miR-122 Continues to Blaze the Trail for MicroRNA Therapeutics

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As with the development of therapeutics based on RNA interference and traditional messenger RNA (mRNA) targeting with antisense, it is a liver-specific target RNA—microRNA-122 (miR-122)—that has emerged as the lead candidate for a microRNA therapeutic that could have the first meaningful clinical impact. miR-122 is by far the most abundant microRNA in the liver, and possibly in the entire human microRNA repertoire.1,2 This early realization has spawned several investigations of the function of this tissue-specific microRNA. These show it to be important for liver cell identity,1,4 lipid metabolism,3,5 and, perhaps curiously, hepatitis C virus (HCV) replication.6 Given the sheer abundance of this microRNA and the multitude of mRNAs that are derepressed following its inhibition,3 it is possible that more functions will emerge. Because the liver is the organ that is most amenable to the delivery of oligonucleotides following systemic administration, antisense-targeting experiments in both mice and nonhuman primates contributed quite early and very significantly to the functional definition of miR-122, especially its role in maintaining cholesterol levels3,7 and in HCV replication in a chimpanzee model.8 As a result of such rapid progress, clinical trials are now under way to investigate the use of miR-122 as an antisense target for the treatment of chronic HCV infection. The phase I safety trials are sponsored by Santaris Pharma and involve healthy adult volunteers.

Setting the stage for targeting miR-122 in chronic HCV infection

It is the fact that this microRNA promotes rather than inhibits the function of a noncellular target RNA that has rendered miR-122 an attractive therapeutic target.6 Through a combination of microRNA inhibition with simple 2′-O-methylated antisense molecules and elegant genetic studies, Jopling and colleagues determined that miR-122 engages two highly conserved sites in the 5′ noncoding region (NCR) of HCV in a seed-dependent manner, so as to facilitate the accumulation of HCV RNA.9 A reduction of approximately 1 log in genotype 1 HCV RNA was observed in both Huh-7 replicon and transient transfection models when miR-122 was sequestered in this fashion.

It was around the same time that the feasibility of miR-122 inhibition in vivo was first demonstrated. Administration of relatively large amounts (three doses of 80 mg/kg on each of 3 consecutive days) of cholesterol-conjugated, 2′-O-methylated antisense oligonucleotides to C57BL/6J mice led to functional miR-122 inhibition within a week, as demonstrated by an approximate 40% reduction in total cholesterol and concomitant increased expression of predicted target genes, such as Aldoa.10 A study published the following year showed similar effects with a phosphorothioate 2′-O-methoxymethyl anti-microRNA, which produced largely comparable results when administered over a more extended period of time (4 weeks).

In 2008, Elmén and colleagues demonstrated the feasibility of functional miR-122 sequestration in nonhuman primates.7 The authors employed an unconjugated, 15-nucleotide phosphorothioate DNA-LNA (locked nucleic acid) mixmer, SPC3649, that was shorter than the previous miR-122 antisense compounds, which were complementary to the entire length of miR-122. This modification was supposed to facilitate cellular uptake, and the use of high-affinity LNAs was expected to compensate for the shortened base-pair complementarity. Indeed, Elmén et al. showed in their initial exploratory Huh-7 tissue culture studies that binding affinities correlate with miR-122 functional inhibition (via luciferase reporter and HCV replication assays) and that SPC3649 was more potent than the cholesterolol-conjugated 2′-O-methyl antigamins in a head-to-head comparison in C57BL/6J mice. These attributes translated into lowering total cholesterol in African green monkeys by 30–40% following three intravenous injections of 10 mg/kg over 5 days. The effect was dose-dependent, maximal at 1–2 weeks after administration of three doses of 10 mg/kg, and the reduction

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in cholesterol was maintained for 7–9 weeks. Importantly, the authors reported no abnormalities in clinical chemistries or other adverse events attributable to the mixmer. Unfortunately, the authors did not monitor the expression of cytokines, which would be important when interpreting the results of the mixmer’s application for the treatment of HCV. In any case, this study established that cholesterol lowering is a consequence of miR-122 sequestration not only in mice but also in primates. Well established in the preclinical models and readily measurable, cholesterol concentrations may prove to be a valuable biomarker for determining the optimal dosage in the first clinical trials to treat HCV infection in humans. However, in all animal models studied, there is a lowering of both the “good” (high-density lipoprotein (HDL)) and the “bad” (low-density lipoprotein) cholesterol, with relatively pronounced HDL lowering at the higher dosages.

**Anti-miR-122 shows considerable HCV antiviral efficacy in chimpanzees**

These studies set the stage for testing the effect of miR-122 inhibition with the LNA-DNA mixmer in the only bona fide animal model for chronic HCV infection in humans: chronically infected chimpanzees. One drawback of this model is the limited availability of chimpanzees in general and HCV-infected chimpanzees in particular, and the associated ethical and financial costs of such studies. In order to derive maximal insight from the use of only four chimpanzees, which were chronically infected with genotype 1 HCV, the study contained a 4-week run-in period, during which saline placebo was administered, to establish baseline HCV burden. Following this, two animals each received either 12 weekly doses of 1 mg/kg or 5 mg/kg anti-miR-122, both of which seemed to be well tolerated when assessed for markers of tissue damage. Notably, there was a reduction of more than 300-fold in HCV burden in the serum of both animals at the high dose, whereas in the low-dose group one animal experienced a 1.3-log reduction in HCV titer and the other showed no measurable response. In the latter animal, there seemed to be a discrepancy between the apparently effective miR-122 inhibition by northern blot analysis and the lack of anti-HCV activity. This may, however, be a simple artifact of duplex formation of the tightly binding anti-miR with the target microRNA, which can occur after RNA isolation and is a general complication when determining the degree of microRNA sequestration by northern blot analysis and real-time quantitative reverse transcription–polymerase chain reaction assays. Maximal HCV responses were observed 2 weeks after the administration of the last dose, which was probably the consequence of the approximate 3-week half-life of the mixmer and indicates that more protracted dosing might further lower HCV titers. HCV titers returned to baseline roughly 12 weeks after the last dose.

However, because the effect on HCV titers was surprisingly potent as compared with the earlier tissue culture results, and because the response in the low-dose group was variable, it was important to rule out a contribution from cytokine-like responses. Although no such elevations were observed, the ability to exclude all such responses will require more complete studies, including more sensitive markers. In addition, even considering the difficulty of performing these studies, it is unfortunate that no control group (even a crossover design) receiving a scrambled mixmer was included, as this would have helped identify any nonspecific responses. Nucleic acid–based therapeutics have been fooled in past studies by extrapolating the efficacy, safety, or both, of an approach from historical controlled studies in unrelated experimental systems and species. Thus, it could be argued that even given the added cost, such controls should be an important component of future studies. Of course, not all human responses are predictable even with the best and most comprehensive preclinical animal studies. An on-target explanation for the remarkable antiviral potency of microRNA sequestration in vivo that does not invoke disruption of the miR-122–HCV RNA interaction is the contribution of miR-122-dependent changes in lipid metabolism, including a 40% lowering of total cholesterol in the chimpanzee model. Such changes are known to affect HCV replication. It is also possible, however, that the apparent discrepancy may be simply a reflection of the differences between the tissue culture and chimpanzee systems.

Remarkably, no viral rebound was observed during treatment with SPC3649, which would have indicated viral escape mutations. These are often observed with single-agent direct antivirals, including those of similar potencies and in the same model system. In agreement, deep sequencing of the 5’ NCR found no evidence for compensatory changes of the sequences around the miR-122 binding site. This is consistent with their high conservation and bodes well for treatment strategies employing the anti-miR-122 molecules. Nevertheless, it will be interesting to study whether subtle changes eventually emerge that increase the affinity of the 5’ NCR–miR-122 interaction, in that this would further establish the on-target nature of the antiviral efficacy. Functional miR-122 sequestration was demonstrated by the enrichment of mRNAs with miR-122 seed matches in the transcript populations whose expression was increased, with the exception of the findings in the animal that did not experience HCV repression. By contrast, the expression of many interferon (IFN)-regulated genes decreased in parallel with HCV titers, leading the authors to speculate that there might be an added beneficial effect due to resetting of the IFN responsiveness of otherwise IFN-resistant patients.

**Future outlook**

In sum, the evidence suggests that anti-miR-122 could be a valuable addition to future HCV antiviral combination therapies. There was a more than 2-log efficacy in the gold-standard animal model for chronic HCV infection, a novel mechanism of action targeting a host factor, and an absence of viral escape mutations during the 12-week treatment period, all of which occurred without significant toxicity. The timing of the inhibition of anti-miR-122 function in vivo was delayed as compared with the results in tissue culture models, especially when using the unconjugated compounds. Because rapid viral response rates are generally predictive of HCV treatment success, future anti-miR-122 candidates may involve the administration of conjugates similar to the cholesterol-conjugated antagonim or nanoparticles/liposomes to more rapidly achieve the required therapeutic concentrations of...
intracellular anti-miR-122 during the loading phase. This may also shorten treatment duration, an important factor for compliance, and reduce the risk for viral escape mutations even further.

Compliance has been a problem with the current standard of care, which consists of 48 weeks of IFN-ribavirin. Even then, only 40–50% of patients with genotype I—the predominant genotype in the Western world—achieve sustained viral responses, i.e., the inability to detect HCV.13 Direct antivirals, most notably protease and polymerase inhibitors, have made good progress in the clinic, and the approval of several HCV protease inhibitors expected within the next 1–2 years will increase cure rates to 60–70% in treatment-naïve patients with genotype I.13 RNAi-based treatments, for use in both synthetic and DNA-directed approaches targeting the viral RNA for nucleolytic cleavage, are in earlier, preclinical stages of development4 and represent yet another promising new mechanism of action for combination therapy. In addition to increasing cure rates for these patients, an important development goal of all these rapidly acting antivirals is to shorten the duration of IFN treatment, if not abolish the need for IFN and ribavirin altogether. They further provide hope for those in whom prior therapies have failed and who are most in need of new therapies. In this setting, an anti-miR-122 may be used to reduce the pool of viruses from which resistance could develop, and to this therapy could be added the protease–polymerase antivirals and a shortened course of IFN. Although preclinical studies have thus far focused on genotype I, based on the conserved miR-122 target sites, all of this should be applicable to essentially all genotypes.

There are legitimate concerns about miR-122 as a therapeutic target. Although the liver specificity of this microRNA eliminates on-target toxicities from non-target tissues, the very high abundance of this microRNA in the liver—approximately 70% of microRNAs in the adult liver—implies an important function. Regulating lipid metabolism, including HDL cholesterol concentrations, seems to be one such function, and clinical vigilance with regard to an increase in cardiovascular events is indicated. In addition, considerable evidence has been accumulating that miR-122 is important for maintaining liver cell identity and that its inhibition could increase the risk of developing hepatocellular carcinoma.4,15 This may be of particular concern in conditions that already predispose toward liver cancer. On the other hand, the expected limited treatment duration of anti-miR-122 in HCV infection and the essentially positive safety profile in the preclinical studies reported thus far indicate that anti-miR-122 should be initially considered for HCV patients who are at the highest risk for developing HCV-related morbidity and mortality. This includes treatment-failure populations, although it could be argued that in the case of the liver cancer danger it is this population that is actually at highest risk. It should be remembered that the revelation of HDL lowering and the hepatocellular carcinoma risk are the result of the many studies on this particular microRNA. Instead of leading to the conclusion that miR-122 is therefore an unsuitable therapeutic target, such information should greatly facilitate careful monitoring for possible toxicities of anti-miR-122 therapeutics both in extended preclinical carcinogenicity and toxicology studies and in the clinic.

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