Nature Biotechnology think that Alnylam delivery technologies, some of which were in-licensed from Tekmira, single it out as the front-runner in the systemic RNAi drug delivery space. Last month, Tekmira settled a legal dispute with Alnylam for $65 million and potentially another $10 million next year.

At the Boston University seminar, the company showed that ALN-TTR02 reduced serum TTR protein by up to 94%, with up to 77% knockdown at day 28 after a single dose. Mutations in TTR cause TTR-mediated amyloidosis (ATTR), a hereditary, systemic disease that causes abnormal amyloid proteins to accumulate and damage body organs and tissue. Silence CSO Klaus Giese thinks the Alnylam results are good news for everyone in the RNAi field. "There are roughly 20,000 genes in the human genome, so there are enough for all of us," he says. Davis says that the Alnylam data give him comfort that patients can be dosed with siRNA at up to 1 mg per kilogram [of body weight]. "No one knew that before," he remarks. The compound entered a phase 2 trial for ATTR on 7 June. Two months ago, Cambridge, Massachusetts–based Genzyme, wholly owned by Paris-based Sanofi, paid $22.5 million up front to Alnylam for Asian rights to its ATTR program.

Ram Mahato, a professor of pharmaceutical sciences at the University of Tennessee Health Sciences Center in Memphis, remains circumspect about progress, however. Unmodified siRNA duplexes are labile in acidic environments (such as in the stomach or, at the cellular level, in endosomes and lysosomes) and show susceptibility to degradation by exonucleases in the gastrointestinal tract or blood, and their strong negative charge (which leads to electrostatic repulsion from cell membranes) and comparatively large size (~13 kDa) limit uptake and endosomal release. Furthermore, he says, even larger pegylated LNPs can reduce target specificity and increase immunostimulatory side effects. As a result, Mahato thinks a marketed product is still some way away, although he is confident in the two main approaches are being explored to make siRNA and microRNA compounds patient ready.

One approach is to improve the oligonucleotides' molecular characteristics by modifying the phosphodiester RNA backbone with phosphorothioate, boranophosphate, methylphosphonate or, in a few cases, even locked nucleic acids (in which a methylene bridge connects the 2′-O with the 4′-C of the ribose). Sugars can also be replaced with 2′-deoxy- or 2′-deoxy-2′-fluoro-, with 2′-fluoro-β-D-arabinose, or with 2′-*O*-(2-methoxyethyl)-, 2′-*O*-alkyl- or 2′-*O*-methyl (2′-*O*-Me) groups. These modifications increase serum stability, cellular uptake and cytosolic release.

Indeed, nearly all companies working in RNAi therapy are chemically modifying their oligonucleotides. For example, Alnylam's siRNAs are modified with 2′-*O*-Me, with a few 2′-deoxy-2′-fluoro–modified nucleotides and a phosphorothioate linkage between the thymidines at the 3′ end. Similarly, Quark, based in Fremont, California, uses 2′-*O*-Me–modified siRNAs (a chemistry licensed from Silence) to protect against nuclease degradation and prevent an innate immune response. Their products are being developed for local injection to the eye and for systemic delivery to target the kidney—a logical organ choice, given that naked RNAs naturally travel to the kidney quickly, before they degrade.

The other approach is to combine siRNA with improved drug delivery vehicles. For example, PEG can be added to cationic lipid nanoparticles to mask the positive charge that causes proinflammatory responses. Overall, a balance is sought between having sufficient charge to prevent self-aggregation and siRNA complex formation, on the one hand, and avoiding nonspecific interactions with cell membranes and plasma proteins and uptake by the reticuloendothelial system, on the other. "Many companies and researchers have made progress, but the technologies are not yet good enough to make therapeutics," Mahato cautions.

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For many delivery technologies, the goal is to not only protect the siRNA from chemical or enzymatic degradation, but also prevent activation of the toll-like receptor 3 double-stranded RNA (dsRNA)-sensing system and enhance siRNA release from endosomes upon arrival at the target site and entry into the RNA-induced silencing complex. Even then, dsRNA liberated into the cytosol may activate nuclear factor κB and interferon-α through its interaction with the retinoic acid–inducible gene I product. GU-rich siRNA sequences (whether dsRNA or single-stranded RNA) may also trigger toll-like receptor 8 interaction and signaling, leading to immune responses. For systemic delivery, particles must be larger than 10 nm to avoid rapid renal clearance but not larger than 150 nm to avoid opsonization by cells of the reticuloendothelial

IN brief

Merck tests needle-free vaccines

In October, the Whitehouse Station, New Jersey–based Merck agreed to license a novel vaccine delivery system from Sydney, Australia–based biotech Vaxxas, for testing with an undisclosed Merck vaccine candidate. The biotech's Nanopatch, needle-free delivery platform is a densely packed array (>20,000/cm^2) of 110-μm-long needles dry-coated with vaccine. The antigens are delivered just below the skin's surface where they target around 50% of the skin's immune cells triggering a strong immune response. The Nanopatch delivered Merck's human papillomavirus (HPV) vaccine Gardasil into mouse-ear epidermal and dermal skin. The vaccine prompted production of virus-neutralizing antibodies, in all mice (*PLos One* **5**, e13460, 2010). With Nanopatch delivery, the vaccine dose could be cut to as little as one-hundredth of that of a traditional vaccine, reducing the cost for high-priced or difficult-to-manufacture antigens and easing the pressure on strained resources in pandemics. The system has other advantages, according to Professor Ian Frazer, director, Diamantina Institute, University of Queensland: "Microneedles use dried vaccine material, so don't need to be kept cold, and could even be posted out for use at home, for example, as traveler's vaccines or in remote areas." *Suzanne Elvidge*

Linked-in for angels

The first dedicated UK national angel network has been launched to help raise much-needed capital for fledgling life sciences companies. Angels for Life Sciences (A4LS), which held its inaugural meeting in London on November 6, stands out by bringing together investors with experience of the sector and generalists who usually shy away from biotech for fear of the unknown. The network is sponsored by the UK's BioIndustry Association, the international law firm Fasken Martineau, UK investment foundation Nesta, one of the UK's first business angel networks OION, and medical research foundation Wellcome Trust. Participating companies are vetted and each has to bring a cornerstone investor, who provides 10–15% of the required cash and acts as a mentor. The sponsors are funding two meetings. The first, which brought together five companies and 30 potential investors, will be followed by one in northwest England next year. Many early-stage UK life sciences firms have found it tough to raise capital, says Simon Kerry, A4LS network manager, and founder and CEO of Karus Therapeutics in Chilworth, UK. One of the difficulties is getting technical complexities across to generalist investors. Myra Waiman, CEO of Romney Consulting and one of the 30 investors in London, says: "I remember when first investing in biotech feeling utterly overwhelmed and intimidated at similar events. This had a very different, friendly feel about it, with all parties talking to each other." *Barbara Casassus*