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Antisense therapy for hepatitis C virus infection

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COMMENTARY ON:

Treatment of HCV infection by targeting microRNA. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R, King BD, Kaupinen S, Levin AA, Hodges MR. N Engl J Med. 2013 May 2;368(18):1685-94. doi: 10.1056/NEJMoa1209026. Copyright 2013. Abstract reprinted by permission from the Massachusetts Medical Society.

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Abstract. *Background: The stability and propagation of hepatitis C virus (HCV) is dependent on a functional interaction between the HCV genome and liver-expressed microRNA-122 (miR-122). Miravirsen is a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122 in a highly stable heteroduplex, thereby inhibiting its function.*

Methods: In this phase 2a study at seven international sites, we evaluated the safety and efficacy of miravirsen in 36 patients with chronic HCV genotype 1 infection. The patients were randomly assigned to receive five weekly subcutaneous injections of miravirsen at doses of 3 mg, 5 mg, or 7 mg per kilogram of body weight or placebo over a 29-day period. They were followed until 18 weeks after randomization.

Results: Miravirsen resulted in a dose-dependent reduction in HCV RNA levels that endured beyond the end of active therapy. In the miravirsen groups, the mean maximum reduction in HCV RNA level (log10 IU per milliliter) from baseline was 1.2 ($P = 0.01$) for patients receiving 3 mg per kilogram, 2.9 ($P = 0.003$) for those receiving 5 mg per kilogram, and 3.0 ($P = 0.002$) for those receiving 7 mg per kilogram, as compared with a reduction of 0.4 in the placebo group. During 14 weeks of follow-up after treatment, HCV RNA was not detected in one patient in the 5-mg group and in four patients in the 7-mg group. We observed no dose-limiting adverse events and no escape mutations in the miR-122 binding sites of the HCV genome.

Conclusions: The use of miravirsen in patients with chronic HCV genotype 1 infection showed prolonged dose-dependent reductions in HCV RNA levels without evidence of viral resistance. (Funded by Santaris Pharma; ClinicalTrials.gov number, NCT01200420.)

Keywords: HCV; miR-122; Antisense; DAA.

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MicroRNAs are small non-coding RNAs encoded by the human genome that transcriptionally and post-transcriptionally modify gene expression. The microRNA-122 (miR-122) forms the dominant microRNA in the liver and is exclusively expressed in hepatocytes. It has been implicated in multiple different processes, including lipid metabolism, cell differentiation, iron metabolism and hepatic circadian regulation [1]. In 2005 Jopling and colleagues identified miR-122 as an essential co-factor for hepatitis C virus (HCV) replication [2]. The 5′ untranslated region (UTR) of HCV is highly conserved across genotypes and contains two miR-122 binding sites, disruption of which blocks HCV replication through unknown mechanisms [3]. Miravirsen, a 15 nucleotide long oligonucleotide complementary to miR-122, can form stable heteroduplexes with miR-122 and interfere with HCV replication. Whether miravirsen exerts its antiviral effects predominantly through sequestration of available miR-122, indirectly through disrupting lipid pathways essential to the viral lifecycle, or through other mechanisms remains under active investigation. Its efficacy against chronic HCV infection was first shown in studies in chimpanzees, the only natural HCV animal model. Chimpanzees that received the highest, 5 mg/kg, dose through a weekly infusion had a marked decrease in plasma and liver HCV RNA [4], which led to clinical testing of miravirsen. Now Janssen and colleagues report on their findings from a phase 2a study in treatment naive non-cirrhotic patients chronically infected with HCV genotype 1 [5]. They enrolled 36 patients who were randomized to 5 weekly subcutaneous injections with three different doses of miravirsen (3, 5 or 7 mg/kg) or placebo, with the possibility of pegylated interferon (PegIFN) and ribavirin (RBV) rescue at defined time points after miravirsen completion and at the investigator's discretion. After the last injection of miravirsen, patients were followed for an additional 14 weeks for viral kinetics and adverse events. They found that HCV RNA showed a dose-dependent decline, with 1 (11%) patient in the 5 mg/kg and 4 (44%) patients in the 7 mg/kg groups reaching undetectable HCV RNA levels, all after the fifth dose of miravirsen. Notably, the individual response curves shown by the authors were quite variable, even with the highest dose. Three of the patients whose HCV RNA became undetectable relapsed 4–5 weeks later and one patient went on to be treated with PegIFN/RBV. The long term outcome in the remaining patient who achieved an undetectable HCV RNA at study week 14 and remained undetectable through week 18 was not reported. Adverse events were generally mild with only injection site reactions being likely related to miravirsen administration.

Treatment for chronic HCV, which until recently was plagued by poor tolerability and suboptimal response rates, is undergoing

a profound paradigm shift with the development of HCV protease, polymerase and NS5A inhibitors and the demonstration of extremely high rates of sustained virologic response with interferon-free combination regimens. Although DAAs currently under clinical investigation generally have high potency, all but nucleotide polymerase inhibitors have low barriers to resistance and therefore combination therapy appears necessary for most HCV genotypes. In contrast to the rapid decline in HCV RNA level within days after starting potent DAAs, there was a <2 log decline at week 4 even with the highest dose of miravirsen. Given our lack of understanding how HCV utilizes miR-122 in its life cycle [6] and by what mechanisms miravirsen blocks HCV replication, it is difficult to account for the different kinetics. Apart from being amongst the first successful antisense oligonucleotide therapies in man, the current study by Janssen and colleagues is notable for additional reasons. First, the strategy of targeting miR122 is distinct from that underlying the three major DAA classes under investigation; in theory, it can be used complementary to DAAs. Similar to strategies that interfere with cyclophilin A [7], miravirsen is an example of the capacity for an inhibitor of a host factor essential for the HCV life cycle to abrogate viremia with no evidence of viral escape. Although theoretical escape mutants can be engineered *in vitro* [8], the high barrier to resistance and the duration of post-treatment viral suppression in some patients (to the end of study in one patient), imply the possibility that miravirsen could lead to viral eradication with a monotherapy. To address this ambitious proposition, the authors mention that longer duration studies with 12 weeks of miravirsen monotherapy are ongoing. Finally, given the highly conserved 5′ UTR and miravirsen's ability to block replication of all HCV genotypes *in vitro* [8], it is likely that the current results with genotype 1 patients will hold true across genotypes. This may make therapy with miravirsen a potential approach for genotypes that may be less susceptible to some of the DAAs in development.

Its attractive features and favorable short-term safety profile notwithstanding, antisense therapy warrants a note of caution. MiR-122 modulates the expression of an estimated 200 hepatocyte proteins, some of which have been implicated in cholesterol metabolism and cancer development. Although not confirmed in the setting of HCV infection, low miR-122 levels expressed in hepatocellular carcinomas (HCCs) appear to predict a poor prognosis [9], as acknowledged by the authors, and decrease susceptibility to sorafenib in cell culture[10]. Furthermore germline deletion of miR-122 was recently shown to lead to steatohepatitis and spontaneous HCC development in mice [11,12]. The lower serum cholesterol levels observed in patients after miravirsen administration [5] illustrate that other miR-122 targets are also affected during therapy. In a population at increased risk for developing HCC, these experimental findings warrant careful scrutiny during further clinical development.

The potential benefits of miravirsen must be weighed in the context of the very high rates of sustained virologic response in recent studies of oral DAA combination regimens. The requirement for parenteral administration is a potential drawback, but this may be mitigated if the authors prove to be correct in their suggestion that the pharmacokinetic profile of the drug makes once monthly dosing feasible. Overall, the results of Janssen *et al.* represent an intriguing proof of concept for a new class of host factor antagonists that combine antiviral potency with a high barrier to resistance. The formulation of a developmental pathway for miravirsen may prove to be challenging in the current environment, but it deserves further study and could have a therapeutic role, particularly if DAA combinations leave unmet needs in some patient populations.

Conflict of interest

I.M.J. received grant/research support, Abbvie, Achillion, Boehringer Ingelheim, Bristol Myers Squibb, Gilead, Novartis, Pfizer, Roche/Genentech, Merck, Tibotec/Janssen, Vertex; is on the speakers' bureau of Bristol Myers Squibb, Gilead, Roche/Genentech and Vertex and act as a consultant/advisor for: Abbvie, Achillion, Boehringer Ingelheim, Bristol Myers Squibb, Enanta, Gilead, Glaxo Smithkline, Idenix, Kadmon, Novartis, Presidio, Roche/Genentech, Merck, Tibotec/Janssen and Vertex.

References

[1] Jopling C. Liver-specific microRNA-122: biogenesis and function. RNA Biol 2012;9:137–142.

[2] Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 2005;309:1577–1581.

[3] Jopling CL, Schutz 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.

[4] Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010;327:198–201.

[5] Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting MicroRNA. N Engl J Med 2013;368:1685–1694.

[6] Fukuhara T, Matsuura Y. Role of miR-122 and lipid metabolism in HCV infection. J Gastroenterol 2013;48:169–176.

[7] von Hahn T, Ciesek S, Manns MP. Arrest all accessories–inhibition of hepatitis C virus by compounds that target host factors. Discov Med 2011;12:237–244.

[8] Li YP, Gottwein JM, Scheel TK, Jensen TB, Bukh J. MicroRNA-122 antagonism against hepatitis C virus genotypes 1–6 and reduced efficacy by host RNA insertion or mutations in the HCV 5′ UTR. Proc Natl Acad Sci U S A 2011;108:4991–4996.

[9] Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009;28:3526–3536.

[10] Bai S, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. J Biol Chem 2009;284:32015–32027.

[11] Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 2012;122:2871–2883.

[12] Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest 2012;122:2884–2897.