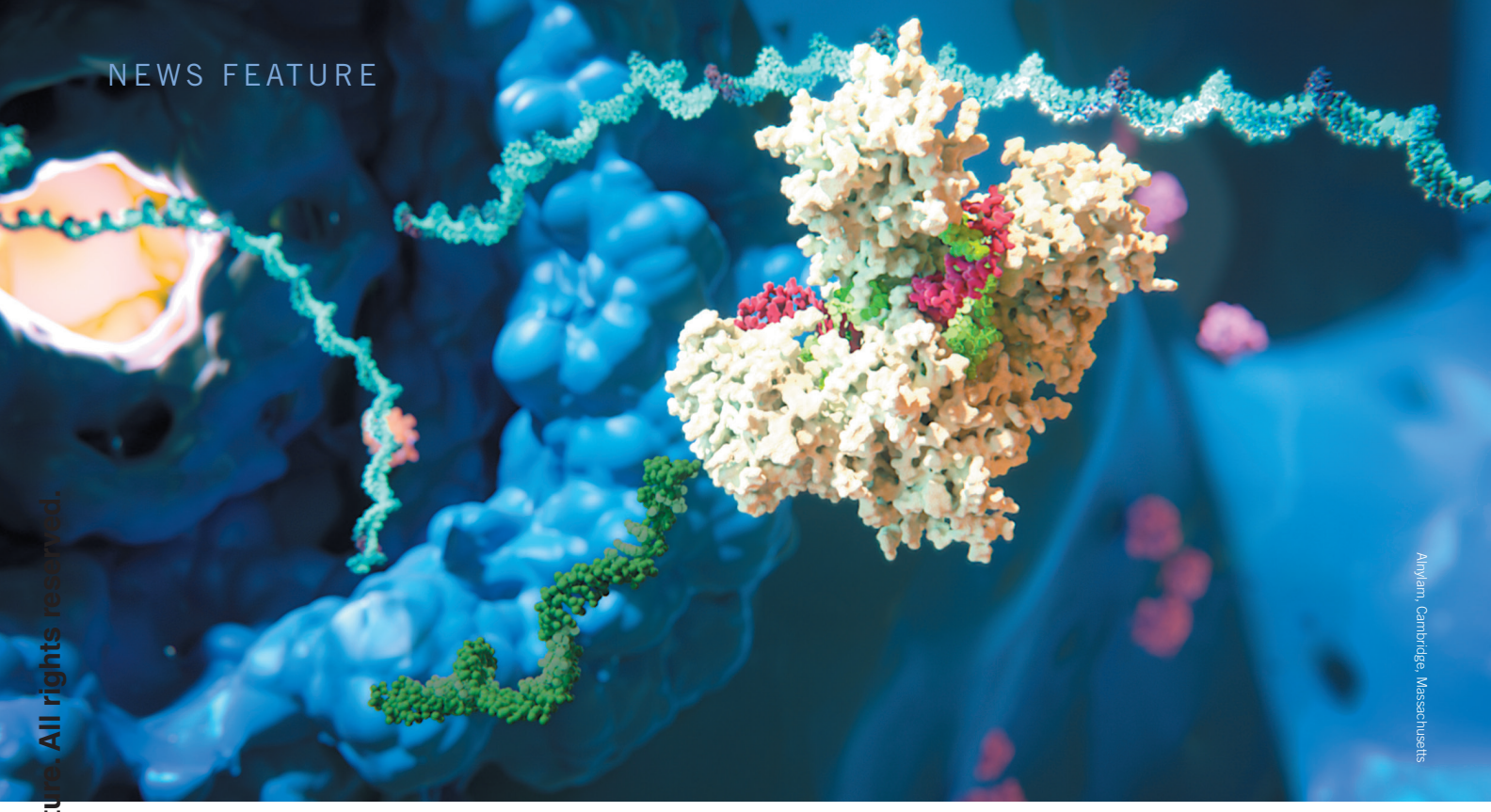


Alnylam, Cambridge, Massachusetts

Worth the RISC?

The long, tortuous race to market a therapeutic based on RNA interference (RNAi) may be nearing its end. Ken Garber reports.

In its 15 years of existence, the fortunes of the RNAi therapeutics field have swung wildly between hype and futility. Over a dozen companies entered the field between 2002 and 2008, at which point big pharma—fearful of losing out on a disruptive technology—went on a buying spree. But these multinationals quickly suffered buyer's remorse once the shortcomings of RNAi delivery systems became clear. Basel, Switzerland–based Roche killed its RNAi program in 2010, after investing $500 million, and the rest of pharma quickly followed. This near-death experience eliminated most companies, but the surviving biotechs eventually generated clinical data that won over skeptics who considered the whole approach ineffective. This reignited investor interest in the field, and now two short interfering RNA (siRNA) oligonucleotides are in phase 3 clinical trials, with one, Cambridge, Massachusetts–based Alnylam's patisiran for transthyretin amyloidosis (ATTR) therapy, set to read out at mid-year (**Table 1**).

Bringing a new therapeutic modality to market is never without reverses, and problems continue for RNAi therapeutics. The clinical programs of Pasadena, California–based Arrowhead Pharmaceuticals and Cambridge, Massachusetts–headquartered Dicerna suffered serious setbacks last year. In October, Alnylam suffered another reverse: an imbalance in deaths between the treatment and placebo arms[1] of its phase 3 trial of revusiran siRNA for hereditary ATTR with cardiomyopathy, which resulted in the study's discontinuation (and a concomitant wiping out of $3.6 billion of market value). The company has since presented positive safety and efficacy data for four different clinical programs. But, "there's a lot of skepticism, and people are waiting to see what happens," says Judy Lieberman, an RNAi researcher at Harvard and a member of Alnylam's scientific advisory board. With pivotal clinical data likely emerging over the next four months, 2017 could prove a tipping point for RNAi drugs.

Expedited delivery

The therapeutic prospect of potent, specific silencing of otherwise 'undruggable' genes by RNA interference—an endogenous, catalytic mechanism—quickly mobilized the biotech industry after siRNA silencing was first shown in mammalian cells in 2001 (ref. 2). The hurdle was always going to be delivery in the body (**Fig. 1**). Some companies took the gene therapy route early on, using viral vectors to deliver DNA encoding short hairpin RNA (shRNA), which the enzyme Dicer converts into siRNA, a short duplex that engages with the RNA-induced silencing complex (RISC), the cell's mRNA cleavage machinery. After multiple clinical failures made manifest the challenge of delivering an RNA 'prodrug', via a gene therapy viral vector, to drug a target via an entirely new therapeutic mechanism, activity and interest in this 'DNA-directed' approach to RNAi eventually died away.

More companies turned to systemic delivery of a chemically synthesized siRNA, avoiding the vagaries of predicting final therapeutic activity in a target tissue from an RNA prodrug, expressed from a DNA plasmid, which had the potential risk of chromosomal integration. But this therapeutic class had its own problems.

Naked, negatively charged siRNAs are too large and too hydrophilic to diffuse across cell membranes alone, and once inside cannot easily escape from endosomes to engage the cytoplasmic RNAi machinery. Unmodified siRNAs are quickly degraded by serum and cytoplasmic nucleases, and they stimulate the innate immune system, triggering inflammatory and other immune responses.

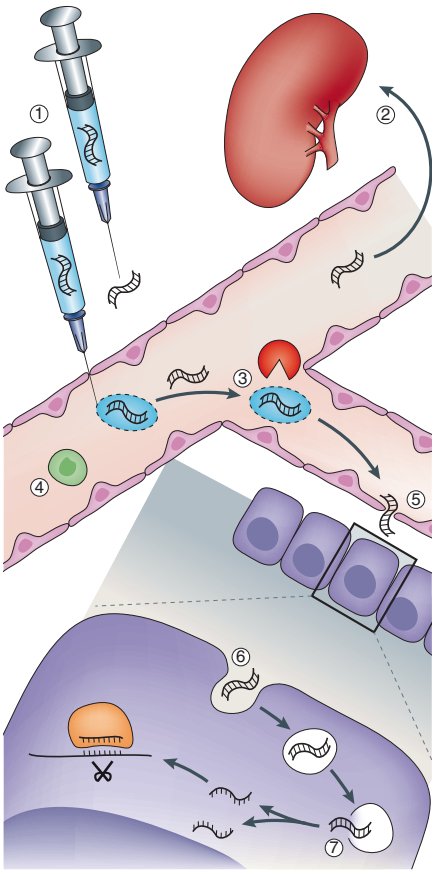
Companies attacked these problems along two lines simultaneously: chemical modifications to the siRNA backbone, and conjugation to (or encapsulation within) targeting molecules. Incorporation of 2′-*O*-methyl or 2′-fluoro modifications to the ribose, and phosphorothioate linkages, with sulfur substituting for one of the non-bridging oxygens in the phosphate backbone, gave protection from nuclease degradation and dampened

innate immune system activation. These modifications (originally developed for antisense oligonucleotides), tweaked in various ways, have worked well, although questions about off-target effects and long-term toxicity are not yet settled. Not enough patients have been treated for a long enough time for anyone to know for sure that these chemically modified oligos are completely safe.

"The chemical modifications buy you a lot," says Lieberman. "They buy you stability, they buy you very dramatically reduced off-target effects, the lack of immune stimulation… and some of the durability. But I think any unnatural product, especially if it's not biodegradable, could be toxic, in some settings."

Conjugation and encapsulation, too, have had mixed results, with ruthless attrition of methods taking place over the years. Encapsulation in polymer or lipid nanoparticles (LNPs) protected siRNAs from nucleases and facilitated tissue-specific (mainly liver) targeting and cell entry. But polymer-conjugated siRNAs have largely failed to date, with clinical trials showing toxicity but little efficacy[3]. Lipids, although still in use—Alnylam's patisiran, poised for phase 3 success, is lipid-encapsulated—have, as a class, never overcome toxicity concerns stemming from innate immune activation, especially with chronic use. Alnylam's LNP-delivered composite of vascular endothelial growth factor and kinesin spindle protein (ALN-VSP), an LNP formulation of vascular endothelial growth factor and kinesin spindle protein oligos, for example, showed dose-dependent cytokine and complement activation in phase 1, with related side effects[4], even though patients were given steroid premedication. The drug, outlicensed, has not returned to the clinic. Patisiran uses a different cationic lipid and has not shown the same increase in inflammatory markers[5], but it still must be given with steroids. "The future is going to be not in lipid nanoparticles," says Lieberman.

Instead, the platform of choice, at least for now, is *N*-acetyl galactosamine (GalNAc). GalNAc is a ligand for the asialoglycoprotein receptor (ASGPR) on hepatocytes, and conjugating GalNAc to the siRNA oligo gets the molecule into these liver cells efficiently through receptor-mediated endocytosis. GalNAc also enables subcutaneous drug delivery, instead of the intravenous (IV) infusions necessary with lipid carriers[6], and there's no need for steroids. "Right now the overall mantra [is] that with development of a GalNAc conjugate to fully metabolically stabilized siRNAs, the problem of liver hepatocyte delivery is solved," says Anastasia Khvorova, an RNAi researcher at the University of Massachusetts in Worcester, Massachusetts.



Nat. Rev. Genet. **16**, 543–552 (2015)

Figure 1 siRNA therapies face many barriers to reaching their targets. They must (1) enter circulation or target tissue; (2) avoid excretion; (3) avoid nuclease degradation; (4) avoid immune recognition; (5) extravasate into tissue; (6) be taken up by cells; and (7) escape endosomes.

Inclisiran, Alnylam's GalNAc-conjugated, chemically modified, anti-proprotein convertase subtilisin/kexin (PCSK) 9 oligo strongly makes this case. (PCSK9 inhibition in liver lowers serum low-density lipoprotein (LDL) cholesterol.) A single dose of the drug, given to healthy volunteers, knocked down circulating PCSK9 by a mean of over 50% for at least six months, and reduced circulating LDL by >40% over the same time span[7]. Interim phase 2 data in hypercholesterolemia, released in November by Alnylam and by Parsippany, New Jersey–based Medicines Company, which is leading clinical development, were similar. The drug has been very well tolerated, with over 500 patients treated in phase 2. "It's clearly kind of a game change[r]," says Khvorova. "Because with this type of efficacy, you can argue that this technology can be competitive oral drugs, because for a lot of indications, the clinician will prefer one injection in half a year rather than making the patient take a drug every single day."

This remarkable durability, following a single dose, defies easy explanation. It's greater than predicted by small animal studies, according to Lieberman. (Primate data have not been released.) "People have theories, but I don't think there's any data" on mechanism, she says. One possibility is that the endosome serves as a long-term slow-release reservoir for the drug in humans. Although that could contribute, says Alnylam chief medical officer Akshay Vaishnaw, Alnylam's unpublished mouse hepatocyte immunoprecipitation experiments show that siRNA antisense strands remain loaded into RISC months after dosing. It's unclear whether siRNAs are being continuously fed to RISC from subcellular organelle depots, or just don't turn over once loaded into RISC. Regardless, after months, the loaded RISC "is busy doing its thing, chopping the target messenger RNA," says Vaishnaw.

Potency's flip side

But Khvorova says it's too early to conclude that liver delivery has been solved. "We really do not know...the long term consequences of modulation of gene expression in humans through the RNAi mechanism, because we are partially disturbing a natural pathway," says Khvorova. When an siRNA enters the cell cytoplasm, its guide strand is loaded into RISC, which enzymatically cleaves mRNAs complementary to the guide strand sequence. RISC is also used by microRNAs, endogenous RNA duplexes, for regulating gene expression and maintaining cellular homeostasis. In 2006, a Stanford group showed that high doses of shRNA saturate the endogenous RNAi machinery and kill recipient mice[8]. "For a long time, for chemically synthesized siRNA, this was not considered to be an issue," says Khvorova. "You hijack a little bit of the RISC complex, it's such a small fraction of the overall machinery that it should be insignificant. Now, when we are talking about the six months' duration of effect with a single injection, it's clear that these compounds are sticking around for quite extended periods of time." So RISC saturation has become a concern again. "If…a significant fraction of the RISC complex is occupied with the artificial sequence, it will result in the introduction of disbalance in the microRNA profile, and any disbalance in the microRNA profile, significant disbalance, is toxic to the cell," says Khvorova. "And we just don't know, there are no data yet."

Off-target effects are another unknown. Although companies use design algorithms to avoid sequences that bind off-target mRNAs, such methods aren't foolproof. "For siRNAs,

Table 1 Selected clinical stage siRNA-based RNAi therapeutics

Company	Agent	Delivery formulation	Indication	Development stage
Alnylam	Patisiran (ALN-TTR02)	LNP	Familial amyloidotic polyneuropathy	Phase 3
Quark	QPI-1002 (I5NP)	Naked siRNA	Post-kidney transplant, post-cardiac surgery	Phase 3 Phase 2
Quark	QPI-1007	Naked siRNA	NAION	Phase 2/3
Sylentis (in Madrid)	SYL1001	Naked siRNA	Dry-eye syndrome	Phase 2 complete
Alnylam, Sanofi Genzyme	Fitusuran (ALN-AT3)	GalNAc conjugate	Hemophilia A&B	Phase 2
Alnylam	Givosiran (ALN-AS1)	GalNAc conjugate	Acute hepatic porphyrias	Phase 1 complete, Phase 3 pending
Medicines Company, Alnylam	Inclisiran (PSCK9si) (ALN-PSCsc)	GalNAc conjugate	Hypercholesterolemia	Phase 2
RXi Pharmaceuticals	RXI-109	Cholesterol conjugate	Dermal scarring after surgery; retinal scarring	Phase 2 Phase 1
Arbutus Biopharma	ARB-1467 (TKM-HBV)	LNP	Chronic hepatitis B infection	Phase 2
Alnylam	ALN-CC5	GalNAc conjugate	PNH	Phase 2
Alnylam	ALN-TTRsc02	GalNAc conjugate	ATTR amyloidosis	Phase 1
Alnylam	ALN-GO1	GalNAc conjugate	Primary hyperoxaluria	Phase 1
Alnylam	ALN-HBV	GalNAc conjugate	Hepatitis B	Phase 1
MD Anderson Cancer Center	siRNA-EphA2-DOPC	LNP	Advanced solid tumors	Phase 1

LNP, lipid nanoparticle; NAION, non-arteritic ischemic optic neuropathy; PNH, paroxysmal nocturnal hemoglobinuria; GalNAc, *N*-acetyl galactosamine.

there is no documented example of off-target mediated effects," says Khvorova. "But the problem is, it's only recently [that] compounds started to be delivered really potently in human beings, particularly in liver."

Another concern: the 2′-fluoro modifications in many siRNAs, including Alnylam's. In 2015 researchers at Ionis Pharmaceuticals in Carlsbad, California, reported that 2′-fluoro phosphorothioate oligonucleotides, introduced into cells, bind to several cellular proteins, triggering their proteasomal degradation, and causing cell death[9]. But Alnylam last year reported *in vitro* data showing that GalNAc-conjugated siRNAs with high 2′-fluoro content were not particularly cytotoxic, whereas 2′-fluoros did confer some toxicity to certain single-strand (antisense) oligos[10]. "It's premature to really say anything about the clinical significance, especially of the Ionis paper," says former RNAi researcher Dirk Haussecker in Rastatt, Germany, founder of the *RNAi Therapeutics* blog. Haussecker hopes these results are replicated in some other laboratories independently.

Thus far, with >1,000 patients treated, Alnylam's drugs have been generally well tolerated, with a 2.2% incidence of clinically significant liver function abnormalities. Injection site reactions affect 15% of patients. But until there is more experience with long-term exposure to siRNAs, especially the newer, more potent chemistries, safety questions will persist. The revusiran trial deaths, and the earlier termination of Alnylam's ALN-AAT (antitrypsin) oligo, for treating AAT deficiency, due to cases of liver enzyme elevation, are worrisome signals.

Alnylam, as of late January, was analyzing the revusiran trial deaths, with drug-related toxicity only one of many possible explanations. Alnylam CEO John Maraganore said at this January's J.P. Morgan Healthcare Conference that there are no platform-wide concerns. Revusiran is "given at much higher doses than any of our other programs," he pointed out. "Moreover, the data to date point to [patient] baseline characteristics as being the potential cause for the mortality events."

Three days after the October 4 revusiran trial unblinding and program termination, patisiran's phase 3 trial data-monitoring committee determined that the overall benefit–risk ratio justified that trial's continuation. In an open-label extension of the phase 2 hereditary ATTR (transthyretin amyloidosis) polyneuropathy trial, there were no clinically significant changes in liver function tests. As for efficacy, 17 of 24 patients showed either stabilization or frank improvement in their neuropathy impairment scores. Top-line data from the 225-patient, randomized, placebo-controlled phase 3 will be reported in the middle of the year. "There's a good chance it'll be approved, that the phase 3 trial will turn out to be positive, with stabilization or even possibly improvement of the disease," says Lieberman. "But of course you never know." Assuming positive data, Alnylam expects to file its 'new drug application' in the US and 'marketing authorization application' in the EU at year's end. Patisiran would be the first approved RNAi therapeutic.

Low-hanging fruit

Among active RNAi therapeutics companies (**Table 1**), Alnylam stands out for its longevity (it was founded in 2002), its resources (over $1 billion in cash at the end of 2016), and its ambitions, with two more phase 3 programs set to launch in 2017, in hemophilia and acute hepatic porphrias. At the American Society of Hematology (ASH) annual meeting in December, Alnylam reported that its fitusiran oligo was able to knock down its target, antithrombin, by a mean of over 80% in its phase 1 hemophilia trial and in that trial's phase 2 extension. Fitusiran treatment reduced the number of bleeding episodes in patients without inhibitors (antibodies to infused clotting factor) from an annualized median of four episodes before treatment, to just one, and in patients with inhibitors from 31 episodes to a median of zero, with most patients bleed-free[11,12]. In November, acting on a previous agreement, Cambridge, Massachusetts–based Sanofi Genzyme elected to co-develop and co-commercialize fitusiran with Alnylam. Also at ASH, Alnylam reported that its siRNA for acute hepatic porphyrias reduced the rate of porphyria attacks by an average annualized 74% rate compared with the pretreatment period[13].

Other RNAi companies, such as Dicerna and Arrowhead, have also adopted GalNAc-based delivery platforms, as have antisense companies Regulus, in San Diego, and Ionis. Last year, Arrowhead dropped its dynamic polyconjugate delivery platform in favor of subcutaneous GalNAc, according to Haussecker—in September, the company signed a $674-million cardiovascular deal with Amgen for its new preclinical candidates. In terms of resources committed, "based on investments, it's Alnylam, then Arrowhead, Dicerna," says Haussecker. Silence Therapeutics (formerly Atugen), based in London, is also pursuing GalNAc conjugates preclinically.

These companies are not only using the same basic platform but are targeting many of the same mRNAs. Both Dicerna and Alnylam, for example, are targeting the enzyme glycolate oxidase in primary hyperoxaluria, an ultrarare disease caused by liver oxalate overproduction. Dicerna is following Alnylam's lead in targeting PCSK9 in lipid disorders, and Arrowhead and Alnylam both have programs in alpha-1 antitrypsin (AAT) deficiency and in hepatitis B. "It's maybe a good idea for the field to have like two or three candidates targeting the same gene for the same indication," says Haussecker. "Because it's quite likely that one or two of them will fail, maybe for off-target reasons." This happened in September, when Alnylam discontinued its leading AAT oligonucleotide after 3 of 15 healthy volunteers experienced dose-dependent liver enzyme elevation, signaling liver toxicity. Alnylam attributed the problem to microRNA-like off-target effects and is advancing a new molecule with a different sequence to the clinic.

Alnylam's most immediate competitive challenge is not from other RNAi therapeutics but from antisense oligos. The antisense field has struggled for over three decades to produce a commercially viable product, and now seems to have broken through with Exondys 51 (eteplirsen) from Sarepta Therapeutics in Cambridge, Massachusetts, approved by the US Food and Drug Administration in September for Duchenne muscular dystrophy, and especially with Ionis's Spinraza (nusinersen), approved in December for spinal muscular atrophy. Ionis has three other liver programs in phase 3, including its IONIS-TTRrx antisense oligo, targeting transthyretin in hereditary ATTR-polyneuropathy. This trial will read out in the second quarter of 2017, ahead of Alnylam's patisiran, setting up a market clash next year, assuming both get approved.

This could be the first of many commercial battles between antisense and RNAi, two technologies that are targeting many of the same diseases. The technologies have similarities. Both hybridize target mRNAs through Watson–Crick base-pairing, and both employ the cellular enzymatic machinery to cleave target mRNA (RNase H1 and Argonaute, respectively). The siRNA field has incorporated many of the chemical modifications promoting nuclease resistance pioneered with antisense, albeit with a focus on ensuring that they do not affect RISC activity.

Antisense has better cellular penetration properties, whereas siRNA is more potent intracellularly, but delivery vehicles have blurred this difference: IONIS-TTRrx is a weekly subcutaneous injection, whereas patisiran is given intravenously once every three weeks. Antisense, unlike siRNA, does not stimulate a strong innate immune response. "We'll be best," said Ionis CEO Stanley Crooke at the J.P. Morgan conference in January. "No need to go to an infusion center, no need to go to the hospital, no need for high-dose steroids, and no risk of infusion reactions that break through despite high-dose steroids." But some cases of severe platelet declines in patients on IONIS-TTrx and on a different Ionis oligonucleotide last year raised safety concerns for the company's platform. (No further cases have been reported as of late January.) Besides a safety edge—patisiran treatment has not caused large platelet declines—Alnylam's Vaishnaw says that patisiran has so far shown greater target knockdown efficiency.

But patisiran, no matter how successful, will be the last of Alnylam's LNP-encapsulated drugs. And the company's GalNAc-conjugated "enhanced stabilization chemistry" (ESC) oligos promise a large improvement in potency and durability over its previous chemistry (which was used to make the now-discontinued revusiran). These incorporate four additional phosphorothioates and include half the 2′-fluoro modifications, replacing them with 2′-*O*-methyls, which together give Alnylam's new compounds the ability to knock down targets with drug exposure that's lower by an order of magnitude or more. Alnylam's second-generation TTR therapeutic, a GalNAc-conjugated ESC siRNA, is potent enough for quarterly subcutaneous dosing. And the inclisiran data are even better. "For the liver I would say that RNAi… looks like the more promising approach, compared to antisense, especially with the long duration of activity," says Haussecker.

Looking beyond liver

For delivery outside the liver, progress has been slow. Because nanoparticle-encapsulated or GalNAc-conjugated siRNAs are efficiently taken up by hepatocytes, liver became the low-hanging fruit. But "Alnylam or some other companies, which are better capitalized now than maybe five years ago, should tackle additional organs," says Haussecker. RNAi otherwise risks becoming a single-organ niche technology, a far cry from its original promise.

One company targeting other organs is the privately held Quark, based in Fremont, California. Its unconjugated, naked siRNAs are blunt-ended 19-mers with alternating 2′-*O*-methyl ribose modifications, and its most advanced program takes advantage of normal siRNA clearance through the kidneys. Quark's p53-targeting oligo, in a phase 3 trial that began last year, is being tested for the prevention of delayed graft function following kidney transplant from older organ donors. (Kidney reperfusion upregulates p53 and triggers apoptosis; temporary inhibition by Quark's oligonucleotide offers a window for kidney recovery.) Novartis in Basel has an option to develop this molecule, which was the first siRNA to be delivered systemically to people, back in 2007. Quark's second-generation naked siRNA, with alternating methyls only on the guide strand, is targeting caspase-2 in a phase 2/3 trial in non-arteritic ischemic optic neuropathy (NAION), a rare complication of cataract surgery. It's delivered by intravitreal injection. Quark hopes to finish this trial in three years. "We are fully funded" by financial institutions in Japan, says Quark chief medical officer Shai Erlich.

Quark also has plans to target the heart, lung, skin, brain, and inner ear with its oligos, in each case using local delivery methods. But local delivery of naked siRNAs is a risky strategy for commercial sustainability, because it typically involves organ-specific surgical or invasive procedures, which can be expensive and logistically complex. And temporary target inhibition in the kidney has limited applications. "I'm not taking them very seriously from a platform perspective," says Haussecker. RXi Pharmaceuticals in Marlborough, Massachusetts, is also employing local delivery for its asymmetric, chemically stabilized, cholesterol-modified siRNAs, which show rapid uptake into various non-liver tissues.

Avidity Biosciences in La Jolla, California, is testing antibody–siRNA conjugates preclinically, for delivery to tumors, heart, muscle, lung, liver, and B cells. "Commercially, this is the next step after GalNAc," says Khvorova. Meanwhile, Solstice Biologics, in San Diego, is developing short interfering ribonucleotide neutrals (siRNNs)[14]. The phosphate backbone incorporates neutral phosphotriester groups, allowing delivery into cells. Once inside, siRNNs are converted by cytoplasmic triesterases into native-charge phosphodiester backbone siRNAs. But there are far fewer RNAi therapeutics companies doing research into novel delivery systems than a decade ago. "It's really thinned out a lot," says Haussecker. "That's also probably the case in academia."

Cancer has been the most attempted non-liver target. Arrowhead, Alnylam, Atugen (now Silence Therapeutics), Tekmira (now Arbutus), and Dicerna all took their shot. But none of their cancer drugs remains in clinical development. Human trials "were pretty disappointing," says Haussecker. "Adverse events, and hardly any signs of clinical efficacy." Trials were based on the premise that tumor blood vessels are leaky, with large pores, and that nanoparticles carrying siRNAs would preferentially diffuse into tumors and accumulate

there following systemic delivery. Despite animal evidence for this "enhanced permeability and retention" effect[15], it did not translate well to humans. "That whole theory about retention and leakiness is really overstated," says Lieberman, who points out tumors are not very well vascularized. "I don't think those particles are going to be the solution." Most companies no longer are interested in cancer; the only currently active trial for an anticancer siRNA is an investigator-sponsored trial at the MD Anderson Cancer Center in Houston.

The trouble with lipids

The MD Anderson program, currently in phase 1 for advanced solid tumors, employs an LNP carrier with a neutral charge. "We feel that it would be a safer modality compared to some of the more charged carriers," says MD Anderson oncologist Anil Sood. Cationic lipids, however, have by far been the most preferred liposomal carrier in clinical trials because they deliver their enclosed siRNAs efficiently into cells. They couple with negatively charged phospholipids in cell membranes, forming bilayer structures that disrupt the membranes, allowing siRNA internalization[16]. But they're toxic, to varying degrees. "With some charged particles, people have seen things like activation of ROS [reactive oxygen species] species in the lungs," says Sood. "People have also reported activation of complement, or nonspecific cytokines being released."

Although cationic lipid carriers aren't themselves proinflammatory, inflammation may result owing to their ability to enhance the crosslinking of siRNAs with intracellular toll-like receptors. The effect is partly related to sequence, so companies carefully screened therapeutic sequences for innate immune stimulation, and also tried various combinations of the same chemical modifications (2′-*O*-methyls and 2′-fluoros) that protect against nuclease degradation. These modifications efficiently prevent modified siRNAs from activating toll-like receptors[17]. But, for unknown reasons, they haven't been completely effective either preclinically[18] or in human trials. Some, like patisiran, have largely avoided severe infusion reactions with steroid pretreatment. But for siRNA delivery, LNPs "are out of fashion," says Haussecker. "There's a lot of clinical data now that show that especially for chronic dosing that you shouldn't be using them."

Arbutus, though, is committed to lipids, at least for now. "LNP is a proven delivery technology," says Arbutus senior vice president for corporate affairs Adam Cutler. The Arbutus LNPs contain four different lipids that facilitate fusion with the siRNA payload during formulation, stabilize the particle in the circulation, and disrupt cell and endosomal membranes to enable payload entry into the cytoplasm. The company, with clinical programs in hepatitis B, Ebola and cancer, shifted over completely to hepatitis B virus (HBV) in July 2015, presumably owing to lack of efficacy in the other diseases.

An improved LNP carrier enclosing three different siRNAs is in phase 2 for chronic HBV. In interim results reported in December, the drug, which targets all four HBV transcripts, achieved a dose-dependent reduction of serum hepatitis B surface antigen, a key marker of biological response. But one of 18 patients receiving multiple doses discontinued treatment because of a transient elevation in liver enzymes. "It's unlikely to be drug-related," says Arbutus CEO Mark Murray.

Murray considers LNP immune effects easily manageable. But even Arbutus is working on GalNAc. "We have our own GalNAc delivery technology in development," Murray says. "If the logic is there to go to GalNAc, we'll have that tool, we'll do it."

LNPs could come back, says Khvorova, if the siRNA chemical modifications now used for GalNAc conjugates prove toxic. Otherwise, once patisiran is approved (or not), the pipeline of GalNAc ESC oligos from Alnylam, Arrowhead, and Dicerna will dictate the field's near-term future. Despite the renewed interest, "there is still a lot of skepticism," Khvorova says. "Negative news has a much higher impact on the field than positive news." For example, the November 13 publication of dramatic inclisiran trial data in *The New England Journal of Medicine*[7] boosted Alnylam's stock by only 7%, with those gains disappearing the next day. "The inclisiran paper should have had an overwhelming, positive impact," says Khvorova. Given past failures and current concerns, it may take more than one drug approval to win over the skeptics.

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Published online 27 February 2017; doi:10.1038/nbt.3810

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