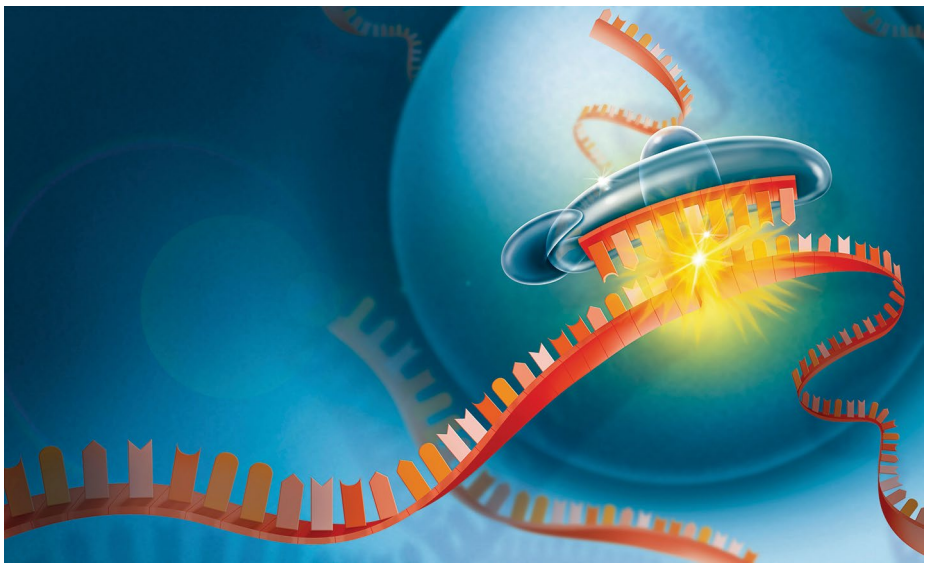
Billion-dollar deal propels RNAi to CNS frontier

Biotech giant Regeneron partners with Alnylam to expand RNAi therapies beyond the liver and into the brain.

Regeneron Pharmaceuticals and Alnylam Pharmaceuticals have allied to develop RNA-interference-based drugs for the central nervous system (CNS) and the eye, opening a new chapter in the development of this rapidly emerging therapeutic modality. Until recently, delivery constraints have meant that indications involving the liver were prioritized for RNAi agents. But after more than a decade researching how to deliver short interfering RNA (siRNA) molecules into the CNS, the latter route is now on the brink of clinical application (Table 1). Also edging towards the clinic is Dicerna Pharmaceuticals, who entered a large-scale licensing deal with Eli Lilly last October, focused on neurodegenerative disease, pain and cardiometabolic disease. "We're optimistic that in this calendar year we may, in collaboration with Lilly, have our first clinical candidate for a CNS indication," Dicerna president and CEO Douglas Fambrough says.

Alnylam's alliance with Regeneron will fuel that expansion. The deal—which involves $800 million in upfront cash and equity investment plus up to $200 million more in near-term milestones—will provide substantial resources for a thorough exploration of siRNA's potential in CNS and ocular indications. Although neither company has specified the targets or the precise timing, a first trial of a CNS-targeted siRNA drug is likely imminent. (The alliance also encompasses C5 complement-mediated diseases and certain undisclosed liver conditions; an existing alliance in nonalcoholic steatohepatitis is ongoing.)

As well as cash, Regeneron brings its scientific strengths in target discovery and disease biology to bear on siRNA's chemistry. The timing of the deal coincides with the conclusion of the research phase of a longstanding alliance between Alnylam and Paris-based Sanofi, a company that, incidentally, has also recently wound down a lengthy relationship with Regeneron in antibody discovery.



The next frontier: CNS-targeted siRNA drugs are edging close to the clinic. Credit: BSIP SA / Alamy Stock Photo

Alnylam has pioneered the development of siRNA as a therapeutic modality. The first approved RNAi-based drug, Alnylam's Onpattro (patisiran), licensed last August for hereditary transthyretin-mediated amyloidosis. "For them [Alnylam], the missing ingredient in taking their platform to the next level was some of the biology and the targets," says Nouhad Husseini, Regeneron's vice president and head of business development. Regeneron's extensive research efforts in mouse genetics and in human population genetics could be the leavening agent. "I don't think the world really appreciates the extent of what Regeneron is doing in discovery," Husseini says. Regeneron, for its part, has long experience of siRNA as a research tool but had been initially slow to embrace the technology as a therapeutic modality. "Frankly there was a lot of skepticism here. We didn't really believe it could be broadly applied," says Husseini.

That is no longer the case, given Alnylam's progress at extending the technology beyond the liver. "The data that we saw were compelling," says Husseini. Last October, Alnylam reported results from experiments in non-human primates, which indicated that a single dose of siRNA, administered by direct injection into the spinal cord, resulted in sustained and robust silencing of β-catenin mRNA, which encodes a Wnt pathway protein that is ubiquitously expressed across the brain and spinal cord. The company stated then that it planned to seek approval to conduct

Table 1 | Selected siRNA drug development programs for ophthalmologic and CNS indications

Developer(s)	Molecule	Target	Indication(s)	Clinical stage
Sylentis (Madrid)	Tivanisiran	Transient receptor potential cation channel, subfamily V, member 1	Dry eye disease	Phase 3
Quark Pharmaceuticals	QPI-1007	Caspase 2	Non-arteritic anterior ischemic optic neuropathy and other optic neuropathies	Phase 3
Dicerna, Lilly	DCR-Neuro1	Undisclosed	Undisclosed neurodegenerative disease	Research
Dicerna, Lilly	DCR-Neuro2	Undisclosed	Undisclosed	Research

a first clinical trial of an siRNA CNS drug candidate by late 2019 or early 2020. In December, it identified as its lead CNS candidate ALN-APP, an siRNA targeting amyloid precursor protein. It is in development for cerebral amyloid angiopathy, a condition characterized by amyloid deposition on blood vessel walls, which can cause brain hemorrhage. Neither company has confirmed whether this program forms part of the alliance, but Alnylam has identified dominantly inherited conditions, such as Alzheimer's disease, amyotrophic lateral sclerosis, frontotemporal dementia, Huntington's disease, Parkinson's disease, prion disease, spinocerebellar ataxia and many orphan genetic conditions with CNS involvement, as being appropriate for siRNA-based therapy.

Dicerna has been a little more forthcoming about its plans. "Alzheimer's is one of the indications we're pursuing with Lilly," says Fambrough. The pharma is among many that have attempted, so far without success, to tackle Alzheimer's disease, pursuing both small-molecule- and antibody-based approaches against a wide array of targets. They are not, at this point, publicly identifying the focus of their RNAi program in Alzheimer's, although Fambrough is unequivocal on one aspect of their approach: "We're not targeting β-amyloid," he says.

That it is now feasible to target CNS tissues with siRNA agents stems largely from technological advances, many of which were originally developed for antisense oligonucleotides (ASOs). Scientists improved the stability of siRNA molecules and reduced their immunotoxicity by painstakingly introducing chemical modifications that did not compromise their ability to interact with Dicer and Argonaute, the enzymes that process long double-stranded RNAi 'triggers' (precursors) into functioning siRNA molecules. Replacing phosphodiester backbones with phosphorothioate linkages protects against nuclease digestion, for example. Adding 2′-*O*-methyl and 2′-fluoro modifications to the ribose sugar groups avoids the activation of innate immune sensors that recognize double-stranded nucleic acids. "We're now able to hone the molecules," says Kevin Fitzgerald, CSO and senior vice president for research at Alnylam. "You can tweak the stability of the molecule, and as you tweak the stability, you tweak the durability."

Dicerna too has made various structural adjustments to its liver-directed molecules. Fambrough says that these modifications have resulted in a 100-fold improvement in intracellular stability, and those same chemical changes are now available to use in additional organ systems. "They're paying additional dividends as we move to other tissues," he says. "Before you had chemically stabilized siRNA that could not have happened," says Si-ping Han, of City of Hope National Medical Center, who is the corresponding author on a recent review of RNAi-based drugs.

The other great advance in siRNA molecule development is in their delivery. To improve specificity and uptake efficiency, developers have switched from using lipid nanoparticles—employed, for example, in Onpattro—to conjugating RNAi triggers to ligands that recognize particular cell-surface receptors. For example, *N*-acetylgalactosamine (GalNAc) binds the asialoglycoprotein receptor, which is highly expressed on hepatocytes and has therefore become the ligand of choice for targeting liver cells. The combination of high cellular uptake and long-term stability means that developers can now routinely expect a single dose of siRNA to remain active for three months and more.

But GalNAc conjugation, because of its specificity for hepatocytes, does not offer siRNA drugs a route into the CNS. ASOs have achieved initial success via intrathecal injections in spinal muscular atrophy, but even with repeated monthly injections, penetration of compounds into deep brain regions still represents a challenge.

Targeting CNS cells—neurons or glia—is a question of selecting an appropriate ligand–receptor pair that will provide the desired level of specificity. Cholesterol is one example of a conjugate that works for local siRNA delivery to the brain, eye and skin, but Alnylam is not at this point disclosing publicly the identity of the ligand–receptor pairs that it will employ in the alliance with Regeneron. The programs involved will generally necessitate a broad uptake of siRNA molecules across all CNS cells, however. "The majority of the targets we're interested in looking at are ubiquitously expressed," Fitzgerald says. They do appear to be amenable to long-term RNAi-mediated silencing. "If anything, the duration of effect in the CNS appears to be longer," says Fitzgerald. "We're certainly beyond quarterly [dosing] at this point."

Dicerna has developed methods for targeting CNS with its proprietary 'Dicer substrate siRNAs' (DsiRNAs), which may not require ligand conjugates. The DsiRNA format, developed by Dicerna's cofounder John Rossi, of City of Hope, differs from classic siRNA molecules, which are designed to bypass the Dicer cleavage step and to enter the RNA-induced silencing complex (RISC) directly, before

Thrive joins liquid biopsy race

Liquid biopsy company Thrive Earlier Detection launched in May with the aim of bringing an inexpensive test for early tumor detection into routine clinical use. Armed with $110 million Series A backing from Third Rock and other investors, Thrive took out an exclusive license to CancerSEEK, a multianalyte blood test developed by researchers at Johns Hopkins University that simultaneously analyzes cancer gene mutations combined with cancer protein biomarkers, all of which are analyzed by machine learning algorithms. CancerSEEK demonstrated greater than 99% specificity in a study published in *Science* across eight solid tumor types. The platform is under evaluation in a prospective trial in collaboration with Geisinger Health System. Thrive's CEO Steve Kafka, also a partner at Third Rock Ventures, was formerly at Foundation Medicine (FMI), whose liquid biopsy test analyzes genetic markers and microsatellite instability to help direct therapy and find clinical trial options for patients. FMI is one of several deep-pocketed firms focused on liquid biopsy. Another is Grail, which tapped private equity investors to the tune of a billion dollars in 2017 to develop a DNA-methylation-based test platform for early detection of multiple cancer types. Guardant Health, which like FMI is aiming its liquid biopsy test at patients with advanced cancer, raised $238 million last October in its IPO. Also taking the AI route to early cancer detection is Freenome, which raised $72 million in a Series A round to develop a blood-based test for colorectal cancer. The company analyzes cell-free DNA, RNA and proteins, which yield not only a snapshot of the tumor itself, but also circulating tumor-specific DNA and RNA and protein fragments from immune cells destroyed by the tumor. Freenome analyzes the resulting complex fragmentation patterns, using them as biomarkers for early tumor detection and to reveal tissue origin.

Published online: 2 July 2019
https://doi.org/10.1038/s41587-019-0188-x

"I've given up trying to predict which things combine with which. In general, anything that stimulates inflammation just broadly speaking ... tends to pair well with Keytruda." Roger Perlmutter, head of R&D at Merck, comments on the company's flagship immuno-oncology drug, as it continues to impress in the marketplace and the clinic. (*STAT*, 29 May 2019)

undergoing further processing to become functional molecules. DsiRNAs are slightly longer and bind to and are cleaved by Dicer, a step, its developers claim, that increases the efficiency of RISC assembly and the subsequent RNAi effect. "We have been able to optimize neural delivery by alteration of the chemical characteristics of the loop end of our molecule," says Fambrough. "We have a free hand to change the chemistry in the loop end in any way we want, without impairing the RNAi function of the molecule."

Alnylam has developed a further general refinement to its ligand-conjugated siRNA technology, which could widen the therapeutic window for its siRNA molecules. The technology, part of its 'Enhanced Stabilization Chemistry+' (ESC+) platform, involves the inclusion of a glycol nucleic acid base at a specific location in the seed region (bases 2–8 from the 5′ end) of siRNAs, which reduces off-target effects that can arise at high doses, without affecting potency. "We're learning how to make sure the molecule hits exactly where we want it to hit and nowhere else," Fitzgerald says. "The ESC+ chemistry is maybe not that important for applications in the liver, where the doses needed to achieve therapeutic effects are pretty low," says Han, who is a postdoctoral researcher in Rossi's lab. "However, if they find themselves needing to apply higher doses to get therapeutic effects in tissues outside of the liver, the ESC+ chemistry may give them an extra safety margin," he says.

Of course, certain questions about the potential utility of a given drug candidate can only be answered in a clinical trial. "No matter how good the data look in animal models, there is always some uncertainty regarding potency, biodistribution and safety in moving to humans for a new tissue type with a new ligand–siRNA combination," says Han. The upcoming clinical trials will not all be positive, but they help to further the evolution of what is currently a niche therapeutic modality into a more generally applicable technology. ❐

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Published online: 11 June 2019
https://doi.org/10.1038/d41587-019-00014-7

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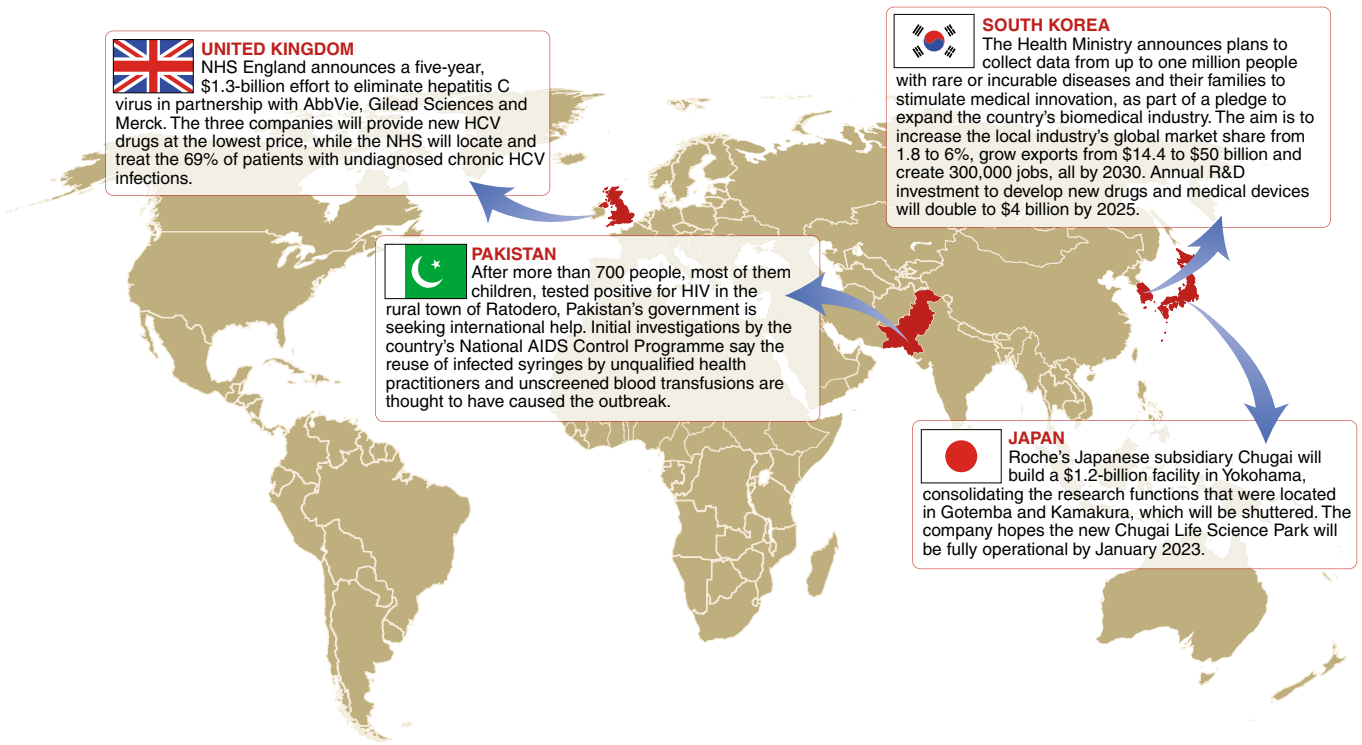

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Published online: 2 July 2019
https://doi.org/10.1038/s41587-019-0185-0