

EDITORIAL



Micromanaging Hepatitis C Virus

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Hepatitis C virus (HCV), a positive-stranded RNA flavivirus, persistently infects approximately 170 million persons worldwide and can lead to cirrhosis and hepatocellular carcinoma. The approval of two HCV protease inhibitors, telaprevir and boceprevir, by the Food and Drug Administration (FDA) in 2011 dramatically improved therapy. These drugs are used in combination with pegylated interferon alfa and ribavirin.¹ Although protease inhibitors have greatly enhanced the sustained viral response rate, these inhibitors are active against only the dominant viral genotype found in North America and Europe (genotype 1), and about a third of patients with genotype 1 infection do not have sustained viral repression. Without concurrent administration of interferon and ribavirin, drug resistance develops rapidly. Moreover, the use of interferon is associated with substantial side effects in many patients, and prolonged treatment (≥ 24 weeks) with these drugs is required to eliminate the virus, after which some patients still have a relapse. The protease inhibitors have nontrivial side effects and interfere with the cytochrome P-450 system, leading to drug–drug interactions.

To address these problems, second-generation protease inhibitors and small-molecule drugs to inhibit other viral enzymes are being evaluated in clinical studies. In the future, drug cocktails that target multiple HCV enzymes simultaneously may overcome the problems of monotherapy, as they have for infection with the human immunodeficiency virus. However, new therapeutic approaches that are less likely to create resistance and are effective against the diverse strains of HCV would be a welcome addition to the drug arsenal.

Janssen et al.² now describe in the *Journal* just such an approach. This strategy arose from the discovery that to survive and replicate in liver cells, HCV depends on the most abundant host liver microRNA, miR-122. MicroRNAs consist of approximately 22-nucleotide RNAs that suppress the expression of messenger RNAs bearing partially complementary sequences by accelerating their degradation and inhibiting their translation into protein.³ The liver-expressed miR-122 binds to two highly conserved sites in HCV RNA, but rather than causing it to be degraded, miR-122 protects it from degradation.^{4–7} All tested strains of HCV depend on miR-122, suggesting that it is a universal HCV Achilles' heel.

The action of a microRNA can be antagonized by introducing small RNAs with a complementary sequence, called antisense oligonucleotides, into cells.^{8,9} However, getting nucleic acids across the cell membrane into the cytosol, where the virus replicates, in vivo and in sufficient amounts for activity without unacceptable toxicity has been challenging.¹⁰ Because the liver is designed to remove toxic substances from the body, the hurdle for nucleic acid delivery to that organ is lowest. In fact, after two decades of research to convert antisense oligonucleotides into useful drugs, the first such drug, mipomersen, which inhibits the expression of apolipoprotein B-100 in the liver, was approved by the FDA to treat familial hypercholesterolemia.¹¹

In the study by Janssen et al., the investigators used a chemically modified antisense oligonucleotide, which has an enhanced binding affinity, is resistant to nucleases, and is able to penetrate cell membranes. As a consequence, miravirsin, the miR-122 antisense oligonucleo-

tide that they tested, enters liver cells and binds tightly and stably to miR-122, preventing the latter from binding to HCV RNA.⁹ A preclinical study in two HCV-infected chimpanzees had shown that miravirsin (at a dose of 5 mg per kilogram of body weight) given weekly for 12 weeks reduced viral load by approximately 2.6 logs without causing drug resistance or serious side effects.¹²

In the phase 2a dose-finding study, Janssen et al. administered subcutaneous weekly doses of miravirsin (3, 5, or 7 mg per kilogram) or placebo for 5 weeks in patients who had not undergone previous therapy for HCV infection. The patients did not receive any interferon or ribavirin but were allowed to add this combination therapy 2 weeks after the last miravirsin treatment in the lowest-dose group and 5 weeks after miravirsin in the higher-dose groups. Miravirsin treatment caused no significant toxic effects, aside from mild injection-site reactions and a transient slight increase in serum liver enzymes, and led to a dose-dependent reduction in viral load. At the highest dose, the viral load declined by approximately 3 logs and was below the level of detection in four of nine patients. There was no sign of drug resistance, as verified by sequencing of the targeted viral sequence. However, after miravirsin was stopped, levels of virus rebounded in patients who had not begun receiving interferon and ribavirin. Since miR-122 normally controls cholesterol levels independently of its effect on HCV, patients receiving miravirsin had a sustained decrease in serum cholesterol levels of approximately 25%, which lasted 14 weeks after the final injection. This result, together with other preclinical and clinical pharmacokinetic and pharmacodynamic findings, suggests that miravirsin (along with other liver-targeting nucleic acid drugs that make use of the RNA interference machinery¹³) has a sustained biologic effect that lasts for weeks. This means that such drugs can be administered infrequently (probably at monthly intervals).

Antagonizing miR-122, alone or in conjunction with other antiviral agents already approved or in development, could provide curative therapy for a large proportion of patients infected with all HCV strains without danger of drug resistance. It could also shorten the treatment time to achieve viral elimination, reduce the rate of

relapse, and offer the possibility of interferon-free regimens. Only further clinical trials can determine whether this promise can be met. A side benefit may be a reduced level of serum cholesterol, which may be especially important in patients with HCV infection because the strong pharmacokinetic interaction between HCV protease inhibitors and statins precludes the dual use of these drugs.

Although short-term administration of miravirsin did not sound any safety alarms, longer-term use could prove to be problematic, since miR-122 is a tumor-suppressor gene for hepatocellular carcinoma. Mice that lack the gene for miR-122 are viable but have a high risk of fatty liver, fibrosis, and hepatocellular carcinoma.^{14,15} Because these conditions are also side effects of HCV infection, careful study design and safety monitoring will continue to be critical. Pending satisfactory answers to the questions regarding safety, one could envisage an HCV RNA drug cocktail composed of miravirsin in combination with other therapeutic agents that might include small interfering RNAs directed against conserved sequences in the viral protease, replication complex, or polymerase genes.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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1. Fox AN, Jacobson IM. Recent successes and noteworthy future prospects in the treatment of chronic hepatitis C. *Clin Infect Dis* 2012;55:Suppl 1:S16-S24.
2. Janssen HLA, Reesink HW, Lawitz EJ, et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med* 2013. DOI: 10.1056/NEJMoa1209026.
3. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215-33.
4. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* 2005;309:1577-81.
5. Jopling CL, Schütz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 2008;4:77-85.
6. Machlin ES, Sarnow P, Sagan SM. Masking the 5' terminal nucleotides of the hepatitis C virus genome by an unconventional microRNA-target RNA complex. *Proc Natl Acad Sci U S A* 2011;108:3193-8.
7. Li Y, Masaki T, Yamane D, McGivern DR, Lemon SM. Competing and noncompeting activities of miR-122 and the 5' exonuclease Xrn1 in regulation of hepatitis C virus replication. *Proc Natl Acad Sci U S A* 2013;110:1881-6.

8. Krützfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs in vivo with 'antagomirs.' *Nature* 2005;438:685-9.
9. Elmén J, Lindow M, Schütz S, et al. LNA-mediated microRNA silencing in non-human primates. *Nature* 2008;452:896-9.
10. de Fougères A, Vornlocher HP, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. *Nat Rev Drug Discov* 2007;6:443-53.
11. Stein EA, Dufour R, Gagne C, et al. Apolipoprotein B synthesis inhibition with mipomersen in heterozygous familial hypercholesterolemia: results of a randomized, double-blind, placebo-controlled trial to assess efficacy and safety as add-on therapy in patients with coronary artery disease. *Circulation* 2012;126:2283-92.
12. Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010;327:198-201.
13. Song E, Lee SK, Wang J, et al. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat Med* 2003;9:347-51.
14. Tsai WC, Hsu SD, Hsu CS, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest* 2012;122:2884-97.
15. Hsu SH, Wang B, Kota J, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest* 2012;122:2871-83.

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