Neutralizing the negatives of RNAi

By Kai-Jye Lou, Senior Writer

Despite years of attempts with liposomes, nanoparticles and conjugation strategies, delivery of siRNA to organs beyond the liver remains the biggest hurdle to using the technology for a host of diseases. A **University of California, San Diego** group has changed the rules with a prodrug strategy that delivers siRNA into cells as an uncharged oligonucleotide, bypassing the limitations of siRNA's negative charge.[1] But the real breakthrough will be to show that the prodrugs work in a range of cells and tissues that standard siRNAs cannot easily reach.

Solstice Biologics LLC obtained an exclusive worldwide license to the technology last year and set up shop to translate the strategy into therapeutics. The company was cofounded by Steven Dowdy, the UCSD investigator behind the invention, and Curt Bradshaw, who is CSO.

Dowdy is a professor in the Department of Cellular and Molecular Medicine at the **University of California, San Diego School of Medicine**.

The goal of Dowdy's strategy was not new as—like others—he wanted to mask the negative charges on the RNA backbone's phosphate groups that have long been known to limit the molecules' cellular uptake and circulating half-lives.

However, what Dowdy managed that others failed to do is find a way to hide the charged groups without irreversibly conjugating them to polymers or sugars or encapsulating the siRNAs in large particles—none of which has yielded efficient delivery of siRNAs to tissues outside the liver.

Instead, he used a reversible chemical modification with phosphotriester groups that neutralize the charge. This enables the molecules to enter cells and undergo cleavage by intracellular thioesterases to release the active siRNA (*see* **Figure 1, "siRNA delivery free of charge"**).

The group dubbed the new molecules short interfering ribonucleic neutrals (siRNNs).

"Our overall goal was to molecularly sculpt the outside of the siRNA to disguise it to look like a protein by introducing these phosphotriester groups," Dowdy told *SciBX*.

In addition, he said, the modifications they used protect the siRNNs from degradation by circulating nucleases. "Proteases cannot digest them either because siRNNs are not actually proteins, and the kidney doesn't filter them out because they do not have a massive negative charge," he said.

Dowdy has been working on siRNA delivery for over a decade and previously cofounded Traversa Therapeutics Inc. on a different strategy to protect and deliver siRNAs.

Traversa's platform involved two different components: one to shelter siRNA from degradation in plasma (a double-stranded RNA binding domain) and the other to allow uptake into targeted cells (a peptide-transduction domain).[2] However, the company failed to secure enough funding to continue operations and filed for bankruptcy in 2012.

The prodrug technology, by contrast, focuses directly on the phosphate backbone that is the source of the charge problem.

"Phosphate chemistry is known to be very problematic, and the phosphate backbone of siRNA has not been very amenable to chemical manipulation," said Dowdy. "It took our lab 8 years and around $7 million to arrive at our current solution. There were eight chemistry problems that needed to be solved in a manner where every solution is compatible with one another."

He added, "What we have done over the years is work out the universal rules to negate the negative charges on the phosphate backbone of siRNA. In aggregate, we've already created a library of over 100 of these charge-neutralizing phosphotriester groups, and our goal is to more than double that."

> **"To me the reversible chemistry is a key component of this work, which I feel can be expanded to other RNA-based therapeutic strategies."**
> ***–John Rossi, Beckman Research Institute at City of Hope***

Dowdy's group had to tinker with multiple variables on the molecules to create compounds that could be formulated as drugs. The siRNNs they finally produced were soluble, stable in serum, nonimmunogenic and had synthetic yield rates above 90%. In addition, unlike siRNAs, the siRNNs bound serum albumin strongly, which suggests they might have slower clearance from plasma than siRNA.

Finally, the team tested the potency of siRNNs *in vivo* versus siRNAs by conjugating constructs of each to hepatocyte-targeting domains. In mice, different formulations of liver-targeted siRNNs against *apolipoprotein B* (*Apob*) decreased mRNA expression up to 60% compared with what was seen in water-treated controls. In contrast, siRNA against *Apob* that was also targeted to the liver decreased mRNA expression by about 20%.

Results were published in *Nature Biotechnology*.

According to John Rossi, the approach Dowdy used to generate siRNNs could accelerate the development of siRNA drugs. "To me the reversible chemistry is a key component of this work, which I feel can be expanded to other RNA-based therapeutic strategies," he told *SciBX*.

Rossi is chair of the Department of Molecular and Cellular Biology at the **Beckman Research Institute at City of Hope**. He has cofounded multiple RNAi therapeutics companies including Calando Pharmaceuticals Inc. (now part of **Arrowhead Research Corp.**), **Dicerna Pharmaceuticals Inc.** and **MiNA Therapeutics Ltd.**

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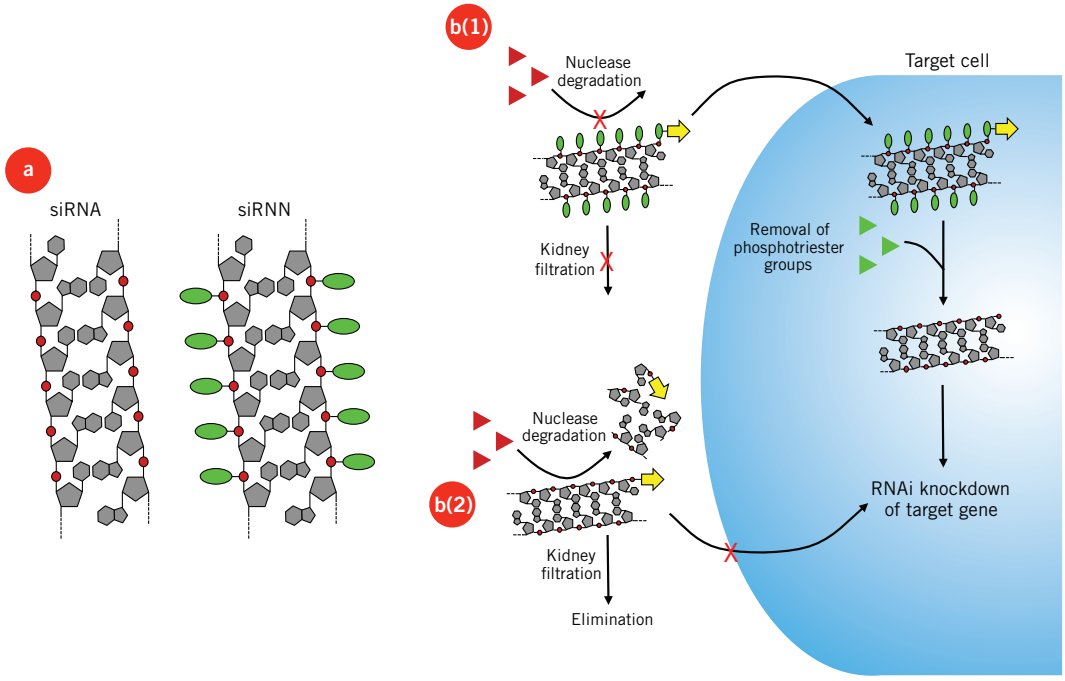

Figure 1. siRNA delivery free of charge. Short interfering ribonucleic neutrals (siRNNs) are a class of RNAi prodrugs that solve the delivery limitations of standard siRNAs and enable knockdown of disease-relevant genes in a range of target cell types.

[**a**] siRNNs are double-stranded siRNAs modified along their phosphate backbones with neutral phosphotriester groups (green ovals) that hide the negatively charged phosphate groups (red circles).

[**b(1)**] siRNNs can be conjugated to a targeting domain (yellow arrow) to deliver them to specific cells. Upon entering a target cell, cytoplasmic thioesterases (green triangles) cleave the phosphotriester groups from siRNNs, leaving siRNAs that can knock down expression of the target gene.

The phosphotriester groups on siRNNs prevent the molecule from being degraded by circulating nucleases (red triangles) or being removed by the kidney. However, the negatively charged phosphate backbone of the oligonucleotide must be accessible for RNAi knockdown activity, so these phosphotriester groups have to be removed from the siRNN after it enters the target cell, as described above.

[**b(2)**] *In vivo* delivery of naked siRNAs is generally ineffective for knockdown of target genes because these oligonucleotides are rapidly degraded by circulating nucleases or filtered out by the kidney. The exposed negatively charged phosphate backbone of siRNAs also hinders the oligonucleotide's ability to cross cellular membranes.

Beyond the liver

For siRNNs to have a major impact on the field, they will have to demonstrate activity in cells and tissues outside the liver.

While that remains to be tested, Rossi believes there is a fair chance of success. "I feel these siRNNs will expand the potential disease applications since most of the lipid carrier formulations favor delivery to the liver while siRNNs should traffic throughout the body," he said.

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> ***–Curt Bradshaw, Solstice Biologics LLC***

Bradshaw added, "These siRNNs are not limited by diffusion like lipid nanoparticles. They can exit the vasculature and penetrate into the tissues and cell types we want to target. Because of the way these siRNNs are derivatized, we believe we can take advantage of many types of targeting domains to deliver siRNNs to different cell types."

Bradshaw said that he does not view other RNAi companies, such as **Alnylam Pharmaceuticals Inc.**, **Tekmira Pharmaceuticals Corp.** and Dicerna, as direct competitors as it is very straightforward with RNAi platforms to go after targets that are not already being pursued by another company.

"We think other RNAi companies like Alnylam could be potential partners," added Solstice president and CEO Lou Tartaglia. "Alnylam is currently focused on the liver, and I think they would very much like to see companies move RNAi technologies beyond liver."

Alnylam did not respond to requests for comment. Tekmira and Dicerna declined to comment.

Tartaglia is not saying what specific indications the company plans to pursue first, but he did note that based on discussions with other members of industry, cells and tissues of interest for strategic alliances include T cells and B cells, as well as muscle, kidney and lung tissues.

Rossi said that he would like to see breast and prostate cancers tested because they have been difficult to target with standard siRNA delivery methods. He added that diseases of the blood such as leukemia and viral infections also could be good indications for siRNNs.

Bradshaw told *SciBX* that Solstice has "pharmaceuticalized" the approach described by Dowdy's group and is continuing to develop the capabilities of the siRNN platform with new siRNN formulations and planning nonhuman primate studies.

UCSD and Solstice have filed patent applications covering the chemistry used to create siRNNs.

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