes an identical SMN protein. However, its pre-mRNA undergoes aberrant splicing, with 90% of mature *SMN2* transcripts lacking exon 7 and producing a truncated, unstable polypeptide. Some individuals with SMA carry multiple copies of *SMN2* and can thus produce higher levels of full-length SMN protein, which reduces the severity and delays the onset of the disease.

The molecular basis of *SMN2* exon 7 skipping was elucidated by several groups, including those of Ravendra Singh at University of Massachusetts Medical School and Adrian Krainer at the Cold Spring Harbor Laboratory, in the late 1990s to early 2000s. *SMN2* contains a synonymous C-to-T substitution in exon 7 that weakens the binding of splicing activators, thereby reducing the efficiency of the 3′ splice site. In 2003, Cartegni and Krainer engineered bifunctional ASOs that operate as synthetic splicing activators: a peptide mimicking a splicing activator was covalently linked to an ASO that hybridized to exon 7. This chimeric effector was able to promote exon 7 inclusion in cell extracts. Those findings prompted C. Frank Bennett, from Isis (later Ionis) Pharmaceuticals, to contact Krainer and initiate a collaboration, as recounted by Rigo et al. in 2012.

Over the next years, the strategy to control exon 7 inclusion was optimized for use in cells and

animal models. ASOs targeting a site near the 5′ splice site in *SMN2* intron 7 could efficiently promote exon 7 inclusion without the need of an appended peptide moiety. They acted by preventing binding of the splicing repressors HNRNPA1 and HNRNPA2. In addition, chemical modifications in the backbone (phosphorothioate) and nucleotides (2′-*O*-methoxyethyl, or 2′-MOE) of the ASOs improved their pharmacological properties.

With promising results in pre-clinical studies, the ASO nusinersen entered clinical trial phase I and II studies in 2011 and 2013–2014, respectively. A multi-centre, randomized, double-blind phase III study took place in 2014–2016 and included 121 infants up to seven months of age who had been diagnosed with SMA before they were six months old (infantile onset). Participants received the drug injected intrathecally (that is, through a lumbar puncture for delivery into the cerebrospinal fluid, to reach targets in the central nervous system). A control group was treated with a mock procedure (skin prick). An interim analysis conducted with 82 patients showed that 40% of those treated with nusinersen showed improvements in motor function milestones, such as head control, sitting, rolling, crawling, standing and walking, compared to none in the control group. These results led to early termination of the trial in August 2016, so that infants in the control group could start receiving the drug. Beneficial effects observed in another trial, with children aged 2–12 with later-onset SMA, also prompted its early termination in November 2016.

> “…a successful collaboration between researchers in academia and industry, with support and assistance from patient advocacy groups and regulatory agencies”

The FDA approved nusinersen only three months after the new drug application (NDA) was filed by Biogen. This occurred with fast-track designation and priority review, and without an advisory committee, as outside expertise was not deemed necessary given the lack of controversial issues, as noted in the agency's summary report. Nusinersen was approved by the European Medicines Agency in May 2017, and it is currently available for treating SMA in more than 40 countries.

Inês Chen, Chief Editor,
Nature Structural & Molecular Biology

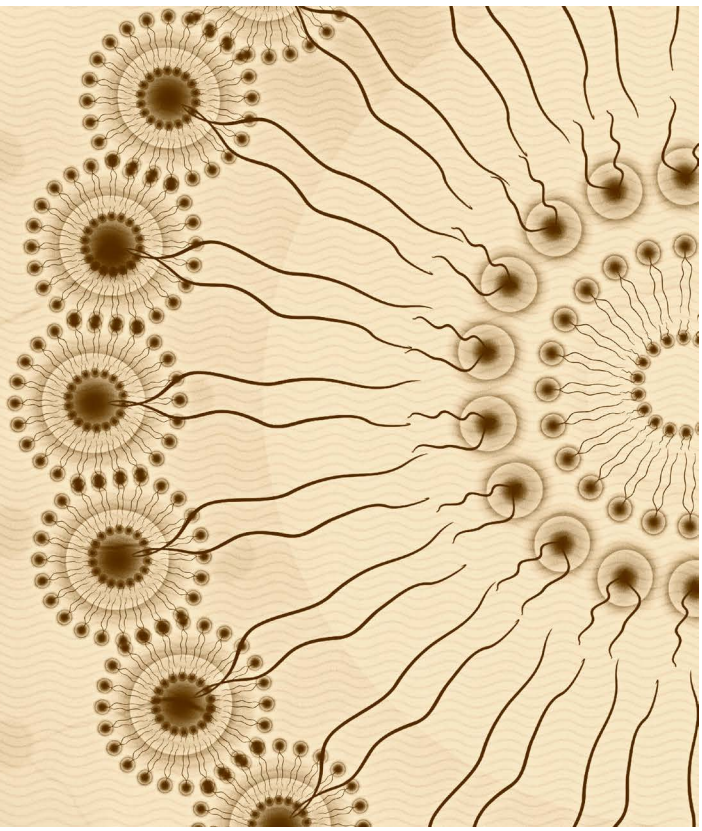
FURTHER READING Lefebvre, S. L. et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155–165 (1995) | Hofmann, Y. et al. Htra2-β1 stimulates an exonic splicing enhancer and can restore full-length *SMN* expression to survival motor neuron 2 (*SMN2*). *Proc. Natl. Acad. Sci. USA* **97**, 9618–9623 (2000) | Cartegni, L. & Krainer, A. R. Disruption of an SF2/ASF-dependent exonic splicing enhancer in *SMN2* causes spinal muscular atrophy in the absence of *SMN1*. *Nat. Genet.* **30**, 377–394 (2002) | Cartegni, L & Krainer, A. R. Correction of disease-associated exon skipping by synthetic exon-specific activators. *Nat. Struct. Biol.* **10**, 120–125 (2003) | Singh, N. K. et al. Splicing of a critical exon of human *Survival Motor Neuron* is regulated by a unique silencer element located in the last intron. *Mol. Cell. Biol.* **26**, 1333–1346 (2006) | Hua, Y. et al. Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects *SMN2* splicing in transgenic mice. *Am. J. Hum. Genet.* **82**, 834–848 (2008) | Singh, N. K. et al. A short antisense oligonucleotide masking a unique intronic motif prevents skipping of a critical exon in spinal muscular atrophy. *RNA Biol.* **6**, 341–350 (2009) | Hua, Y. et al. Peripheral SMN restoration is essential for long-term rescue of a severe SMA mouse model. *Nature* **478**, 123–126 (2011) | Rigo, F. et al. Antisense-based therapy for the treatment of spinal muscular atrophy. *J. Cell Biol.* **199**, 21–25 (2012) | Chiriboga, C. A. et al. Results from a phase 1 study of nurinersen (ISIS-SMN_{Rx}) in children with spinal muscular atrophy. *Neurology* **86**, 890–897 (2016) | Wadman, M. Updated: FDA approves drug that rescues babies with fatal neurodegenerative disease. *Science* https://doi.org/dbg2 (2016) | Finkel, R. S. et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *The Lancet* **388**, 3017–3026 (2017) | Finkel, R. S. et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N. Engl. J. Med.* **377**, 1723–1732 (2017) | Mercuri, E. et al. Nusinersen versus sham control in later-onset spinal muscular atrophy. *N. Engl. J. Med.* **378**, 625–635 (2018) | Bastings, E. *Division Director Summary Review* (FDA, 2016); https://go.nature.com/2lP28zc

MILESTONE 15

A new dawn for RNAi drugs

The 10th of August, 2018 marked a new era for the field of RNA therapeutics, with the first approval of an RNA interference (RNAi)-based drug by the United States Food and Drug Administration. The drug — patisiran (Onpattro) — is approved for the treatment of polyneuropathy in people with hereditary transthyretin-mediated amyloidosis (hATTR). This rare and devastating neurodegenerative disease is caused by deposition of amyloid fibrils formed by misfolded transthyretin protein. A double-stranded small interfering RNA composed of two modified 21-mer oligonucleotides and encapsulated in a lipid nanoparticle formulated for hepatocyte uptake, patisiran silences transthyretin mRNAs in the liver to reduce serum levels of the protein. The approval of patisiran brings new hope to patients with hATTR who previously had no effective treatment options.

FURTHER READING Garber, K. Alnylam launches era of RNAi drugs. *Nat. Biotechnol.* **36**, 777–778 (2018) | Setten, R. L. et al. The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* **18**, 421–446 (2019)



Credit: GiroScience / Alamy Stock Photo