Family seeds

By Amy Donner, Senior Editor

It has been long assumed that the specificity of microRNA inhibitors is determined by length—dogma held that anti-miRNAs should be more than 16 nucleotides and approximate the length of the actual miRNA targets. Now, an international team has *in vivo* proof of concept that using anti-miRNAs of only eight nucleotides in length does not compromise selectivity and provides a simple tool for blocking multiple miRNAs at once.¹

The researchers are planning a Phase I trial following additional toxicology studies in animals.

miRNAs are small noncoding RNAs that regulate gene expression by binding to specific mRNAs via complementary base pairing. Typically, a miRNA regulates one or several different mRNAs. In addition, an mRNA can be targeted by several miRNAs. This regulatory redundancy makes it challenging to intervene therapeutically.

Affinity and specificity of binding between miRNAs and mRNAs is driven in large part by complementary base pairing interactions. Thus, most companies developing anti-miRNAs have opted to focus on molecules that approximate the length of their miRNA targets to maximize such interactions.

Santaris Pharma A/S has the only disclosed anti-miRNA in the clinic. The company's miravirsin, an anti-miRNA targeting miR-122, is in Phase II trials to treat HCV.

Several companies have preclinical anti-miRNA programs, including **Regulus Therapeutics Inc.**'s anti-miRNA that simultaneously targets miR-33a and miR-33b, which control genes involved in regulating cholesterol.²

Regulus is developing its miR-33-targeting anti-miRNA preclinical program in collaboration with **AstraZeneca plc**.

Recently, researchers at Santaris, **Cold Spring Harbor Laboratory** and **Aalborg University** proposed an alternative strategy for simultaneously inhibiting multiple miRNAs by using 8mer anti-miRNAs against a region of 6–8 nucleotides called the seed region that is responsible for mRNA target selection.³

Seed regions tend to be conserved among redundant miRNAs. The approach was shown to work *in vitro* and in mice.⁴

One major advantage of developing shorter, seed-targeting anti-miRNAs is that they can be rationally designed. In contrast, their longer counterparts need to be developed empirically.

Now, a group led by Anders Nääär has tested the 'shorter is better' strategy in a nonhuman primate model and shown that it has therapeutic

potential. The team used an 8mer anti-miRNA oligonucleotide that targets the seed region of miR-33a and miR-33b.

Nääär is a professor of cell biology at **Harvard Medical School** and the **Massachusetts General Hospital Cancer Center**.

First, Nääär's team screened an anti-miRNA library and isolated a locked nucleic acid (LNA)-modified, 8mer anti-miRNA that inhibited miR-33a and miR-33b activity in binding and gene expression assays.

Next, the group tested the activity and specificity of the anti-miR-33a/b in cells and mice. The 8mer de-repressed miR-33a and miR-33b at the RNA and protein level in a human liver cell line, and it increased high-density lipoprotein (HDL) levels in mice compared with saline.

In obese African green monkeys fed a high-fat diet, subcutaneous injection of anti-miR-33a/b increased HDL up to 39% compared with vehicle. Liver biopsies showed that anti-miR-33a/b de-repressed several miR-33a and miR-33b targets.

Anti-miR-33a/b was well tolerated for more than 108 days and had a plasma half-life of 17.5 days, which is comparable to half-lives reported for longer anti-miRNAs. There were no injection site reactions or adverse effects.

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—Sakari Kauppinen,
Aalborg University Hospital

These results were similar to nonhuman primate data reported by Regulus showing the effects of longer anti-miRNAs targeting miR-33a and miR-33b. Regulus researchers also saw increased hepatic expression of miR-33a and miR-33b targets and higher plasma levels of HDL cholesterol compared with using mismatch control anti-miRNA.⁴

The new study was published in *Science Translational Medicine*. The team included researchers from Santaris, **Aalborg University Hospital**, **Duke University Medical Center**, **RxGen Inc.** and **Weill**

Cornell Medical College in Qatar.

Santaris was involved in every stage of the study but did not respond to requests for comment. RxGen carried out the nonhuman primate studies.

"The nonhuman primate study is an important step toward development of therapeutics inhibiting entire miRNA families sharing the same seed sequence," said study coleader Sakari Kauppinen, a professor of hematology at Aalborg University Hospital.

Targeting the seed

Nääär's group now plans to run toxicology studies in rodents and nonhuman primates prior to a Phase I safety trial. The next step would be testing anti-miR-33a/b therapy in patients with familial hypercholesterolemia.

According to Nääär, about 47% of all conserved miRNAs are members of families sharing the same seed sequence, suggesting that this approach could be useful in other diseases associated with miRNA families. The ability to target multiple miRNAs safely and efficaciously using a single 8mer therapeutic represents an important alternative approach for the field.

Neil Gibson, CSO of Regulus, said that papers about 8mers "establish proof of concept of therapeutic potential. The latest study provides

additional compelling evidence that the miRNA-targeting approach is safe and well tolerated.”

Gibson added that targeting the seed is not the only strategy for hitting families of miRNAs.

“All miRNAs in a family do not necessarily interact with the same mRNAs. The way you target the family may be strongly influenced by the mRNAs you are trying to modulate. With longer anti-miRNAs you can differentially regulate members of the same family, taking advantage of both seed and nonseed sequences. Integration of chemical modifications can also help you control selectivity of anti-miRNAs for miRNAs,” he said.

Massachusetts General Hospital has filed for patents in the U.S., Europe and Japan covering the therapeutic targeting of miR-33a and miR-33b to treat cholesterol-related disorders and for regulating lipid metabolism. The patent applications include anti-miRNAs from 8mer up to full complementarity with miR-33a and miR-33b.

Regulus’ patent portfolio includes patents and patent applications covering the sequences and complementary sequences of anti-miR-33a/b, composition of matter covering various chemically modified anti-miRNAs and method of use for targeting miR-33a and miR-33b to treat cardiovascular disease.

Näär acknowledged that “the miR-33 IP landscape is complex. I think MGH is on solid ground, but anyone interested in licensing our miR-33 IP will obviously do their own due diligence.”

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