ORIGINAL ARTICLE

Treatment of HCV Infection by Targeting MicroRNA

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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 ($\log_{10}$ 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.)

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Approximately 170 million persons worldwide are chronically infected with the hepatitis C virus (HCV).[1] Chronic HCV infection is a major cause of liver cirrhosis, liver failure, and hepatocellular carcinoma and is the leading indication for liver transplantation in many Western countries.[2] Sustained eradication of HCV infection has been associated with a reduced risk of liver-related morbidity and all-cause mortality.[3-5]

Despite the recent registration of protease inhibitors for the treatment of chronic HCV genotype 1 infection, current therapeutic regimens remain dependent on the administration of pegylated interferon and ribavirin for 24 to 48 weeks.[6,7] Thus, anti-HCV therapy continues to be associated with substantial side effects. In addition, there is the risk of drug interactions mediated by cytochrome P-450 3A, drug resistance, unknown sustainability of response, and reduced efficacy against certain HCV genotypes and subtypes.[8]

MicroRNAs (miRNAs) are small, endogenous, noncoding RNAs that direct posttranscriptional regulation of gene expression by binding to partially complementary sites within the 3′ untranslated region of target messenger RNAs (mRNAs), resulting in translational repression or mRNA deadenylation and degradation.[9] MiRNAs have been implicated in the regulation of a wide range of important biologic processes, such as cellular growth and differentiation, developmental timing, apoptosis, and modulation of host response to viral infection.[10]

MicroRNA-122 (miR-122) is a highly abundant miRNA expressed in the liver and is essential to the stability and propagation of HCV RNA.[11,12] MiR-122 binds to two closely spaced target sites (S1 and S2) in the highly conserved 5′ untranslated region of the HCV genome, thereby forming an oligomeric miR-122–HCV complex that protects the HCV genome from nucleolytic degradation or from host innate immune responses.[12-14] A third potential miR-122 binding site in the 3′ untranslated region of the HCV genome does not appear to have any functional relevance.[11] The miR-122 binding sites are conserved across all HCV genotypes and subtypes.[15] MiR-122 could thus represent a host target for antiviral therapy.

Miravirsen is a 15-nucleotide locked nucleic acid–modified antisense oligonucleotide complementary to and with a high affinity and specificity for the 5′ region of mature miR-122. Miravirsen can sequester and thus inhibit miR-122 (Fig. 1). The administration of miravirsen to chimpanzees with chronic HCV infection provided long-lasting viral suppression without evidence of resistant mutations at the two miR-122 binding sites of the 5′ untranslated region of the HCV genome, and no adverse events were observed in phase 1 studies in healthy volunteers.[16,17] Here, we report on the safety and activity of miravirsen in patients with chronic HCV infection.

METHODS

STUDY POPULATION

Patients who had not undergone previous therapy for chronic HCV genotype 1 infection were enrolled at seven international sites. Eligible patients were 18 to 65 years of age and were required to have compensated liver disease with a plasma HCV RNA level of more than 75,000 IU per milliliter. Patients with other causes of chronic liver disease, cirrhosis (as diagnosed on previous biopsy), or decompensated liver disease were excluded. Patients were also required to be seronegative for hepatitis B surface antigen and antibodies to human immunodeficiency virus and to have an absolute neutrophil count of 1500 or more per cubic millimeter, a platelet count of 100,000 or more per cubic millimeter, normal values for serum creatinine and for total and direct bilirubin, and alanine aminotransferase levels less than 3 times the upper limit of the normal range.

The study was approved by the institutional review board or ethics committee at each participating center and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulations. All patients provided written informed consent before enrollment in the study.

STUDY DESIGN

From September 2, 2010, to November 16, 2011, we enrolled 36 patients in a randomized, double-blind, placebo-controlled, sequential-series, ascending multiple dose–ranging study. The patients underwent central randomization with the use of a Web-based system in a 3:1 ratio to receive either miravirsen (in doses of 3 mg, 5 mg, or 7 mg per

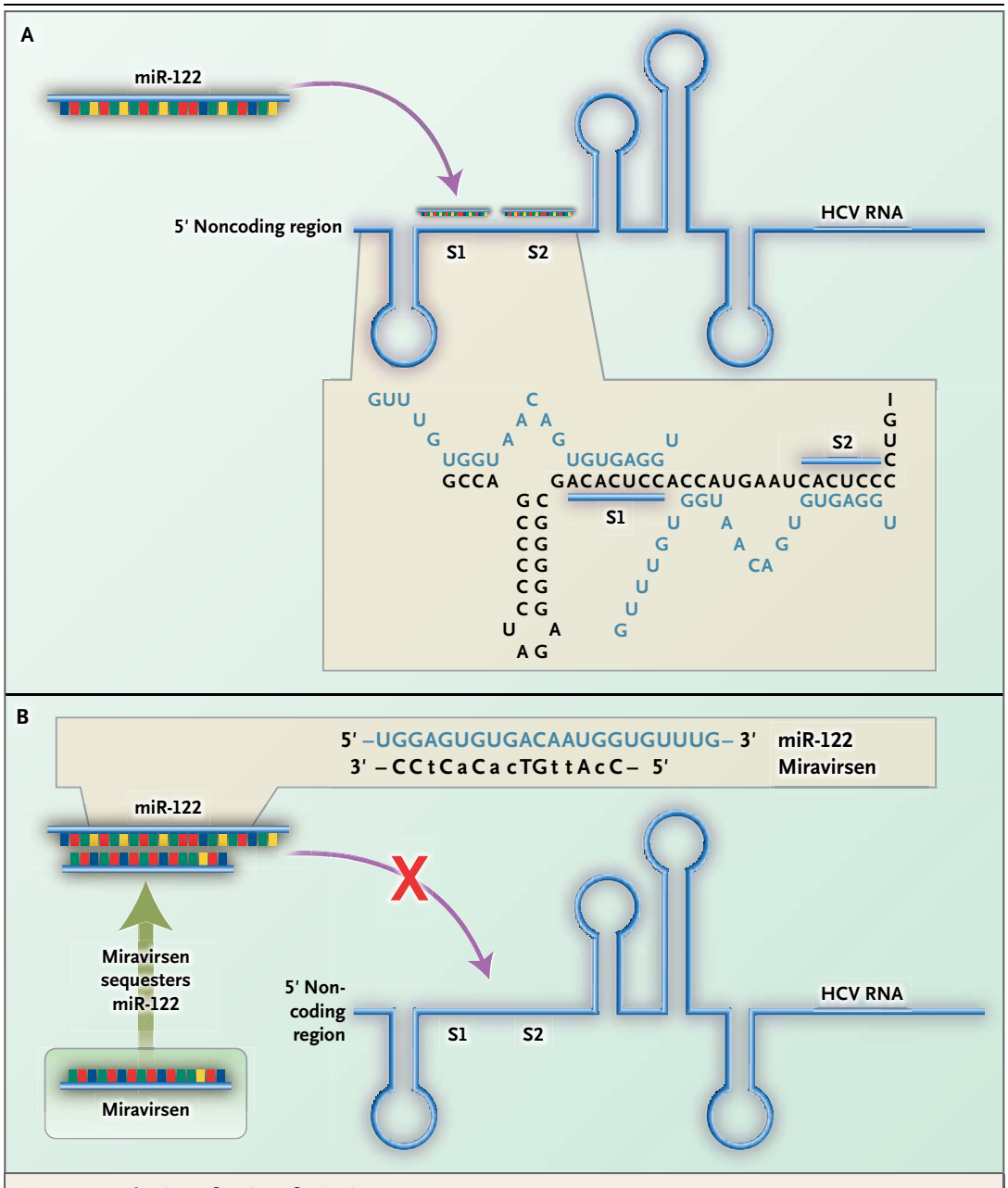

Figure 1. Mechanism of Action of Miravirsen.

In Panel A, microRNA-122 (miR-122) binds to two closely spaced target sites (S1 and S2) in the 5′ noncoding region of the HCV genome and thereby promotes the propagation of HCV RNA.[13] In Panel B, miravirsen, a locked nucleic acid–modified antisense oligonucleotide, sequesters mature miR-122 in a highly stable heteroduplex, which results in the functional inhibition of miR-122.

kilogram of body weight) or placebo. An independent review committee evaluated safety data for the 3-mg group before the study drug was administered to the 5-mg group; the same process was used for the 7-mg group.

Miravirsen was reconstituted to a concentration of 150 mg per milliliter and was administered subcutaneously in five weekly doses over a 29-day period (Fig. 1 in the Supplementary Appendix, available with the full text of this article

at NEJM.org). Placebo injections contained normal saline (0.9% sodium) and were administered at a volume equivalent to that for miravirsen. After the administration period, patients returned for weekly follow-up visits until week 8, for visits every 2 weeks until week 14, and for a final visit at week 18. At the investigators' discretion, patients were allowed to initiate therapy with pegylated interferon and ribavirin at study week 7 (for patients receiving 3 mg of miravirsen per kilogram) or week 10 (for those receiving 5 mg or 7 mg per kilogram).

STUDY OVERSIGHT

The study protocol (available at NEJM.org) was designed and developed by the sponsor, Santaris Pharma, along with the principal investigator at each study site and representatives of Duke Clinical Research Institute. All authors participated in the collection of the data, had complete access to data, participated in the data analysis, and were involved in the preparation and content review of the final manuscript. Duke statisticians performed the statistical analyses. The publication committee, consisting of the first author and several coauthors (including a representative of the sponsor), made the decision to submit the manuscript for publication. All authors vouch for the completeness and accuracy of this report as well as the fidelity of the report to the study protocol.

EFFICACY, SAFETY, AND PHARMACOKINETICS

At every patient visit, we measured the plasma HCV RNA levels using the Abbott RealTime HCV assay, with a reported lower limit of detection and quantification of 12 IU per milliliter. Samples with HCV RNA levels below the lower limit of detection or quantification were considered to have undetectable levels. The HCV RNA genotype was assessed by an in-house genotype-specific polymerase-chain-reaction (PCR) assay that had been developed with the use of the nucleotide sequence of the hypervariable region 1 of the E2 protein of HCV. Direct sequencing was used to confirm the genotype in the event that the result obtained on PCR assay was ambiguous.

Assessment of resistance-associated mutations was performed in all patients at baseline and at week 5 and at the time of viral rebound in the event that the HCV RNA level exceeded 1000 IU per milliliter and pegylated interferon and ribavirin were not initiated. Amplification and sequence analysis of miR-122 binding sites within the 5′ and 3′ HCV RNA untranslated regions was accomplished by site-specific primed reverse-transcriptase PCR, followed by population-based sequencing with the use of the Applied Biosystems 3730xl Genetic Analyzer (for details, see the Methods section in the Supplementary Appendix).

We performed physical examinations and serum biochemical and hematologic laboratory tests at all study visits. We asked the patients open-ended questions to determine whether new adverse events, both general and according to organ system, had emerged since the previous visit; all adverse events were classified according to terms used in the *Medical Dictionary for Regulatory Activities*. Adverse events were considered to be mild if they were transient in nature and generally did not interfere with normal activities, moderate if they were sufficiently discomforting to interfere with normal activities, and severe if they prevented normal activities. Twelve-lead electrocardiography was performed at screening and periodically throughout the study. Plasma samples for miravirsen pharmacokinetic assessment were collected before the administration of each dose of a study drug and 2 hours after each dose. Miravirsen levels were determined with the use of a hybridization enzyme-linked immunosorbent assay.[17]

STATISTICAL ANALYSIS

The primary analyses included all patients who underwent randomization and who received at least one dose of a study drug. We compared the rates at which HCV RNA levels declined using HCV RNA measurements obtained throughout the study period or until the initiation of therapy with pegylated interferon and ribavirin. To gauge the decline in viral load associated with miravirsen alone, we excluded from the analyses HCV RNA results after the initiation of therapy with pegylated interferon and ribavirin. To report all potential toxicity during or after the administration of miravirsen, we reported side effects and biochemical safety profiles on all data that were collected from baseline to week 18, regardless of whether patients were receiving pegylated interferon and ribavirin.

For each patient, we determined the maximum change in the HCV RNA level from baseline and used two-sample t-tests to compare the means of these values in each miravirsen-dose group with

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Miravirsen			Placebo (N=9)
	3 mg/kg (N=9)	5 mg/kg (N=9)	7 mg/kg (N=9)	
Median age (range) — yr	35 (26–66)	46 (33–65)	48 (31–61)	56 (42–66)
Body-mass index — median (range)†	28 (18–31)	26 (19–38)	29 (21–38)	27 (18–37)
Male sex — no. (%)	5 (56)	8 (89)	6 (67)	3 (33)
Race — no. (%)‡				
White	9 (100)	8 (89)	7 (78)	7 (78)
Black	0	1 (11)	1 (11)	2 (22)
Asian	0	0	1 (11)	0
Alanine aminotransferase — IU/liter	74.3±38.7	69.1±21.4	81.3±71.8	92.7±37.0
Total bilirubin — μmol/liter	10.2±5.1	11.8±8.6	9.9±3.6	12.4±5.1
Serum albumin — g/liter	44.9±3.5	43.0±2.8	42.6±3.3	43.3±2.7
Platelet count — per mm^3	267,000±70,000	250,000±38,000	245,000±51,000	189,000±69,000
Prothrombin time — sec	11.1±0.4	12.6±1.0	12.2±1.4	11.7±1.7
Subtype of HCV genotype 1 — no. (%)§				
1a	5 (56)	7 (78)	5 (56)	6 (67)
1b	2 (22)	1 (11)	3 (33)	2 (22)
1a/1b	2 (22)	0	1 (11)	1 (11)
1a/3a	0	1 (11)	0	0
HCV RNA — $\log_{10}$ IU/ml	6.0±0.7	6.2±0.3	5.9±0.6	6.2±0.4
HCV RNA ≥800,000 IU/ml — no. (%)	5 (56)	8 (89)	6 (67)	7 (78)
IL28B CC genotype (rs12979860) — no. (%)	2 (22)	4 (44)	4 (44)	2 (22)
Interferon-inducible protein 10 — pg/ml	1209±1283	1069±787	798±501	1390±881

* Plus–minus values are means ±SD. There were no significant differences among groups. To convert the values for bilirubin to milligrams per deciliter, divide by 17.1. HCV denotes hepatitis C virus.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ Race was self-reported.
§ Several patients had a mixture of HCV genotype 1a and HCV genotype 1b. One patient had a mixture of HCV genotype 1a and HCV genotype 3a.

the placebo group. A P value of 0.05 was considered to indicate statistical significance; all tests were two-sided, with no adjustment for multiple testing. We assessed the dose–response relationships to HCV RNA levels using a linear trend test, assuming an equal space among the four study groups. All analyses were performed with the use of SAS software, version 9.2 (SAS Institute).

RESULTS

PATIENTS

A total of 36 patients with chronic HCV genotype 1 infection were enrolled in the study, with 9 patients in each miravirsen group (receiving 3 mg, 5 mg, or 7 mg per kilogram) and in the placebo group (Fig. 2 in the Supplementary Appendix). Baseline characteristics were similar among the four study groups (Table 1). Of the 36 patients, 12 started therapy with pegylated interferon and ribavirin during follow-up.

EFFICACY

The reduction in HCV RNA levels was dose-dependent and sustained beyond the administration period for miravirsen (Fig. 2). In the miravirsen groups, the mean of the maximum reduction in HCV RNA levels ($\log_{10}$ 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

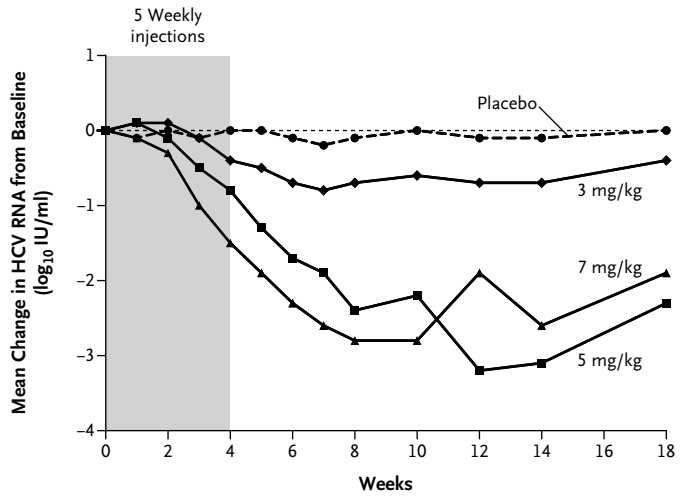

Figure 2. Change from Baseline in HCV RNA Levels.

Shown are the mean changes in HCV RNA levels from baseline for patients receiving 3 mg, 5 mg, or 7 mg of miravirsen per kilogram of body weight, as compared with placebo. Miravirsen was administered in five weekly subcutaneous injections during the first 29 days of the study (gray shading). The dashed line indicates no change from baseline. The HCV RNA levels during the use of pegylated interferon and ribavirin in some patients were not included in this analysis.

5 mg per kilogram, and 3.0 (P=0.002) for those receiving 7 mg per kilogram, as compared with a decline of 0.4 in the placebo group. A reduction in the HCV RNA level of at least 2 log_{10} IU per milliliter occurred in one patient (11%) in the group receiving 3 mg of miravirsen per kilogram and in six patients each (67%) in the groups receiving 5 mg or 7 mg of miravirsen per kilogram, as compared with none of the patients in the placebo group. Corresponding dose-dependent pharmacokinetic profiles are shown in Figure 3 in the Supplementary Appendix.

Figure 3 shows the reductions in HCV RNA levels among individual patients during the administration of miravirsen and after initiation of pegylated interferon and ribavirin. In five patients, the use of miravirsen alone resulted in undetectable HCV RNA (i.e., <12 IU per milliliter). Of these patients, one who received 5 mg of miravirsen per kilogram had undetectable HCV RNA at study week 14 but subsequently had an HCV RNA level of 3180 IU per milliliter at week 18. The other four patients, who received 7 mg of miravirsen per kilogram, had undetectable HCV RNA at study week 5 (in one patient), week 6 (in one patient), or week 14 (in two patients). In the two patients with undetectable HCV RNA at weeks 5 and 6, the HCV RNA levels remained undetectable up to and including week 10. Subsequently, one patient had a detectable HCV RNA level of 6230 IU per milliliter at week 12, and the other started therapy with pegylated interferon and ribavirin at week 10 and remained without detectable HCV RNA through the end of study (week 18). Of the two patients with undetectable HCV RNA levels at week 14, one continued to have undetectable HCV RNA throughout the study, and the other had a low HCV RNA level of 80 IU per milliliter at week 18.

Virologic rebound, which is defined as an increase exceeding 1 log_{10} in the HCV RNA level over nadir, occurred after the discontinuation of miravirsen in 1 patient receiving 3 mg of the drug per kilogram (at week 18), in 5 patients receiving 5 mg of the drug per kilogram (3 patients at week 10, 1 at week 14, and 1 at week 18), and in 3 patients receiving 7 mg of the drug per kilogram (at weeks 12, 14, and 18). We did not detect resistance-associated mutations in the two miR-122 seed sites of the HCV genome in any of the 36 patients. We observed no apparent relationships between the declines in HCV RNA levels and factors that have previously been associated with such declines, including the *IL28B* genotype (rs12979860), baseline serum interferon-inducible protein 10 (IP-10) levels, baseline viral load, the presence of HCV genotypes 1a or 1b, and other baseline characteristics of the patients.

SAFETY

Of the 112 adverse events that were recorded for patients receiving miravirsen, 93 were grade 1, 17 were grade 2, and 2 were grade 3. Of the 31 adverse events recorded for patients receiving placebo, 23 were grade 1, 7 were grade 2, and 1 was grade 3 (thrombocytopenia, which was assessed by the investigator as moderate in severity). There were no dose-limiting toxic effects or treatment discontinuations because of adverse events (Table 2).

During the 18-week study period, five patients in the miravirsen groups (two receiving 3 mg per kilogram, 1 receiving 5 mg per kilogram, and two receiving 7 mg per kilogram) and two patients in the placebo group had adverse events of moderate severity. These events included single occurrences of headache, otitis externa, pelvic

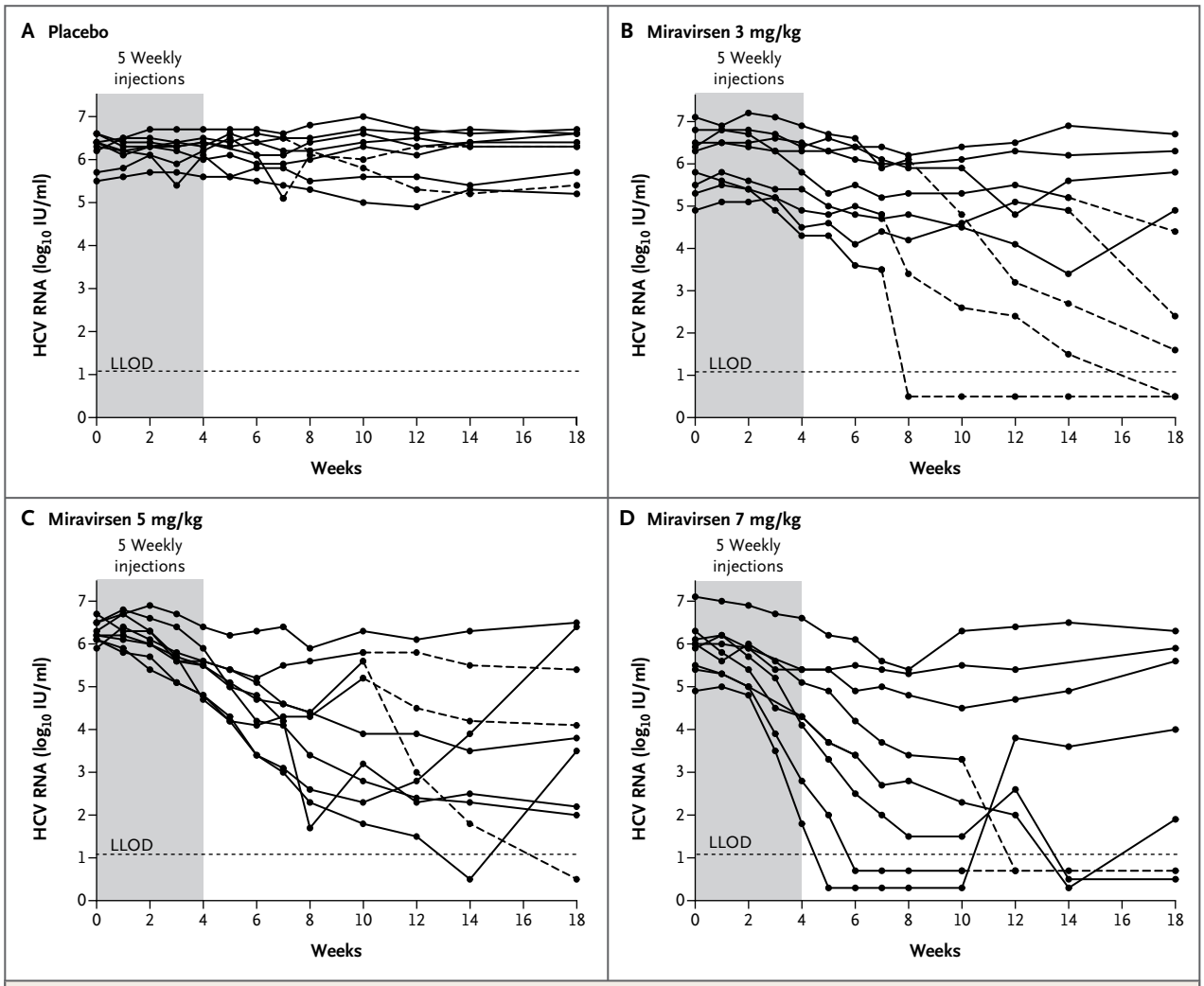

Figure 3. HCV RNA Levels for Individual Patients, According to Study Group.

Miravirsen or placebo was administered in five weekly subcutaneous injections during the first 29 days of the study (gray shading). The dashed curves in the data points represent HCV RNA levels after the initiation of therapy with pegylated interferon and ribavirin in 12 patients. The lower limit of detection (LLOD) was 12 IU (or 1.08 $\log_{10}$ IU) per milliliter. In the group receiving 3 mg of miravirsen per kilogram, two patients had decreases in HCV RNA levels below the LLOD after the initiation of pegylated interferon and ribavirin. In the group receiving 5 mg of miravirsen per kilogram, undetectable HCV RNA levels occurred at week 14 in one patient who was treated with miravirsen alone and at week 18 in one patient who started pegylated interferon and ribavirin after week 10. In the group receiving 7 mg of miravirsen per kilogram, four patients had undetectable HCV RNA levels with miravirsen alone: one at week 5, one at week 6, and two at week 14. Therapy with pegylated interferon and ribavirin was initiated in two patients receiving placebo (both after the week 7 visit), in five patients receiving 3 mg of miravirsen per kilogram (two after week 7, one after week 8, and two after week 14), in three patients receiving 5 mg of miravirsen per kilogram (all after the week 10 visit), and in two patients receiving 7 mg of miravirsen per kilogram (also all after the week 10 visit).

bone injury after a fall, syncope, and flulike symptoms (after starting pegylated interferon and ribavirin) among the miravirsen-treated patients, and headache and a hand abscess among the placebo-treated patients. The only severe event during the study was loss of consciousness in one patient 9 weeks after the last dose of 7 mg of miravirsen per kilogram, which occurred after a fall and also resulted in pelvic bone injury. This severe event was designated as a serious adverse event, since the patient was hospitalized overnight for observation.

Injection-site reactions that included a combination of erythema, pruritus, persistent induration,

Table 2. Adverse Events.*

Event	Miravirsen			Placebo (N=9)
	3 mg/kg (N=9)	5 mg/kg (N=9)	7 mg/kg (N=9)	
Any adverse event				
Patients — no. (%)	8 (89)	7 (78)	8 (89)	7 (78)
Events — no.	29	32	51	31
Moderate or severe adverse event				
Patients — no. (%)	2 (22)	1 (11)	2 (22)	2 (22)
Events — no.	2	1	3	2
Most common adverse events — no. of patients (%)†				
Headache	3 (33)	2 (22)	4 (44)	3 (33)
Fatigue	1 (11)	3 (33)	4 (44)	3 (33)
Nasopharyngitis	3 (33)	2 (22)	1 (11)	2 (22)
Nausea	0	1 (11)	3 (33)	1 (11)
Rash	0	2 (22)	2 (22)	1 (11)
Diarrhea	2 (22)	0	0	1 (11)
Myalgia	0	2 (22)	1 (11)	1 (11)
Flulike symptoms	2 (22)	0	1 (11)	1 (11)
Pruritus	0	1 (11)	2 (22)	1 (11)
Injection-site event‡				
Classic reaction	0	0	2 (22)	0
Other event	1 (11)	0	3 (33)	0

* Listed are all adverse events that were reported throughout the 18-week study period regardless of whether patients were receiving pegylated interferon and ribavirin along with the study drug.
† The events listed in this category occurred in at least 15% of the patients in any study group.
‡ Classic reactions are those that are characteristic of oligonucleotide drugs; these include erythema, pruritus, persistent induration, and a burning sensation. Other events include any other injection-site reactions, including pain and hematoma.

or a burning sensation are characteristic of oligonucleotide drugs and were reported in two patients in our study, both in the group receiving 7 mg of miravirsen per kilogram. These injection-site reactions were self-limited or resolved with minimal treatment. No systemic allergic reactions were observed. There were no deaths.

Among patients receiving miravirsen, biochemical safety profiles indicated a sustained decrease in levels of serum alanine aminotransferase (Fig. 4A in the Supplementary Appendix), aspartate aminotransferase, and γ-glutamyl transpeptidase. Clinically insignificant increases in levels of serum alkaline phosphatase and creatinine that were not dose-dependent were noted among most patients in all three groups receiving miravirsen. None of these increases exceeded the criteria for grade 1 toxicity, according to the Common Terminology Criteria for Adverse Events, version 4.0. No clinically significant changes in hemoglobin levels or total white-cell counts were noted during miravirsen administration. There were mean increases of 8 to 10% above baseline in platelet counts among patients receiving miravirsen, but there were no clinically significant changes in prothrombin time or activated partial thromboplastin time. No patient stopped miravirsen treatment or required a dose reduction because of laboratory abnormalities. As expected, a decrease in the serum total cholesterol level was found (Fig. 4B in the Supplementary Appendix). There were no changes in the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol.

DISCUSSION

In this study, five weekly injections of miravirsen, an antisense inhibitor of miR-122, produced a dose-dependent and prolonged decrease in HCV RNA levels in patients with chronic HCV genotype 1 infection. In some patients, undetectable HCV RNA levels were achieved. We observed no evidence of viral resistance.

Patients receiving miravirsen had reductions in aminotransferase levels, in contrast to the increased levels reported in those receiving phosphorothioate antisense compounds in previous studies.[18] Miravirsen treatment did not result in clinically significant changes in renal function or increases in the activated partial thromboplastin time. A gradual and prolonged non–dose-dependent reduction in cholesterol levels was observed in accordance with the effects of miR-122 antagonism on cholesterol homeostasis, consistent with data from previous studies in mice and nonhuman primates.[17,19-21]

Studies of miravirsen in animals have not indicated any adverse effects associated with the sequestration of miR-122 and subsequent upregulation of miR-122–regulated target mRNAs, suggesting that short-term inhibition of miR-122 is safe.[17,19,22] The degree of modulation of most miR-122–regulated target mRNAs is relatively

small and could explain the good side-effect profile. In contrast, the effects of miR-122 sequestration appear to result in a more substantial change in HCV RNA levels. The expression of several miR-122–regulated host genes has been implicated in the development of hepatocellular carcinoma, suggesting that miR-122 has tumor-suppressive effects. Although a direct causal relationship between sustained loss of miR-122 function and hepatocellular carcinoma remains to be determined, down-regulation of miR-122 has been described in hepatocellular carcinoma, with lower miR-122 levels correlating with a poor prognosis.[23-26] However, Varnholt et al.[27] have reported the up-regulation of miR-122 in HCV-induced hepatocellular carcinoma, suggesting that the role of miR-122 in HCV-derived hepatocellular carcinoma could be different from that in hepatocellular carcinomas not associated with HCV. Regardless, short-term inhibition of miR-122 by miravirsen was shown to be reversible.

Reduced intrahepatic miR-122 levels, possibly related to higher expression of interferon-regulated genes, have been observed in patients who did not have a virologic response to interferon-based therapy.[28] However, other studies have not shown any association between intrahepatic interferon-regulated genes and miR-122 expression.[29] We did not find a clear association between virologic response to miravirsen and baseline IP-10 levels, *IL28B* genotype, or any other host and viral factors assessed in this study. However, the power of our study to identify factors associated with virologic response was limited because of the small sample size.

Our results are relevant to the consideration of miravirsen as a potential treatment for HCV infection. First, the miR-122 HCV binding sites are highly conserved, allowing the use of miravirsen in all HCV genotypes.[15] Second, we have not observed evidence of escape mutations in HCV RNA in primates or humans treated with miravirsen, indicating a high genetic barrier to resistance.[17] Third, the pharmacokinetic profile of miravirsen, with a gradual increase in trough levels representing hepatic accumulation and a prolonged tissue clearance half-life, allows once-monthly regimens, favoring patient compliance. Unlike the currently approved protease inhibitors, miravirsen is not a substrate for cytochrome P-450 and is therefore not expected to have significant drug–drug interactions.

Five patients receiving short-term miravirsen alone had undetectable HCV RNA levels, indicating the potential of miravirsen as monotherapy for chronic HCV infection. However, four of these five patients had a rebound in viral levels at the end of the study, indicating that four weeks of administration of miravirsen (at a weekly dose of 7 mg per kilogram) was insufficient to achieve a sustained virologic response in these patients. It is not clear whether regimens of miravirsen of longer duration could achieve a sustained virologic response; we are currently testing the effect of a 12-week regimen (ClinicalTrials.gov number, NCT01727934). A sustained virologic response has been achieved in several patients treated with an interferon-free regimen that combined direct-acting antiviral agents.[8,30] It is possible that miravirsen could be used as a host-targeting agent to increase the antiviral efficacy of such combination regimens by providing a continuous barrier to viral breakthrough, an approach that would seem worth testing, given the rapid clearance of direct-acting antiviral agents, potential issues of compliance with increased pill burden of current treatment regimens, and selection of resistance-associated mutations.

Because of the wide applicability of antisense therapy, the strategy we have described here may also be relevant for diseases other than chronic HCV infection. Within the field of hepatology, the inhibition of miR-122 has been associated with an improvement of steatosis in a mouse model of diet-induced obesity, suggesting a role for miR-122 antagonism in the treatment of nonalcoholic fatty liver disease.[20] Within other fields, therapeutic silencing of disease-associated miRNAs in preclinical studies of cancer and of cardiovascular and autoimmune disorders has delivered results that warrant clinical investigation.[31-33]

In conclusion, miravirsen administered in five weekly subcutaneous injections over 29 days to patients with chronic HCV infection resulted in significant virologic responses. With this study, we have shown a therapeutic effect by targeting a noncoding host miRNA.

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

REFERENCES

1. World Health Organization. Initiative for vaccine research (http://www.who.int/vaccine_research/diseases/viral_cancers/en/index2.html).
2. Thuluvath PJ, Guidinger MK, Fung JJ, Johnson LB, Rayhill SC, Pelletier SJ. Liver transplantation in the United States, 1999-2008. Am J Transplant 2010;10:1003-19.
3. Backus LI, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A sustained virologic response reduces risk of all-cause mortality in patients with hepatitis C. Clin Gastroenterol Hepatol 2011;9(6):509.e1-516.e1.
4. Morgan TR, Ghany MG, Kim HY, et al. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. Hepatology 2010;52:833-44.
5. van der Meer AJ, Veldt BJ, Feld JJ, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA 2012;308:2584-93.
6. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1195-206.
7. Sherman KE, Flamm SL, Afdhal NH, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. N Engl J Med 2011;365:1014-24. [Erratum, N Engl J Med 2011;365:1551.]
8. Lok AS, Gardiner DF, Lawitz E, et al. Preliminary study of two antiviral agents for hepatitis C genotype 1. N Engl J Med 2012;366:216-24.
9. Ambros V. The functions of animal microRNAs. Nature 2004;431:350-5.
10. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281-97.
11. Henke JI, Goergen D, Zheng J, et al. microRNA-122 stimulates translation of hepatitis C virus RNA. EMBO J 2008;27:3300-10.
12. 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.
13. 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.
14. Shimakami T, Yamane D, Jangra RK, et al. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. Proc Natl Acad Sci U S A 2012;109:941-6.
15. 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;108:4991-6.
16. Hildebrandt-Eriksen ES, Bagger YZ, Knudsen TB, et al. A unique therapy for HCV inhibits microRNA-122 in humans and results in HCV RNA suppression in chronically infected chimpanzees: results from primate and first-in-human studies. Hepatology 2009;50:Suppl:228A. abstract.
17. 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.
18. Kwoh TJ. An overview of the clinical safety experience of first- and second-generation antisense oligonucleotides. In: Crooke ST, ed. Antisense drug technology: principles, strategies, and applications. 2nd ed. Boca Raton, FL: CRC Press, 2008:365-99.
19. Elmen J, Lindow M, Silahtaroglu A, et al. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res 2008;36:1153-62.
20. Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;3:87-98.
21. Krützfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs in vivo with 'antagomirs.' Nature 2005;438:685-9.
22. Elmén J, Lindow M, Schütz S, et al. LNA-mediated microRNA silencing in non-human primates. Nature 2008;452:896-9.
23. Budhu A, Jia HL, Forgues M, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology 2008;47:897-907.
24. 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;28:3526-36.
25. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006;25:2537-45.
26. Tsai WC, Hsu PWC, Lai TC, et al. MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. Hepatology 2009;49:1571-82.
27. Varnholt H, Drebber U, Schulze F, et al. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology 2008;47:1223-32.
28. Sarasin-Filipowicz M, Krol J, Markiewicz I, Heim MH, Filipowicz W. Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy. Nat Med 2009;15:31-3.
29. Urban TJ, Thompson AJ, Bradrick SS, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology 2010;52:1888-96.
30. Chayama K, Takahashi S, Toyota J, et al. Dual therapy with the nonstructural protein 5A inhibitor, daclatasvir, and the nonstructural protein 3 protease inhibitor, asunaprevir, in hepatitis C virus genotype 1b-infected null responders. Hepatology 2012;55:742-8.
31. Garchow BG, Bartulos Encinas O, Leung YT, et al. Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. EMBO Mol Med 2011;3:605-15.
32. Ma L, Reinhardt F, Pan E, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol 2010;28:341-7.
33. Montgomery RL, Hullinger TG, Semus HM, et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation 2011;124:1537-47.