Gastroenterology 2014;146:63–66

BRIEF REPORT

Formulated Minimal-Length Synthetic Small Hairpin RNAs Are Potent Inhibitors of Hepatitis C Virus in Mice With Humanized Livers

Han Ma,[1,*] Anne Dallas,[2,*] Heini Ilves,[2,*] Joshua Shorenstein,[2] Ian MacLachlan,[3] Klaus Klumpp,[1] and Brian H. Johnston[2]

[1]*Hoffmann-La Roche, Nutley, New Jersey;* [2]*SomaGenics, Inc, Santa Cruz, California;* [3]*Tekmira Pharmaceuticals, Burnaby, British Columbia, Canada*

Short synthetic hairpin RNAs (sshRNAs) (SG220 and SG273) that target the internal ribosome entry site of the hepatitis C virus (HCV) were formulated into lipid nanoparticles and administered intravenously to HCV-infected urokinase plasminogen activator–severe combined immunodeficient mice with livers repopulated with human hepatocytes (humanized livers). Weekly administration of 2.5 mg/kg of each sshRNA for 2 weeks resulted in a maximal mean reduction in viral load of 2.5 $\log_{10}$ from baseline. The viral load remained reduced by more than 90% at 14 days after the last dose was given. The sshRNAs were well tolerated and did not significantly increase liver enzyme levels. These findings indicate the in vivo efficacy of a synthetic RNA inhibitor against the HCV genome in reducing HCV infection.

Keywords: Short shRNA; uPA-SCID Chimeric Mice; RNA Interference; siRNA.

Hepatitis C virus (HCV) infection is a leading cause of chronic liver diseases including cirrhosis and hepatocellular carcinoma.[1] The vast number of HCV quasispecies present in any infected individual results in a high potential for developing resistance to direct-acting antiviral agents, which can lead to treatment failure.[2,3] Thus, most investigational new treatments of chronic HCV infection use a combination of molecules with complementary mechanisms and nonoverlapping resistance profiles. RNA interference (RNAi) potentially offers such a therapeutic approach by directly targeting multiple sites on viral RNA. Here, we report the potent, durable, and specific inhibition of HCV infection in vivo by 2 short synthetic hairpin RNAs (sshRNAs) formulated with lipid nanoparticles (LNPs).[4]

The sshRNAs (SG220 and SG273), whose in vitro activity was characterized previously,[5,6] target a conserved region within the internal ribosome entry site (IRES) of the HCV genome (Supplementary Figure 1) with perfect target complementarity to genotypes 1a, 1b, 4a, and 5a, a single mismatch to genotype 2a,[7] and more than one mismatch to genotypes 3 and 6. sshRNAs induce RNAi by a noncanonical mechanism in which intact hairpins are loaded into the RNA-induced silencing complex (RISC) and are activated by Ago2-mediated slicer processing of the passenger arm without cleavage by the enzyme Dicer.[8] sshRNAs also differ from small interfering RNAs (siRNAs) in consisting of a single molecular entity and having a loop that hinders off-target activity by the passenger strand.[8] Both sshRNAs induced potent knockdown of IRES-directed firefly luciferase reporter gene activity in Human Embryonic Kidney 293FT cells without significant in vitro or in vivo immune-stimulatory activity,[5,9] and LNP-formulated SG220 was shown to effect prolonged knockdown in an HCV IRES-dependent bioluminescence mouse model.[9,10]

Chimeric urokinase-type plasminogen activator-severe combined immunodeficiency (uPA-SCID) mice with human hepatocyte reconstitution have been used previously to show the antiviral potency of various HCV inhibitors known to be effective in HCV-infected patients.[11–13] To test sshRNAs in this model, we first examined the uptake of the LNP-formulated sshRNA SG220 in chimeric uPA-SCID mice and compared it with that of regular SCID mice. Results obtained from 3 chimeric and 3 regular SCID mice showed similar levels of sshRNA in the livers of the chimeric and regular SCID mice, as determined by a ribonuclease protection assay (RPA) (Figure 1, lanes 4–6 and 8–10). The specificity of the RPA for SG220 was shown by the lack of protection of the probe by total liver RNA from the phosphate-buffered saline (PBS)-treated mice (Figure 1, lane 2). By comparing the band intensities of the protected probe with those of SG220 standards (Figure 1, lanes 11–14),

*Authors share first authorship.

Abbreviations used in this paper: **HCV, hepatitis C virus; IRES, internal ribosome entry site; LNP, lipid nanoparticle; PBS, phosphate-buffered saline; RNAi, RNA interference; RPA, ribonuclease protection assay; sshRNA, short synthetic hairpin RNA; uPA-SCID, urokinase-type plasminogen activator-severe combined immunodeficiency.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2013.09.049

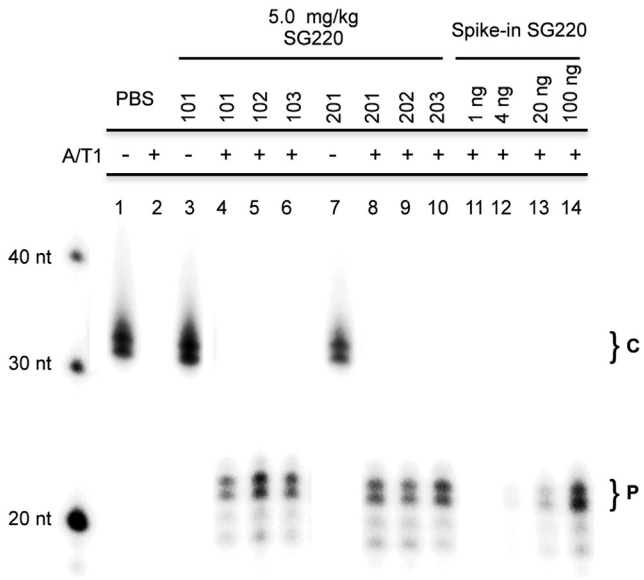


Figure 1. Liver uptake of sshRNA in chimeric mice and regular SCID mice. RNAse protection assay using total liver RNA from chimeric mice (101–103, *lanes 3–6*) or SCID mice (201–203, *lanes 7–10*). Indicated amounts of SG220 were spiked into 50 μg total RNA from untreated mice (*lanes 11–14*) to generate a calibration curve for quantification of liver uptake of SG220. C, undigested probe; P, RNAse digested probe.

it was determined that 5.9% ± 1.4% (mean ± SD) of the total injected SG220 remained in the chimeric mouse liver 48 hours after treatment, compared with 5.1% ± 0.7% in the SCID mouse liver (Supplementary Table 1). The presence of abundant sshRNA in the liver 48 hours after treatment suggests that the human hepatocytes in the chimeric mouse liver can efficiently take-up LNP-formulated sshRNA and that significant amounts remain intact. This is consistent with previous reports in which LNPs designed for efficient delivery to liver have been shown to accumulate 70%–80% of the total injected dose in the liver of mice within the first 24 hours after intravenous administration[14] and to be readily taken up by hepatocytes by virtue of receptor-mediated endocytosis mediated by the in vivo adoption of endogenous apolipoprotein E, a ligand for the low-density lipoprotein–receptor family of cell surface receptors.[15]

We next assessed the activity of the 2 sshRNAs against HCV replication in chimeric uPA-SCID mice that had high proportions of human hepatocytes (serum human albumin level, ≥8 mg/mL in all mice) and were stably infected with genotype 1a HCV (serum HCV-RNA level, $\geq 4.0 \times 10^6$ IU/mL in all mice) (Supplementary Table 2). Four mice in each group received 2 intravenous injections, given 1 week apart, of LNP-formulated SG220, SG273, a combination of SG220 and SG273, or an irrelevant (scrambled) sshRNA at 2.5 or 5.0 mg/kg (Figure 2*A* and *B*). Serum HCV viral titer was monitored twice every week. All mice receiving HCV-specific sshRNAs had more than a 90% (1 $\log_{10}$) reduction in serum HCV-RNA concentration after a single dose. Treatment with 2.5 mg/kg SG220 or SG273 led to a 1.8 $\log_{10}$ or 1.2 $\log_{10}$ mean reduction in HCV-RNA concentration, respectively, at 72 hours after the first injection ($P < .01$; Figure 2*A* and *B*). A higher dose of SG220 (5 mg/kg) did not lead to a further reduction in viral load (Figure 2*A*). The combination of 2.5 mg/kg SG220 and 2.5 mg/kg SG273 provided the strongest inhibition of HCV replication, with a 2 $\log_{10}$ reduction in HCV-RNA concentration observed 72 hours after the first dose and an additional 0.5 $\log_{10}$ reduction 7 days after the second dose ($P < .01$; Figure 2*B*). The inhibition of HCV replication by HCV sshRNA was specific because the serum HCV viral load in mice treated with the LNP-formulated irrelevant control sshRNA at 5 mg/kg did not change significantly at any time point during the study.

The mean viral load reduction in all HCV sshRNA-treated groups was durable. In mice treated with either 2.5 mg/kg SG220 or the combination of SG220 and SG273, the HCV serum viral load remained significantly lower ($P < .01$) than the pretreatment level, up to the last study time point 3 weeks after the second and last doses (Supplementary Table 3). In the case of 2 mice whose pretreatment serum HCV RNA levels were less than 10^7, viral load remained 1.5 and 2.6 $\log_{10}$ below pretreatment levels at 3 weeks after treatment (Supplementary Figure 2). In the mice treated with SG273 alone, the HCV serum viral load remained significantly lower relative to the pretreatment level up to 2 weeks after the second dose ($P < .05$) (Figure 2*B*, Supplementary Table 3).

LNP-formulated sshRNAs were well tolerated. There was no treatment-related increase of liver alanine aminotransferase or aspartate aminotransferase levels in any of the treatment groups or a reduction in serum human albumin concentration (Figure 2*C*), indicating the absence of significant hepatocyte toxicity during the dosing and follow-up periods. There was also no treatment-associated body weight loss and no observations of morbidity or mortality in the study animals. These results, together with the lack of antiviral effect of the irrelevant sshRNAs, suggest that the reduction of serum HCV-RNA concentration by the HCV-targeting sshRNAs was specific.

There have been reports of RNAi-mediated inhibition of HCV in HCV subgenomic replicon systems and cell culture–based infectious HCV systems.[16–18] However, it is not known if RNAi can achieve clinically significant levels of antiviral efficacy against chronic HCV infection in vivo. Our results show that the LNP-formulated[4] HCV targeting sshRNAs efficiently inhibit HCV replication in the uPA-SCID chimeric mice, a model that supports high levels of HCV replication. The RNAi effect of LNP-formulated sshRNA treatment was long-lasting, suggesting that such agents could be effective even with infrequent dosing. The fact that SG220 showed higher potency than SG273 both in vitro and in vivo is

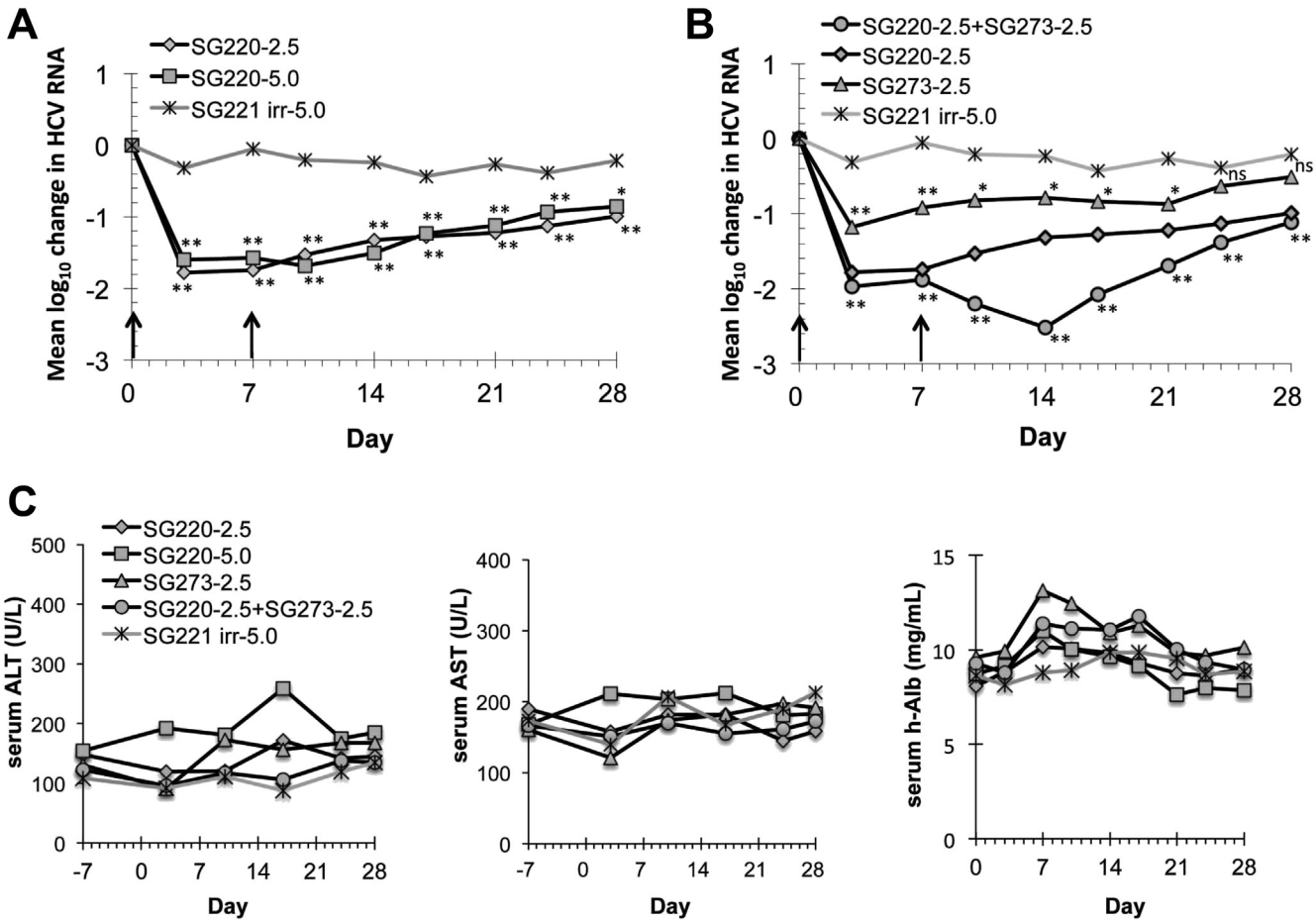


Figure 2. Specific and durable inhibition of HCV replication in chimeric mice. Chimeric mice (n = 4) infected with genotype 1a HCV were dosed intravenously with each of the indicated sshRNAs on days 0 and 7 (*arrows*). (*A*) Mean reduction in serum HCV-RNA level from 2.5 and 5.0 mg/kg doses of SG220. (*B*) Comparison of SG220, SG273, and a combination of both. (*C*) Lack of effect of sshRNA treatments on liver alanine aminotransaminase (ALT), aspartate aminotransferase (AST), and human albumin (h-Alb) levels. $^{**}P < .01$; $^{*}P = .01–.05$.

consistent with a direct-acting antiviral mechanism of action.[5,6,8] This study reports inhibition of HCV virus replication by synthetic RNAs through an RNAi mechanism in a robust HCV infection model and adds HCV to the small group of viruses shown to be targetable with RNAi.[19]

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.09.049.

References

1. Perz JF, et al. J Hepatol 2006;45:529–538.
2. Martell M, et al. J Virol 1992;66:3225–3229.
3. Sun JH, et al. Hepatology 2012;55:1692–1699.
4. Jeffs LB, et al. Pharm Res 2005;22:362–372.
5. Ge Q, et al. RNA 2010;16:118–130.
6. Ge Q, et al. RNA 2010;16:106–117.
7. Kronke J, et al. J Virol 2004;78:3436–3446.
8. Dallas A, et al. Nucleic Acids Res 2012;40:9255–9271.
9. **Dallas A, Ilves H**, et al. Mol Ther Nucleic Acids 2013;2:e123.
10. Kolli S, et al. Bioconjug Chem 2013;24:314–332.
11. Kamiya N, et al. J Gen Virol 2010;91:1668–1677.
12. Shi N, et al. Gut 2013;62:1055–1061.
13. Meuleman P, et al. Antiviral Res 2008;80:231–238.
14. MacLachlan I. Liposomal formulations for nucleic acid delivery. In: Crooke ST, ed. Antisense drug technology: principles, strategies, and applications. 2nd edition. CRC Press, 2007:237–270.
15. Akinc A, et al. Mol Ther 2010;18:1357–1364.
16. Kanda T, et al. J Virol 2007;81:669–676.
17. Wilson JA, et al. J Virol 2005;79:7050–7058.
18. **Yokota T, Sakamoto N**, et al. EMBO Rep 2003;4:602–608.
19. Shah PS, et al. Pharm Res 2011;28:2966–2982.

Author names in bold designate shared co-first authorship.

Received May 22, 2013. Accepted September 22, 2013.

Reprint requests
Address requests for reprints to: Brian H. Johnston, PhD, SomaGenics, Inc, 2161 Delaware Avenue, Santa Cruz, California 95060. e-mail: bjohnston@somagenics.com; fax: (831) 420-0685.

Acknowledgments
The authors thank Palanikumar Ravindran for statistical analysis, Suryanarayana Sankuratri for management of the collaboration, Sergei A. Kazakov for advice in development of the short synthetic hairpin RNA detection assay, and Yoshio Morikawa and colleagues at PhoenixBio Co., for conducting the studies in the chimeric mice and hepatitis C virus–RNA titer determination.

Conflicts of interest
The authors disclose the following: Han Ma and Klaus Klumpp are employees of Hoffmann-La Roche, Inc; Brian Johnston, Anne Dallas, Heini Ilves, and Joshua Shorenstein are employees of SomaGenics, Inc; and Ian MacLachlan is an employee of Tekmira Pharmaceuticals.

Funding
Supported by National Institutes of Health grants R44AI056611, R44AI074256, and R43AI074214 (B.H.J.).

Supplementary Materials and Methods

LNP Encapsulation of sshRNA

Monomeric sshRNAs were formulated into LNPs (Supplementary Figure 3) by the process of step-wise ethanol dilution and spontaneous particle formation as previously described[1,2] (Supplementary Figure 4). An ethanolic solution of lipids 3-*N*-(-methoxy poly(ethylene glycol)2000)carbamoyl-1,2-dimyristyloxy-propylamine (PEG-C-DMA) (0.82 mg/mL), 3-(dilinoleylmethoxy)-N,N-dimethylpropan-1-amine (DLin-MP-DMA) (7.4 mg/mL), cholesterol (2.8 mg/mL), and dipalmitoylphosphatidylcholine (DPPC) (1.1 mg/mL) was mixed with a PBS solution containing 4.5 mg of nucleic acid. The resulting LNPs subsequently were concentrated and diafiltered against 20 wash volumes of PBS (pH 7.4) using a cross-flow ultrafiltration cartridge (GE Healthcare, Piscataway, NJ) and finally sterile-filtered through Acrodisc 0.8/0.2-μm Supor filters (Pall Corp, Ann Arbor, MI). The degree of NA encapsulation (92%–98%) was determined using RiboGreen (Invitrogen, Carlsbad, CA) and a Varian Cary Eclipse Fluorimeter (Santa Clara, CA). Particle sizes (85–90 nm) and polydispersity values ($<$0.1) were determined using a Malvern Nano Series Zetasizer (Malvern, UK).

Cryo-Transmission Electron Microscopy

Cryo-transmission electron microscopy of LNPs was performed at Uppsala University (Sweden) using a Zeiss EM 902A (Carl Zeiss Microscopy GmbH, Munich, Germany). Samples were incubated in a climate chamber (25°C, 98% humidity) for 20–30 minutes before use. Sample solution (0.5 μL) then was deposited on a copper grid, excess was removed by blotting, and the sample was vitrified in liquid ethane. Images at 100,000$\times$ total magnification were captured (Supplementary Figure 5), and the diametric size of the particles was calculated by number averaging.

Treatment of Human Liver–uPA-SCID Chimeric Mice

Male uPA$^{+/+}$/SCID mice with human albumin levels of 7 mg/mL or greater and HCV (genotype 1a) RNA titer between 4.0×10^6 and 7.8×10^7 IU/mL were chosen for the study. All mice received 2 intravenous injections via tail vein of the LNP-formulated HCV sshRNAs or an irrelevant sshRNA at the indicated doses on days 0 and 7. At the indicated time points before or after the sshRNA dosing, blood samples were collected via a retro-orbital route under isoflurane anesthesia. On day 28, all animals were anesthetized with isoflurane and a minimum of 300 μL of blood was collected via cardiac puncture, after which the animals were killed via exsanguination. Animal husbandry and all animal experimental procedures used in this study have been approved by the animal ethnics committee of PhoenixBio (Higashi-Hiroshima City, Japan) in accordance with appropriate Japanese regulatory authorities.

HCV-RNA Extraction, Amplification, and Quantification

HCV RNA was extracted from 5 μL of mouse serum using a SepaGene RV-R RNA extraction system (Sanko Junyaku, Tokyo, Japan; Life Technologies Corp, Carlsbad, CA). The HCV-RNA titer was determined by quantitative real-time polymerase chain reaction using primers and a TaqMan probe (Life Technologies Corporation, Foster City, CA) as previously described.[3] Briefly, one fourth of the extracted RNA of each sample was added to the reverse-transcription polymerase chain reaction performed with the TaqMan EZ reverse-transcription polymerase chain reaction core reagent (Life Technologies Corporation) and an ABI Prism 7500 sequence detector system (Life Technologies Corporation). The thermal reaction was initiated with 2 minutes at 50°C, followed by 30 minutes at 60°C for reverse transcription, and 5 minutes at 95°C; subsequent polymerase chain reaction amplification was performed with 50 cycles of 20 seconds at 95°C and 1 minute at 62°C. Duplicate reactions of each sample were conducted and an average value was used for data analysis. The lower quantification limit of the assay is 7.3×10^3 IU/mL (4.0×10^4 copies/mL). The conversion of HCV-RNA titer from copies/mL to IU/mL was determined by testing 10-fold dilutions of a World Health Organization HCV-RNA standard with a known titer in international units per milliliter (National Institute for Biological Standards and Control, code 96/798) in the polymerase chain reaction, and the titer in copies per milliliter was derived from the standard curve generated with 10-fold dilutions of internal HCV RNA reference with a predetermined titer in copies per milliliter. The conversion factor represents the mean of 3 independent experiments of 8 individual samples at each dilution. The conversion factor is 1 IU/mL = 5.5 copies/mL.

Determination of Blood Human Albumin Concentration and Serum Alanine Aminotransferase and Serum Aspartate Aminotransferase Concentrations

Blood human albumin concentration was determined based on latex agglutination immunonephelometry (LX reagent Eiken Alb II; Eiken Chemical, Co, Ltd, Tokyo, Japan) according to the manufacturer's instructions. Serum total alanine aminotransferase and aspartate aminotransferase activities were measured using Drichem 3500 (Fujifilm, Tokyo, Japan).

Determination of Liver Uptake of sshRNA in Human Liver–uPA-SCID Chimeric Mice and SCID Mice

Forty-eight hours after the intravenous administration of sshRNA, whole livers were harvested and weighed at necropsy. Four hundred milligrams of the left lateral lobe was immediately cut into approximately 0.125-cm^3 pieces and immersed in RNAlater solution (cat#AM7024; Ambion, Austin, Texas) overnight at 4°C. The RNAlater solution then

was removed and liver slices were stored at -80°C until analysis. Total RNA was isolated from the livers of human liver–uPA-SCID chimeric mice and SCID mice using a FastPrep instrument (FastPrep-24; MP Biomedicals, Solon, OH) to disrupt tissue. Approximately 100 mg of liver tissue from each mouse preserved in RNAlater (Ambion) was added to 1.2 g lysing Matrix "D" beads (MP Biomedicals) and 1 mL of Qiazol (Qiagen, Hilden, Germany). Samples were processed in the FastPrep-24 homogenizer twice for 60 seconds at a setting of 6 m/s. Samples were maintained on ice for 5 minutes between processing steps. Cellular debris was removed by centrifugation at 12,000 × g at ambient temperature. The liver homogenate was extracted with chloroform, and the aqueous layer was precipitated with isopropanol. The resulting RNA pellet was washed with 70% ethanol, resuspended in 100 μL of RNAse-free H_2O, and quantified by A_{260} measurement.

RNAse Protection Assay

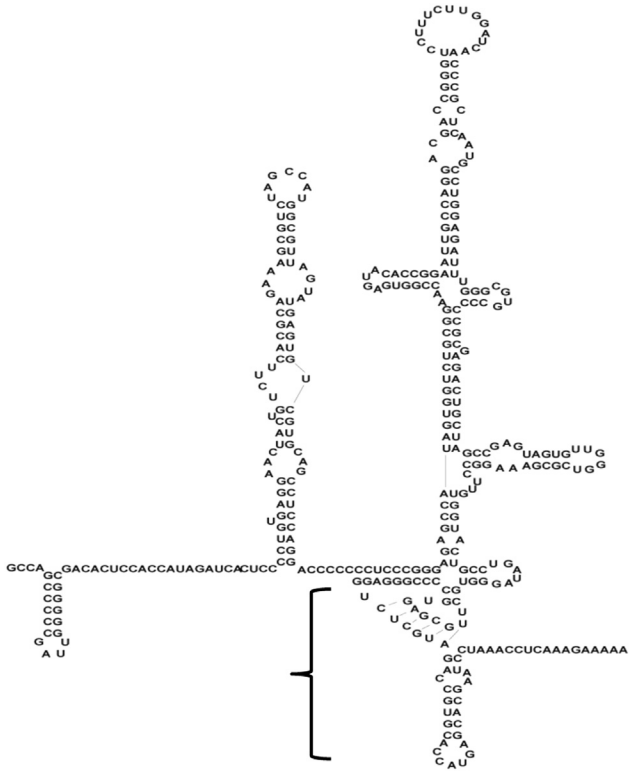
A ^{32}P-labeled probe specific for the detection of SG220 was prepared by in vitro transcription using T7 RNA polymerase (Promega, Madison, WI) in the presence of [α^{32}P]-cytidine triphosphate (Perkin-Elmer, Waltham, MA). After transcription, the probe was purified by a G-50 spin column (Amersham/GE Healthcare, Piscataway, NJ) and 10% denaturing polyacrylamide gel electrophoresis. For each sample, approximately 100,000 cpm ^{32}P-labeled probe was co-precipitated with 50 μg of total RNA isolated from each mouse liver. Hybridization was performed at 42°C for 12 hours, followed by cooling to 38°C for 2 hours, and finally to 32°C for an additional 2 hours (model 400 hybridization oven; Robbins Scientific, Sunnyvale, CA). RNAse protection assays were performed using the RPA III kit (Ambion) according to the manufacturer's instructions with nondigested controls. Samples were analyzed by 10% denaturing polyacrylamide gel electrophoresis (8 mol/L urea). Protected bands were visualized and quantified with the Molecular Imager FX instrument (Bio-Rad, Hercules, CA). In parallel, a standard curve was generated by analyzing spiked-in synthetic SG220 to final amounts of 1, 4, 20, and 100 ng per 50 μg total RNA. Quantification of SG220 sshRNA was performed by comparing the intensities of the protected bands with those of the spiked-in standards to estimate the percentage of SG220 present in the mouse livers 48 hours after injection relative to the total amount dosed.

Statistical Analysis

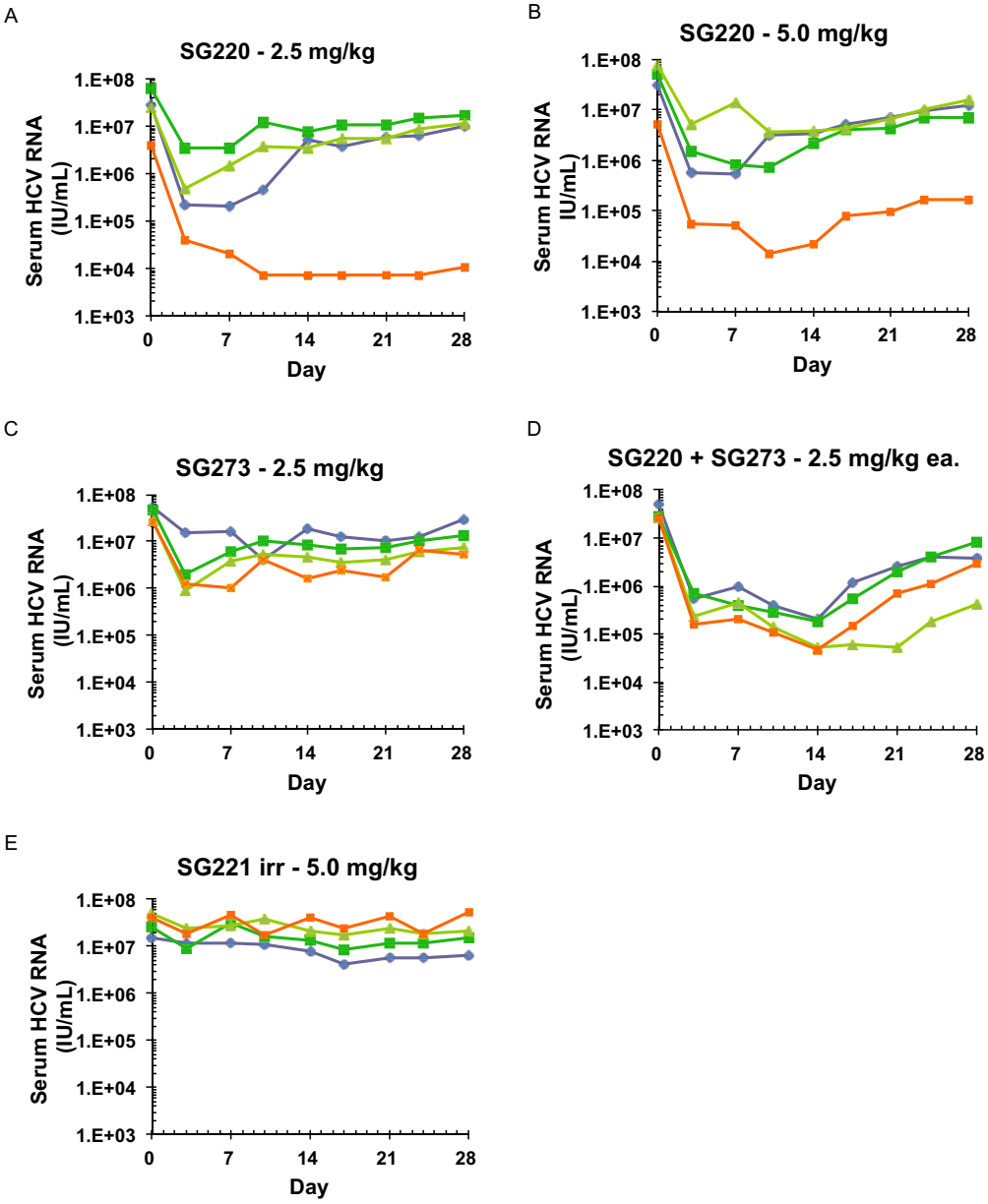
Statistical significance of the difference between the HCV viral load during treatment and follow-up period and the baseline (pretreatment, day 0) level was determined by 2-way analysis of variance followed by post hoc comparisons that were adjusted using Bonferroni correction. Statistically significant differences were defined by *P* values less than .05.

Supplementary References

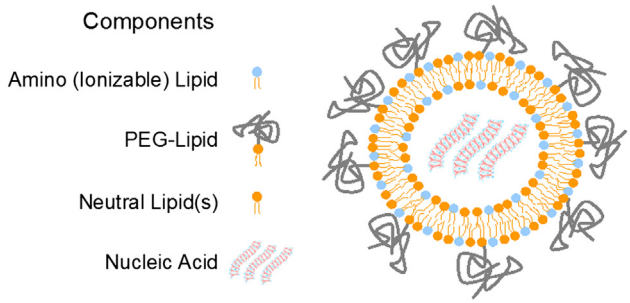
1. Jeffs LB, et al. Pharm Res 2005;22:362–372.
2. Semple SC, et al. Nat Biotechnol 2010;28:172–176.
3. Kamiya N, et al. J Gen Virol 2010;91:1668–1677.



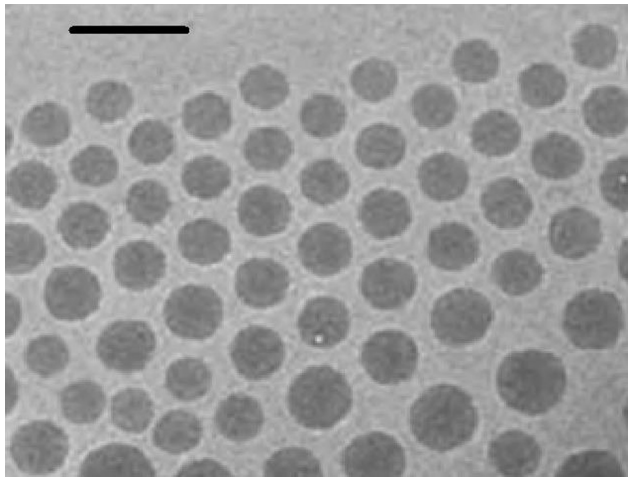
Supplementary Figure 1. Secondary structure of the HCV IRES with the region targeted by sshRNAs SG220 and SG273 indicated by the bracket.



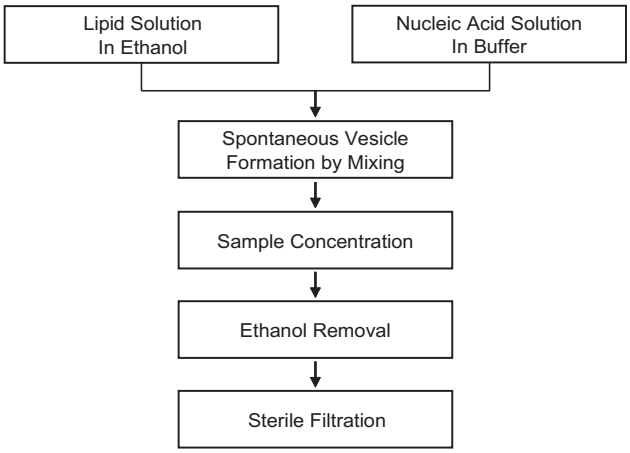
Supplementary Figure 2. Inhibition of HCV replication by LNP-formulated sshRNAs in individual chimeric human liver mice. Four HCV-infected chimeric mice in each group were injected intravenously with formulated sshRNAs on days 0 and 7. Serum HCV RNA was monitored at the indicated time points (IU/mL). Each *broken line* represents an individual mouse, with treatment groups receiving (*A*) SG220 at 2.5 mg/kg; (*B*) SG220 at 5.0 mg/kg; (*C*) SG273 at 2.5 mg/kg; (*D*) a combination of SG220 and SG273 at 2.5 mg/kg each; and (*E*) irrelevant sshRNA SG221 at 5.0 mg/kg.



Supplementary Figure 3. Schematic illustration of a LNP. LNPs are a multicomponent system comprising a mixture of titratable amino lipids (DLin-MP-DMA), PEG lipids (PEG-C-DMA), and neutral lipids (cholesterol and DPPC) fully encapsulating their nucleic acid payload. For the purposes of illustration, this figure shows lipids arranged in a bilayer configuration, however, LNPs, including those in this work, may be prepared in a manner that results in the formation of the class of nanoparticles, shown in Supplementary Figure 5, that lack a canonical bilayer.



Supplementary Figure 5. A representative cryo-transmission electron micrograph image of the LNP formulation used in this study. Scale bar = 100 nm.



Supplementary Figure 4. LNP formation by the process of step-wise ethanol dilution and spontaneous particle formation. An ethanolic solution of lipids is mixed with aqueous nucleic acid solution. The resulting LNPs are concentrated and diafiltered against buffer using a cross-flow ultrafiltration followed by sterile filtration. No physical particle sizing steps are required.

Supplementary Table 1. Liver Uptake of sshRNA in Chimeric Human Liver Mice and SCID Mice

Group	Liver sshRNA uptake at 48 hours, % mean ± SD
Chimeric human liver–uPA-SCID mice (3 mice)	5.9 ± 1.4
Regular SCID mice (5 mice)	5.1 ± 0.7

NOTE. Three chimeric human liver–uPA-SCID mice and 5 SCID mice were administered one intravenous injection of LNP-formulated SG220 at 5.0 mg/kg. sshRNA in the liver at 48 hours after the dosing was detected and quantified by a RNAse protection assay as described in the Materials and Methods section. Liver uptake was presented as a percentage of the initial dose. Mean values and standard deviations are shown.

Supplementary Table 2. Pretreatment HCV-RNA Titer in Each of the Chimeric Mouse Groups Receiving sshRNA Treatment

Group	HCV RNA titer pretreatment, mean (range), IU/mL
SG220, 2.5 mg/kg	3.1E+07 (4.0E+06–6.4E+07)
SG220, 5.0 mg/kg	4.1E+07 (5.1E+06–7.8E+07)
SG273, 2.5 mg/kg	3.8E+07 (2.5E+07–5.5E+07)
SG220 + SG273, 2.5 mg/kg	3.3E+07 (2.7E+07–4.9E+07)
SG221-irr, 5.0 mg/kg	3.3E+07 (1.5E+07–4.9E+07)

irr, irrelevant.

Supplementary Table 3. Statistical Significance of the Changes in HCV Viral Load During the Study Relative to Pretreatment Levels

	P value							
Days in treatment	3	7	10	14	17	21	24	28
irr sshRNA, 5.0	NS	NS	NS	NS	NS	NS	NS	NS
SG220 + SG273, 2.5 ea	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
SG220, 2.5	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
SG220, 5.0	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[b]
SG273, 2.5	[a]	[a]	[b]	[b]	[b]	[b]	NS	NS

irr, irrelevant.
[a] $P < .01$.
[b] $P = .01$–$.05$.