Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study

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Summary

Background **Direct-acting antiviral drugs have a high cure rate and favourable tolerability for patients with hepatitis C virus (HCV). Shorter courses could improve affordability and adherence. Sofosbuvir and ledipasvir with ribavirin have high efficacy when taken for 8 weeks but not for 6 weeks. We assessed whether the addition of a third direct-acting antiviral drug to sofosbuvir and ledipasvir would allow a shorter treatment duration.**

Methods **In this single-centre, open-label, phase 2A trial, we sequentially enrolled treatment-naive patients with HCV genotype 1 infection into three treatment groups: 12 weeks of sofosbuvir and ledipasvir; 6 weeks of sofosbuvir, ledipasvir, and GS-9669; or 6 weeks of sofosbuvir, ledipasvir, and GS-9451. Patients and investigators were not masked to treatment assignment. The primary endpoint was the propotion of patients with sustained viral response at 12 weeks after treatment completion (SVR12), assessed by serum HCV RNA concentrations lower than 43 IU/mL (the lower limit of quantification). We did an intention-to-treat analysis for the primary endpoint and adverse events. This study is registered with ClinicalTrials.gov, number NCT01805882.**

Findings **Between Jan 11, 2013, and Dec 17, 2013, we enrolled 60 patients, and sequentially assigned them into three groups of 20. We noted an SVR12 in all 20 patients (100%, 95% CI 83–100) allocated to sofosbuvir and ledipasvir for 12 weeks; in 19 (95%, 75–100) of the 20 patients allocated to sofosbuvir, ledipasvir, and GS-9669 for 6 weeks (one patient relapsed 2 weeks after completion of treatment); and in 19 (95%, 75–100%) of the 20 patients allocated to sofosbuvir, ledipasvir, and GS-9451 for 6 weeks (one patient was lost to follow-up after reaching sustained viral response at 4 weeks). Most adverse events were mild and no patients discontinued treatment. Two serious adverse events occurred (pain after a post-treatment liver biopsy and vertigo), both unrelated to study drugs.**

Interpretation **In this small proof-of-concept study, two different three-drug regimens that were given for 6 weeks resulted in high cure rates for HCV infection with excellent tolerability. Addition of a third potent direct-acting antiviral drug can reduce the duration of treatment required to achieve sustained viral response in patients with chronic HCV genotype 1 infection without cirrhosis.**

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Introduction

About 185 million people worldwide are infected with hepatitis C virus (HCV).[1] Up to 20% of these patients develop cirrhosis and a quarter of those will progress to end-stage liver disease or hepatocellular carcinoma.[2] Until late 2013, the treatment for hepatitis C genotype 1 was combination therapy with pegylated interferon, ribavirin, and, more recently, a direct-acting antiviral drug, for up to 1 year.[3–5] These regimens are difficult to tolerate because of adverse effects associated with each constituent of the triple-drug regimens, with efficacy of 56–88%.[4,5] In 2013, the US FDA licensed two new direct-acting antiviral drugs, sofosbuvir and simeprevir, for the treatment of HCV infections as part of combination regimens. In addition, 91–100% of patients given sofosbuvir and the antiviral drug ledipasvir as a one pill per day regimen for 12 weeks had sustained viral response (SVR).[6,7] Regimens with a short duration, low pill burdens, and few adverse effects could improve patient adherence.

An attempt to reduce the duration of therapy to 6 weeks through the addition of ribavirin to sofosbuvir and ledipasvir resulted in many patients relapsing after treatment.[8] We postulated that addition of a third direct-acting antiviral drug instead of ribavirin to sofosbuvir and ledipasvir could allow for shorter, more efficacious, treatment. We did a three-group clinical trial in a predominantly male black population, aiming to compare SVR prevalence for patients given sofosbuvir and ledipasvir for 12 weeks, sofosbuvir and ledipasvir plus GS-9669 (a non-nucleoside NS5B thumb site 3 inhibitor of HCV polymerase[8,9]) for 6 weeks, or sofosbuvir and ledipasvir plus GS-9451 (an inhibitor of the HCV NS3/4A protease[10]) for 6 weeks.

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See Online for appendix

Methods

Participants

We enrolled patients at the Clinical Research Center of the National Institutes of Health (NIH), Bethesda, MD, USA. We report enrolment and follow-up data from Jan 11, 2013, to Dec 17, 2013. Eligible participants were aged 18 years or older, infected with chronic HCV genotype 1 (serum HCV RNA concentration ≥2000 IU/mL). We excluded patients with cirrhosis from treatment groups that received 6 weeks of treatment. We assessed the presence or absence of cirrhosis with liver biopsy or with a combination of FibroSURE (LabCorp, Burlington, NC, USA) test plus aspartate aminotransferase to platelet ratio (APRI). Liver biopsy was required in cases of equivocal FibroSURE results. Full eligibility criteria are included in the appendix. We obtained written or oral informed consent from all participants. The study was approved by the institutional review board of the National Institute of Allergy and Infectious Diseases (NIAID) and was done in compliance with the Good Clinical Practice guidelines, the Declaration of Helsinki, and regulatory requirements.

Procedures

Patients were sequentially enrolled into three groups (1:1:1). We contacted patients for screening visits in the order in which patients initially contacted the NIAID study team with interest. We started the study drug in the order in which participants completed eligibility requirements. In the first group, patients were allocated tosofosbuvir and ledipasvir for 12 weeks. In the second group, patients were allocated to sofosbuvir, ledipasvir, and GS-9669 for 6 weeks. In the third group, patients were allocated to sofosbuvir, ledipasvir, and GS-9451 for 6 weeks.

For the **NIAID toxicity table** see http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/daidsaegradingtable.pdf

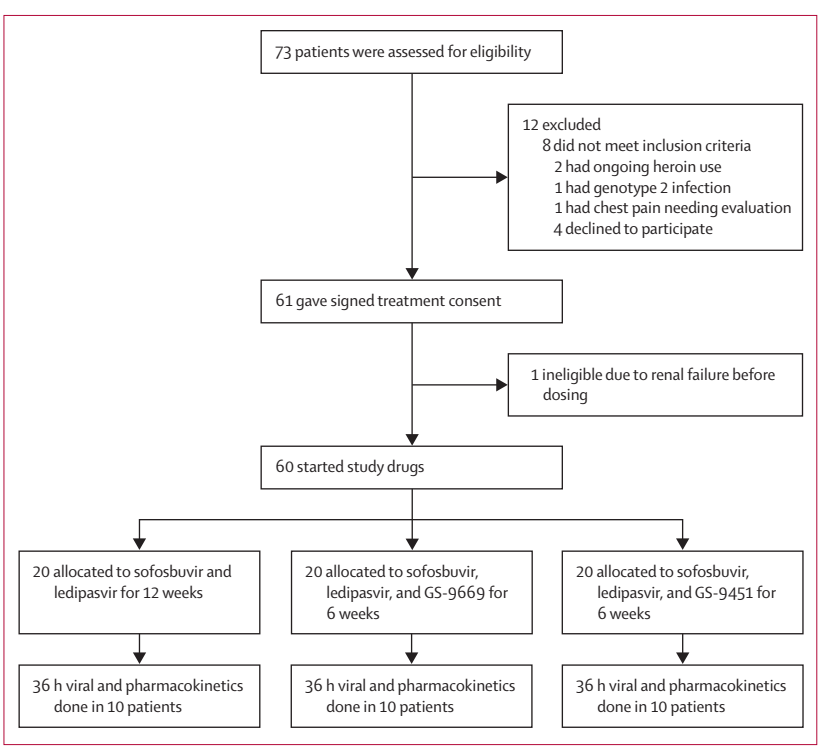

Figure 1: **Trial profile**

Sofosbuvir (400 mg) and ledipasvir (90 mg) were given as a combination tablet taken once a day. GS-9669 was given as two 250 mg tablets once a day and GS-9451 (80 mg) as one tablet once a day. We enrolled 10 patients per treatment group into a substudy to measure early viral kinetics and pharmacokinetics. Criterion for stopping study drugs was failure to achieve a greater than 2 $\log_{10}$ decline in HCV RNA levels at week 4 after the first dose. Additional criteria were HCV RNA greater than lower limit of quantification after two previous consecutive HCV RNA values less than the lower limit of quantification and greater than a 2 $\log_{10}$ increase in HCV RNA from nadir. The protocol permitted participants who failed treatment the option of treatment with the standard of care, which at the time of the study was pegylated-interferon, ribavirin, and sofosbuvir. This protocol was later amended with an option for retreatment with sofobuvir and ledipasvir for 12 weeks for patients who failed the 6 week treatment regimens. Neither patients nor investigators were masked to treatment allocation.

We measured plasma HCV RNA concentrations using the real-time HCV Assay (Abbott Laboratories, Abbott Park, IL, USA), with a lower limit of quantification of 12 IU/mL and a lower limit of detection of 3 IU/mL. We also measured serum HCV RNA concentrations using the COBAS TaqMan HCV RNA assay, version 2.0 (Roche Diagnostics, Indianapolis, IN, USA), with a lower limit of quantification of 43 IU/mL and a lower limit of detection of 15 IU/mL.

We recorded adverse events and clinical laboratory results throughout the study. Adverse events were graded from one (mild) to four (severe) according to the NIAID Division of AIDS toxicity table (version 1.0). Pill counts were done at several timepoints during treatment.

During the first month of treatment, we measured plasma HCV RNA concentrations at day 0, 1, 3, 5, 7, 10, 14, 21, and 28 in all patients. Data for very early viral kinetics were obtained in a subset of 29 of 60 participants (ten in group one, ten in group two, and nine in group three) by measuring plasma HCV RNA concentrations 0, 1, 2, 4, 8, 12, 24, and 36 h after administration of the first dose of study drugs.

Whole blood was collected using PAXgene Blood DNA tubes (Qiagen, Valencia, CA, USA) and stored at –80°C until DNA extraction was done with use of the PAXgene blood DNA kit (PreAnalytiX, a Qiagen/ Becton Dickinson Company). We established *IL28B and IFNL4* genotype for DNA specimens using the 5′ nuclease assay with *IL28B and IFNL4* allele specific TaqMan probes (ABI TaqMan allelic discrimination kit, Roche Diagnostics, Indianapolis, IN, USA) and the ABI 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). Genotyping of variants at the rs12979860 (referred to as

IL28B genotype) and rs368234815 (*IFNL4*) loci was done with custom TaqMan assays as previously described.[7] We offered an optional liver biopsy sample after treatment completion (within 2 weeks of drug cessation) to all participants who had a pretreatment liver biopsy for staging and who underwent eligibility assessment at the NIH Clinical Center. Histopathological assessments of post-treatment liver biopsies were done by one pathologist (DEK) with liver expertise in a non-blinded fashion at the time of biopsy and was staged according to the Knodell histological activity index.[11] We did viral kinetic modelling with a multiscale model[12,13] in all participants who participated in the study (appendix). Deep sequencing of the HCV *NS5A* and *NS5B* genes was done by DDL (DDL Diagnostics Laboratory, Rijswijk, Netherlands) in samples collected at baseline and time of virologic failure in patients who relapsed.

Outcomes

The primary endpoint was the proportion of participants with plasma HCV viral load below the lower level of quantification 12 weeks after treatment completion (SVR12). The main safety endpoint was the frequency and severity of adverse events. Secondary endpoints were proportion of participants with unquantifiable HCV viral load at specified timepoints during and after treatment, discontinuations due to adverse events, safety laboratory changes, and the occurence of HCV-resistance mutations. Other uncompleted secondary endpoints are not reported. We also did a post-hoc comparison of viral kinetics between treatment groups. We report outcomes up to 12 weeks, with follow-up of patients up to 48 weeks post-treatment continuing.[11]

Statistical analyses

The primary endpoint and the main safety endpoint were based on an intention-to-treat population (all patients who received at least one dose of study drug). We calculated sample size to provide a sufficiently high probability of observing at least one adverse event of probability 10% or more and with prespecified CIs for estimates of efficacy assuming 20 patients in each treatment group. If the true probability of an adverse event due to a regimen was 10% or more, then a sample size of 20 allows an 88% chance of observing at least one such adverse event. With a sample size of 20, if all patients achieved SVR12, then the 95% CI for that estimate is 83–100, and, if 19 patients achieved SVR 12, then the 95% CI for that estimate is 75–100. We compared baseline demographics using the Kruskall-Wallis test for continuous outcomes and χ^2 tests for binary outcomes. We compared estimated fall in HCV viral load between groups using a Kruskal-Wallis test and for significant values, multiple comparisons were made between samples using the Conover-Inman[14] procedure including correction for multiple tests. Statistical analyses were done with BiAS, Prism 6.0, SAS, STAT-CRUNCH, and S-Plus 8.0.

Role of the funding source

Data collection, review, and analysis were done by NIH investigators. All funders participated in the study design and writing of the report. NIH-affiliated investigators had full access to all data in the study, and AK and the corresponding author had final responsibility for the decision to submit for publication. The Regulatory Compliance and Human Participants Protection Branch of the National Institute of Allergy and Infectious Diseases (NIAID) served as the study sponsor and was involved in the review and approval of the study via the usual peer-review process as well as the study management. The Regulatory Compliance and Human Participants Protection Branch did not have a role in the design of the study, data collection and analysis, interpretation of the data, preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. Gilead Sciences provided drug and scientific advice.

	12 weeks of sofosbuvir and ledipasvir (n=20)	6 weeks of sofosbuvir, ledipasvir, and GS-9669 (n=20)	6 weeks of sofosbuvir, ledipasvir, and GS-9451 (n=20)	p value
Age (years)	57 (8)	54 (7)	54 (9)	0·28
Men	14 (70%)	13 (65%)	16 (80%)	0·56
Race*				0·32
Black	16 (80%)	19 (95%)	18 (90%)	..
White	4 (20%)	1 (5%)	2 (10%)	..
Ethnic origin*				0·36
Hispanic	1 (5%)	0	0	..
Non-Hispanic	19 (95%)	20 (100%)	20 (100%)	..
Body-mass index (kg/m^2)	25 (4)	28 (7)	28 (6)	0·16
HCV genotype				0·12
1a	11 (55%)	14 (70%)	17 (85%)	..
1b	9 (45%)	6 (30%)	3 (15%)	..
Plasma HCV RNA levels >800 000 IU/mL	15 (75%)	13 (65%)	14 (70%)	0·79
IL28B genotype				0·66
CC	5 (25%)	2 (10%)	5 (25%)	..
CT	9 (45%)	10 (50%)	7 (35%)	..
TT	6 (30%)	8 (40%)	8 (40%)	..
IFNL4 genotype				0·68
TT/TT	3 (15%)	3 (15%)	5 (25%)	..
ΔG/TT	10 (50%)	10 (50%)	6 (30%)	..
ΔG/ΔG	7 (35%)	7 (35%)	9 (45%)	..
Knodell HAI, Metavir, or FibroSURE fibrosis score†				0·16
0–2	12 (60%)	15 (75%)	15 (75%)	..
3	5 (25%)	5 (25%)	5 (25%)	..
4	3 (15%)	0	0	..

Data are mean (SD) or n (%). HCV=hepatitis C virus. HAI=Histology Activity Index. *Race and ethnic origin were self-reported. †For eligibility assessment, 2 (3%) patients had a score from FibroSURE and aminotransferase to platelet ratio index, 9 (15%) patients had biopsy samples scored with Metavir system, and 49 (82%) patients had biopsy samples scored using Knodell HAI system.

Table 1: **Baseline demographics and clinical characteristics**

	12 weeks of sofosbuvir and ledipasvir (n=20)		6 weeks of sofosbuvir, ledipasvir, and GS-9669 (n=20)		6 weeks of sofosbuvir, ledipasvir, and GS-9451 (n=20)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
During treatment						
Day 7	2	10% (1–31)	0	..	5	25% (9–49)
Week 2	6	30% (12–54)	6	30% (12–54)	9	45% (23–68)
Week 4	17	85% (62–97)	20	100% (83–100)	20	100% (83–100)
Week 6	..	..	20	100% (83–100)	20	100% (83–100)
Week 8	20	100% (83–100)	..	..	..	..
Week 12	20	100% (83–100)	..	..	..	..
After treatment						
Week 4	20	100% (83–100)	19	95% (75–100)	20	100% (83–100)
Week 12	20	100% (83–100)	19	95% (75–100)	19†	95% (75–100)

When no data are reported, data were not collected. *The limit of quantification for concentrations of hepatitis C virus RNA was 43 IU/mL. †One patient who achieved sustained viral response at 4 weeks was incarcerated into prison after this visit. Although records were available at 12 weeks after therapy, the *NIAID* institutional review board, including a prisoner representative, decided that data from subsequent timepoints could not be reported.

Table 2: **Proportion of patients with plasma hepatitis C virus RNA concentration lower than the quantification limit***

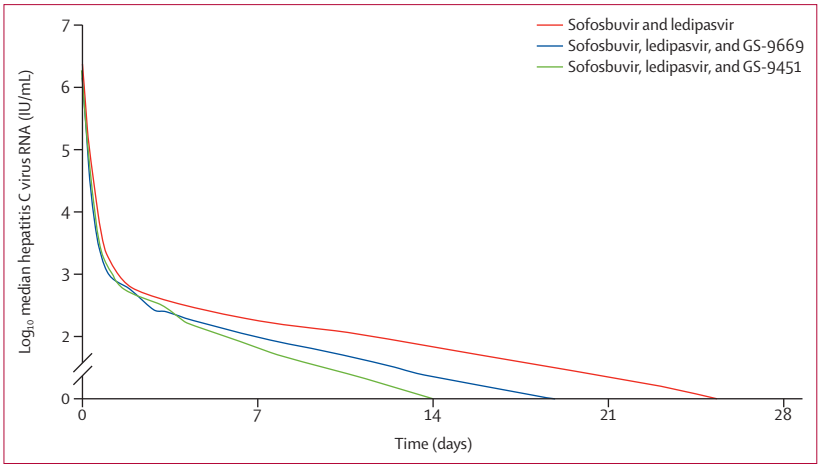

Figure 2: **Decline in median hepatitis C viral load**
Hepatitis C virus RNA decreases were significantly more (p<0·05) at days 7, 14, and 28 in patients who received sofosbuvir, ledipasvir and GS-9451 (green) compared with in those who received sofosbuvir, ledipasvir, and GS-9669 (blue) and in those who received sofosbuvir and ledipasvir (red).

Results

Between Jan 11, 2013, and Dec 17, 2013, 73 participants were screened and 60 were enrolled (figure 1). Baseline characteristics were similar between treatment groups (table 1). Participants were mainly black (53, 88%), men (43, 72%), had *IL28* non-CC genotype (48, 80%), were infected with HCV genotype 1a (42, 70%), and had high baseline concentrations of plasma HCV RNA (>800 000 IU/mL; 42, 70%). 15 (25%) patients had stage 3 liver disease. Of patients treated with sofosbuvir and ledipasvir, three (15%) had stage 4 disease (table 1), no patients had stage 4 disease in the other groups.

Of the patients allocated to receive sofosbuvir and ledipasvir, all 20 (100%, 95% CI 83–100) had an unquantifiable HCV RNA 12 weeks after completion of treatment—ie, had SVR12. 19 (95%, 75–100) patients allocated to sofosbuvir, ledipasvir, and GS-9669, and 19 (95%, 75–100) patients allocated to sofosbuvir, ledipasvir, and GS-9451, had unquantifiable serum HCV RNA levels 12 weeks after completion of therapy.

One patient (5%) allocated to sofosbuvir, ledipasvir, and GS-9669 for 6 weeks relapsed 2 weeks after completion of treatment. This patient was infected with HCV genotype 1a, had stage 3 liver disease, a baseline HCV viral load of 1922 287 IU/mL and an *IL28B* CT genotype. Additionally, 7% of this patient's baseline virus had the M28T *NS5A* mutant and 13% had the Q30H *NS5A* mutant. At relapse, a double-mutant M28T and Q30H was detected, which is associated with a more than 1000-fold reduced susceptibility to ledipasvir. Neither sofosbuvir-resistance variant *NS5B* S282T nor GS-9669-resistance variants were detected in this patient at relapse. The patient took 98% of his drugs, as established by pill counts. One patient treated with sofosbuvir, ledipasvir, and GS-9451 was incarcerated into prison after having SVR after their 4 week follow-visit after completion of therapy. A special session of the NIAID institutional review board deemed that data obtained after incarceration could not be included for publication.

17 (85%) of participants treated with sofosbuvir and ledipasvir had an unquantifiable level (<43 IU/mL of HCV RNA) by week 4 of treatment (table 2). All participants treated with the combination of sofosbuvir, ledipasvir and GS-9669, or that of sofosbuvir, ledipasvir, and GS-9451, had an unquantifiable concentration of HCV RNA by week 4 of treatment (table 2). We noted a rapid, sustained decrease in HCV RNA in patients in all three groups. Viral kinetic modelling shows a three-phase decrease, with a rapid first phase, moderate decay during an intermediate phase, and then a slower third phase (figure 2). This form of decay can be modelled and fitted for every patient (appendix). We assessed whether there was a significantly faster HCV decrease with three drugs compared with two drugs. Fitted HCV RNA concentrations were significantly lower at early timepoints in patients given sofosbuvir, ledipasvir, and GS-9451 than in those given the two other regimens (p<0·05 at days 7, 14, and 21; appendix).

Using our model, we estimated the effect of overall treatment on HCV clearance by taking into account the effectiveness of the regimen in blocking HCV production ($\varepsilon 1$ and $\varepsilon 2$) and intracellular decay (κ). Median overall treatment effect for all three regimens did not differ significantly (p>0·05; 99·977% for sofusbuvir and ledipasvir, 99·954% for sofosbuvir, ledipasvir, and GS-9669, and 99·984% for sofosbuvir, ledipasvir, and GS-9451; appendix). Nevertheless, more patients in the group given sofosbuvir, ledipasvir, and GS-9451 had a maximum threshold effect of 99·95% or higher in clearance of plasma HCV than did those in the group given the other regimens (p=0·09).

All 60 patients completed treatment. The most common adverse events were diarrhoea, headache, and fatigue

(table 3), and most adverse events were mild. Two grade 3 adverse events occurred—pain related to a post-treatment research liver biopsy and an episode of vertigo that caused admission into hospital in a patient who had a history of severe intermittent episodes of vertigo; both patients were allocated to sofosbuvir, ledipasvir, and GS-9451. No grade 4 laboratory abnormalities were reported. 11 grade 3 laboratory abnormalities occurred in nine patients (table 3). Grade 3 hyperglycaemia and hypoglycaemia occurred in one patient who had a history of insulin-dependent diabetes mellitus. Two patients had asymptomatic hypophosphataemia. One patient who had a history of anaemia had transiently decreased haemoglobin concentrations (89 g/L), which improved to baseline concentrations (≥100 g/L) before completion of treatment. Three patients had transiently raised serum creatinine concentrations. Two increases occurred after completion of study drugs: one patient with baseline renal insufficiency reported dehydration 8 weeks after treatment (glomerular filtration rate [GFR] 56 mL/min per 1·73 m² at baseline; 25 mL/min per 1·73 m² at week 8; 68 mL/min per 1·73 m² at week 10 without intervention) and another in a patient who initiated 1600 mg per day of ibuprofen for arthritis (GFR 96 mL/min per 1·73 m² at baseline; 35 mL/min per 1·73 m² at week 6; 90 mL/min per 1·73 m² at week 7). A third patient with baseline renal insufficiency had transient worsening of renal function on treatment that resolved without intervention (GFR 66 mL/min per 1·73 m² at baseline; 35 mL/min per 1·73 m² at day 7; 59 mL/min per 1·73 m² at day 9). One patient treated with sofosbuvir and ledipasvir developed an isolated increase in alanine transaminase (peak 94 U/L at week 4) and aspartate aminotransferase (peak 230 U/L at week 5) with normal bilirubin concentrations. Investigations for autoimmune, infectious, and toxin-induced causes had normal findings; however, at week 5, the patient revealed that they had been eating several grapefruits every day for the previous 4 weeks, which has a potential interaction with the atypical antipsychotic lurasidone that the patient was also taking. The patient stopped eating grapefruits and alanine transaminase and aspartate aminotransferase concentrations decreased to pretreatment concentrations by week 6 without interruption of study drug.

	12 weeks of sofosbuvir and ledipasvir (n=20)	6 weeks of sofosbuvir, ledipasvir, and GS-9669 (n=20)	6 weeks of sofosbuvir, ledipasvir, and GS-9451 (n=20)
Adverse events			
Any adverse event during treatment	20 (100%)	20 (100%)	20 (100%)
Any serious adverse event during treatment	0	0	2 (10%)
Common adverse events†			
Night sweats	0	2 (10%)	0
Constipation	0	2 (10%)	1 (5%)
Vomiting	1 (5%)	2 (10%)	0
Nausea	1 (5%)	2 (10%)	1 (5%)
Shoulder pain	2 (10%)	0	0
Common cold	4 (20%)	1 (5%)	1 (5%)
Fatigue	2 (10%)	2 (10%)	4 (20%)
Diarrhoea	1 (5%)	5 (25%)	3 (15%)
Headache	5 (25%)	5 (25%)	0
Rash	3 (15%)	1 (5%)	0
Abdominal pain	2 (10%)	2 (10%)	0
Back pain	2 (10%)	0	1 (5%)
Laboratory abnormalities			
Any grade 3 abnormality during treatment	4 (20%)	2 (10%)	4 (20%)
Grade 3 abnormalities			
Hypophosphataemia	0	2 (10%)	0
Raised serum creatinine	0	0	3 (15%)
Decreased haemoglobin	0	0	1 (5%)
Raised alanine transaminase	1 (5%)	0	0
Raised aspartate aminotransferase	1 (5%)	0	0
Raised LDL	1 (5%)	0	0
Hyperglycaemia	1 (5%)	0	0
Hypoglycaemia	1 (5%)	0	0

Data are n (%). *Treatment period from first study drug to 30 days after discontinuation. †Occurring in 10% of patients or more.

Table 3: **Adverse events and abnormalities on laboratory tests during treatment**

Discussion

In our study, patients with chronic HCV genotype 1 infection without cirrhosis were successfully treated with a 6 week course of three oral direct-acting antiviral drugs. The regimens were well tolerated, rapidly suppressed HCV viraemia in patients, and resulted in high rates of SVR12. Treatment for HCV infection is rapidly changing to avoid need for parenteral interferon and oral ribavirin, both of which are associated with many toxic effects, including teratogenicity with ribavirin. Single direct-acting antiviral drugs, including sofosbuvir, were initially used in combination with pegylated-interferon and ribavirin for the treatment of HCV genotype 1 infection, improving efficacy.[3,15,16] Interferon-free and ribavirin-free regimens have been shown to be efficacious (panel).[6,17–20] Some interferon-free regimens have been remarkably well tolerated, but the long duration of therapy (12–24 weeks) has raised concerns about adherence and cost.[31] Attempts to shorten the duration of therapy to 6 weeks with combination of sofosbuvir, ledipasvir, and ribavirin in a small study resulted in lower SVR rates (68%, 95% CI 47–85) than in patients give 8 weeks (SVR 90–97) or 12 weeks (SVR 92–98) of sofosbuvir and ledipasvir alone.[21] Two studies have assessed regimens of 8 weeks' duration.[21,22] In the first study,[21] 8 weeks of sofosbuvir and ledipasvir was non-inferior to 12 weeks of sofosbuvir and ledipasvir. In the second smaller study,[22] 8 weeks of ABT-450 and ritonavir, ABT-267, ABT-333, and ribavirin resulted in SVR12 in 86% of patients with HCV genotype 1b infection and in 96% of patients with HCV genotype 1a infection. The investigators did not do a formal comparison of 8 weeks versus 12 weeks with this combination. Although SVR rates were high in both trials,

Panel: Research in context

Systematic review

We searched PubMed on May 27, 2014, for articles in English using a combination of the MeSH search terms "HCV treatment" and "antiviral agent" and consulted the hepatis C virus (HCV) treatment guidelines for phase 2 and 3 clinical trials of treatments for patients with genotype-1 hepatitis C virus. We used no date restrictions. We also searched the reference list of articles from our search for additional reports that met our inclusion criteria. 16 clinical trials have been published of interferon-free regimens for patients with HCV genotype 1. These trials have shown promising safety and efficacy using combination direct-acting antiviral drugs, with or without ribavirin for 8–24 weeks.[7,8,17–30] One other study assessed a regimen of 6 weeks' duration and showed sustained virological response at 12 weeks in 68% of patients.[8]

Interpretation

Although our study is small, we showed high rates of sustained viral reponse at 12 weeks with use of regimens given for only 6 weeks, which supports the possibility that a short, 6 week treatment duration might be effective for some patients. Further development of these short duration therapies is warranted and studies of even shorter treatment durations for HCV infection with combination direct-acting antiviral drugs should be pursued.

more people relapsed in the groups treated for 8 weeks than in groups treated for 12 weeks, suggesting that efficacy might diminish with these regimens at shorter durations.[21,22] It has not been clear what combinations of antiviral drugs and what duration of therapy are most efficacious, tolerable, and cost effective for patients with a range of host and viral factors.

In our study, the use of three direct-acting drugs with different mechanisms of action in two therapeutic groups from a monoinfected urban population allowed for a shorter duration of treatment and resulted in high cure rates and excellent tolerability. Furthermore, viral kinetic modelling suggests that the three-drug regimen of sofosbuvir, ledipasvir, and GS-9451—targeting three different stages of the HCV lifecycle—resulted in enhanced HCV clearance compared with other regimens that target only two stages.

We anticipated that detectable plasma HCV RNA at the end of treatment would be predictive of relapse; however, six patients with quantifiable HCV RNA at the end of treatment (range 14–64 IU/mL) later went on to have SVR12. The exact mechanism by which NS5A inhibitors such as ledipasvir work to suppress HCV virus is not known; however, it has been postulated that the drug leads to the production of non-infectious virus particles.[13] This theory could possibly explain the detection of quantifiable HCV at the end of treatment in patients who subsequently had SVR12 without continued treatment. Another plausible explanation would be that the host innate or adaptive immune system has a role in elimination of residual HCV in-vivo after completion of therapy.

Although 12 weeks of therapy was effective in all patients, one patient treated for 6 weeks relapsed 2 weeks after treatment completion. The patient had advanced stage 3 liver disease and a high baseline HCV viral load, both of which are predictors of poor response to some therapeutic regimens that contain only direct-acting antiviral drugs.[12,18] Additionally, this patient had a double *M28T* and *Q30H* mutation in the NS5A region after treatment, which is associated with a more than 1000-fold reduced susceptibility to ledipasvir compared with those with wild-type HCV virus. Patients with cirrhosis were not included in the 6 week triple-drug groups because these patients can be more difficult to treat.[18] In view of the small numbers of patients with stage 3 liver disease in this study and the exclusion of patients with cirrhosis from the 6 week treatment groups, further studies are needed to determine whether the 6 week regimens that we assessed can be used successfully in patients with cirrhosis.

Although treatment regimens were generally well tolerated, one patient assigned to sofosbuvir and ledipasvir developed raised alanine transaminase and aspartate aminotransferase concentrations. This patient was eating several grapefruits a day (which can lead to CYP3A4 inhibition), while also taking lurasidone, an atypical antipsychotic for chronic bipolar disorder. Inhibition of CYP3A4 can increase concentrations of lurasidone and is thought to have led to this effect because neither sofosbuvir or ledipasvir are metabolised by CYP3A4.[32] However, toxic effects due to study drug cannot be excluded completely without data for study drug concentrations, which are not available.

Our study suggests that use of combinations of direct-acting antiviral drugs for 6 weeks leads to reasonable treatment outcomes, findings that should be validated in larger trials. Whether regimens could be further shortened for patients with specific host or viral factors and comorbidities should be tested. Limitations of the study include its sequential, non-randomised enrolment and that it was a single-site trial. Confidence in the estimates of efficacy is limited by the few patients included and ability to use only historical comparisons for efficacy. Additionally, all patients received intensive nursing support and monitoring, which might be hard to replicate in community-based treatment programmes for hepatitis C.

In view of the confines of the population studied, we can now speculate that a 6 week course of three direct-acting antiviral drugs can result in SVR12 in at least 75%, and perhaps nearly 100%, of people infected with HCV. This quick and simple treatment for HCV might prove relevant for the global elimination of hepatitis C, in which simple and well tolerated therapy of short duration is needed to ensure adherence.

Contributors

SK and AK had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. AK and SK searched the published literature. AK, SK, AN, MP, ASF, MAP, and HM contributed to the study design. AK, ZS, MMM, SS, TP, KT, EP, AN, D'AE, RS, CK, CH, TJ, RK, GD, AO, LLB, EGM, RK, MS, GT, RT, JC, SA, DK, and BJW collected data. AK, ZS, MMM, SS, DB, EH, and MP analysed the data. AK, AO, LLB, EGM, RK, MS, DB, EH, DK, BJW, ASF, MAP, and HM interpreted the data. ZS, MMM, SS, TP, KT, DB, and EH contributed to the figure design. AK wrote the first draft of the manuscript and all authors participated in the review of the Article.

Declaration of interests
WTS, PSP, GMS, and JM are employed by Gilead. RT has served as a speaker for Merck and does research funded by Vertex. JC is a member of the regional advisory boards for Abbott, Bristol-Myers Squibb, and Gilead. GT serves on the Gilead and Merck advisory boards and is a speaker for Gilead.

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References

1 Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57:** 1333–42.
2 Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; **20:** 17–35.
3 Kowdley KV, Lawitz E, Crespo I, et al. Sofosbuvir with pegylated interferon alfa-2a and ribavirin for treatment-naive patients with hepatitis C genotype-1 infection (ATOMIC): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2013; **381:** 2100–07.
4 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.
5 Kwo PY, Lawitz EJ, McCone J, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376:** 705–16.
6 Sulkowski M, Gardiner DF, Rodriguez-Torres M, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med* 2014; **370:** 211–21.
7 Lawitz E, Poordad FF, Pang PS, et al. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383:** 515–23.
8 Gane EJ, Stedman CA, Hyland RH, et al. Efficacy of nucleotide polymerase inhibitor sofosbuvir plus the NS5A inhibitor ledipasvir or the NS5B non-nucleoside inhibitor GS-9669 against HCV genotype 1 infection. *Gastroenterology* 2014; **146:** 736–43. e1.
9 Lawitz E, Hazan L, Gruener D, et al. 1189 GS-9669, a novel NS5B non-nucleoside thumb site II inhibitor, demonstrates potent antiviral activity, favorable safety profile and potential for once-daily dosing. *J Hepatol* 2012; **52** (suppl 2)**:** S471.
10 Lawitz EJ, Hill JM, Marbury T, et al. A phase I, randomized, placebo-controlled, 3-day, ascending-dose study of GS-9451, an NS3/4A protease inhibitor, in genotype 1 hepatitis C patients. *Antivir Ther* 2013; **18:** 311–19.
11 Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1:** 431–35.
12 Guedj J, Neumann AU. Understanding hepatitis C viral dynamics with direct-acting antiviral agents due to the interplay between intracellular replication and cellular infection dynamics. *J Theor Biol* 2010; **267:** 330–40.
13 Guedj J, Dahari H, Rong L, et al. Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. *Proc Natl Acad Sci USA* 2013; **110:** 3991–96.
14 Conover WJ. Practical nonparametric statistics, 3rd edn. Hoboken: Wiley, 1999.
15 Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368:** 1878–87.
16 Fried MW, Buti M, Dore GJ, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naive genotype 1 hepatitis C: the randomized PILLAR study. *Hepatology* 2013; **58:** 1918–29.
17 Gane EJ, Stedman CA, Hyland RH, et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med* 2013; **368:** 34–44.
18 Osinusi A, Meissner EG, Lee YJ, et al. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. *JAMA* 2013; **310:** 804–11.
19 Lawitz E, Poordad F, Kowdley KV, et al. A phase 2a trial of 12-week interferon-free therapy with two direct-acting antivirals (ABT-450/r, ABT-072) and ribavirin in IL28B C/C patients with chronic hepatitis C genotype 1. *J Hepatol* 2013; **59:** 18–23.
20 Everson GT, Sims KD, Rodriguez-Torres M, et al. Efficacy of an interferon- and ribavirin-free regimen of daclatasvir, asunaprevir, and BMS-791325 in treatment-naive patients with HCV genotype 1 infection. *Gastroenterology* 2014; **146:** 420–29.
21 Kowdley KV, Gordon SC, Reddy KR, et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med* 2014; **370:** 1879–88.
22 Kowdley KV, Lawitz E, Poordad F, et al. Phase 2b trial of interferon-free therapy for hepatitis C virus genotype 1. *N Engl J Med* 2014; **370:** 222–32.
23 Afdhal N, Reddy KR, Nelson DR, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370:** 1483–93.
24 Afdhal N, Zeuzem S, Kwo P, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370:** 1889–98.
25 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:** 742–48.
26 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.
27 Poordad F, Lawitz E, Kowdley KV, et al. Exploratory study of oral combination antiviral therapy for hepatitis C. *N Engl J Med* 2013; **368:** 45–53.
28 Suzuki Y, Ikeda K, Suzuki F, et al. Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options. *J Hepatol* 2013; **58:** 655–62.
29 Zeuzem S, Buggisch P, Agarwal K, et al. The protease inhibitor, GS-9256, and non-nucleoside polymerase inhibitor tegobuvir alone, with ribavirin, or pegylated interferon plus ribavirin in hepatitis C. *Hepatology* 2012; **55:** 749–58.
30 Zeuzem S, Soriano V, Asselah T, et al. Faldaprevir and deleobuvir for HCV genotype 1 infection. *N Engl J Med* 2013; **369:** 630–39.
31 Hill A, Khoo S, Fortunak J, Simmons B, Ford N. Minimum costs for producing hepatitis C direct acting antivirals, for use in large-scale treatment access programs in developing countries. *Clin Infect Dis* 2014; **58:** 928–36.
32 Sunovion. Latuda (lurasidone): package insert. Marlborough: Sunovion, 2010.