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Efficacy and safety of 3-week response-guided triple direct-acting antiviral therapy for chronic hepatitis C infection: a phase 2, open-label, proof-of-concept study

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Summary

Background—To shorten the course of direct-acting antiviral agents for chronic hepatitis C virus (HCV) infection, we examined the antiviral efficacy and safety of 3 weeks of response-guided therapy with an NS3 protease inhibitor and dual NS5A inhibitor–NS5B nucleotide analogue.

Methods—In this open-label, phase 2a, single centre study, Chinese patients with chronic HCV genotype 1b infection without cirrhosis were randomly allocated by a computer program to one of

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See Online for appendix

Contributors

GL, YB, and RFS designed the study. JC randomly assigned the patients. GL, YB, RFS, GC, JLi, QS, DJ, FL, BL, JLi, JH, JS, CW, VW, AW, and YW recruited the patients or did the study. CLPW, STYT, YW, LB, ST, YJ, and H-MH did the laboratory work, RK and ASP did the biostatistics analysis and viral kinetic mathematical modelling. GL and RFS wrote the manuscript and all authors contributed to the final version of the manuscript.

Declaration of interests

RFS was involved in the discovery of sofosbuvir. All other authors declare no competing interests.

three treatment groups (sofosbuvir, ledipasvir, and asunaprevir; sofosbuvir, daclatasvir, and simeprevir; or sofosbuvir, daclatasvir, and asunaprevir) until six patients in each group (1:1:1) achieved an ultrarapid virological response (plasma HCV RNA <500 IU/mL by day 2, measured by COBAS TaqMan HCV test, version 2.0). Patients with an ultrarapid virological response received 3 weeks of therapy. Patients who did not achieve an ultrarapid response were switched to sofosbuvir and ledipasvir for either 8 weeks or 12 weeks. The primary endpoint was the proportion of patients with a sustained virological response at 12 weeks (SVR12) after treatment completion, analysed in the intention-to-treat population. All patients who achieved an ultrarapid virological response were included in the safety analysis. This trial is registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02470858), number NCT02470858.

Findings—Between April 5, 2015, and April 15, 2015, 26 eligible patients were recruited. 12 patients were assigned to sofosbuvir, ledipasvir, and asunaprevir; six to sofosbuvir, daclatasvir, and simeprevir; and eight to sofosbuvir, daclatasvir, and asunaprevir. Six patients in each group achieved an ultrarapid virological response (18 [69%]). All patients with an ultrarapid virological response who were given 3 weeks of triple therapy achieved SVR12. The most common adverse events were fatigue (one [17%] of six patients receiving sofosbuvir, ledipasvir, and asunaprevir; one [17%] of six patients receiving sofosbuvir, daclatasvir, and simeprevir; and two [33%] of six patients receiving sofosbuvir, daclatasvir, and asunaprevir) and headache (one [17%] patient in each group). No patients experienced any serious adverse events.

Interpretation—In this proof-of-concept study, all patients with chronic HCV without cirrhosis who achieved an ultrarapid virological response on triple direct-acting antiviral regimens by day 2 and received 3 weeks of treatment were cured, with excellent tolerability. By shortening the duration of therapy from the currently recommended 12 weeks to 3 weeks, we could drastically reduce the cost of therapy and the rate of adverse events. Further large-scale studies should be done to confirm our findings.

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Introduction

Treatment of hepatitis C virus (HCV) infection has entered a new era with the emergence of direct-acting antiviral agents. By 2015, the US Food and Drug Administration (FDA) and EU had approved three new direct-acting antiviral drugs—sofosbuvir, simeprevir, and daclatasvir—for the treatment of HCV infection as part of combination regimens.^{1,2} 91–100%^{3–5} of individuals infected with HCV genotype 1 treated with 8–12 weeks of sofosbuvir and ledipasvir once daily, and 98–100%⁶ of patients who received 12–24 weeks of sofosbuvir and daclatasvir once daily, achieved sustained virological response at 12 weeks (SVR12). The cost of such regimens is onerous⁷ and this has adversely affected treatment access and drug compliance, and has encouraged drug counterfeiting.^{8,9} A major challenge is to reduce treatment cost without affecting efficacy by shortening the duration of treatment.^{10,11} Attempts 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,¹²

but the addition of an experimental NS3/4A protease inhibitor (GS-9451) or an experimental non-nucleoside polymerase inhibitor (GS-9669) yielded an SVR12 in 95% of patients.¹³

Mathematical modelling of HCV RNA changes during therapy suggests that a more rapid second-phase viral decline should allow for a shorter treatment duration.¹⁴ Nucleoside analogue inhibitors do not generate fast second-phase declines such as those with HCV protease inhibitors.^{14–17} Thus, we postulated that addition of an approved protease inhibitor, such as simeprevir or asunaprevir to sofosbuvir and ledipasvir or daclatasvir might induce a more rapid second-phase HCV RNA decline, allowing for a shorter treatment duration.

Therefore, we did a proof-of-concept, response-guided therapy clinical study to investigate the efficacy and the safety of 3 weeks of triple direct-acting antiviral therapy, containing NS5B, NS3, and NS5A inhibitors, in Chinese patients with chronic HCV genotype 1b infection without cirrhosis who achieved an ultrarapid initial viral response (uRVR), defined by a serum HCV RNA lower than 500 IU/mL within the first 2 days of dosing. We focused on patients infected with genotype 1b because this is the predominant strain in Asian populations (estimated at about 50 million), although this genotype can also be found in other populations.^{18,19}

Methods

Study design and participants

This was an open-label, proof-of-concept, phase 2a study done at a single centre (Humanity and Health Medical Centre, Hong Kong SAR, China). Participants were identified using the Beijing 302 Hospital of PLA–Hong Kong Humanity and Health Medical Group, Hepatitis C Diagnosis and Treatment Centre database, which had records for 503 patients infected with HCV by the time of the study. People who satisfied all the inclusion and exclusion criteria and who consented were consecutively enrolled. Key inclusion criteria were: older than 18 years; documented chronic HCV genotype 1b infection for more than 6 months; a baseline plasma HCV RNA concentration of 10^4 – 10^7 IU/mL; and absence of cirrhosis as assessed by liver biopsy or by liver stiffness measurement less than 12.5 kPa. Key exclusion criteria were: hepatitis B virus (HBV) or HIV infection; chronic liver disease of a non-HCV aetiology; hepatocellular carcinoma or other malignancy; drug or alcohol misuse; pregnant or nursing woman; known hypersensitivity to pharmaceutical products used in this study; and any other medical disorders or clinical conditions (eg, substantial cardiopulmonary, neurological, renal, haematological, autoimmune disorders, and any malignancy) that could interfere with the study. Treatment-experienced patients had been exposed to interferon-based therapy previously.

Written informed consent was obtained from all patients. The study was approved by the independent ethics committee at the study centre and was done in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. An independent data and safety monitoring committee reviewed the progress of the study. The full protocol is included in the appendix.

Randomisation and masking

All eligible patients were randomly allocated by a computerised system to one of three treatment groups (sofosbuvir, ledipasvir, and asunaprevir; sofosbuvir, daclatasvir, and simeprevir; or sofosbuvir, daclatasvir, and asunaprevir) until six patients achieved a uRVR in each group. The computer sequence was generated by a biostatistician (JC) who assigned them to trial groups but was not involved in the rest of the trial.

The trial was open label; patients and investigators were aware of group assignment.

Procedures

Patients received sofosbuvir, ledipasvir, and asunaprevir; sofosbuvir, daclatasvir, and simeprevir; or sofosbuvir, daclatasvir, and asunaprevir. Doses were as follows: sofosbuvir 400 mg once daily; ledipasvir 90 mg once daily; daclatasvir 60 mg once daily; simeprevir 150 mg once daily, and asunaprevir 100 mg twice daily. Patients with a uRVR were treated for 3 weeks. Patients who did not achieve a uRVR were switched to sofosbuvir and ledipasvir for either 8 weeks or 12 weeks and followed up (although not included in subsequent studies). A plasma HCV RNA threshold of less than 500 IU/mL by day 2 was chosen because in China this is the most commonly used initial screening criteria, although more sensitive assays are available. Additionally, our preliminary data (not shown) suggested that using this threshold at 48 h was a surrogate for SVR12 with 3 weeks of pan-oral direct-acting antiviral therapy, irrespective of previous interferon-based therapy response.

Screening assessments included measurement of the plasma HCV RNA concentration, *IL28B* genotyping with a TaqMan genotyping assay (Applied Biosystems, Foster City, CA, USA) for the rs12979860 single-nucleotide polymorphism, *IFNL4* (rs368234815) genotyping, and standard laboratory tests. Plasma HCV RNA concentration was measured at baseline (0 h), 1 h, 2 h, 4 h, 8 h, and 24 h after initial dosing, and at days 2, 4, 7, 14, and 21 or at the end of treatment. After the end of treatment all patients had plasma HCV RNA measured at 4-week intervals until week 12. Plasma HCV RNA concentrations were measured by a COBAS TaqMan 48 analyser, version 2.0 (Roche Molecular Systems, Branchburg, NJ, USA), with a lower limit of quantification of 25 IU/mL and a lower limit of detection of 6 IU/mL. HCV genotype and subtype were determined at screening using the Versant HCV Genotype INNOLiPA 2.0 assay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Population sequencing was used to determine resistance-associated variants in all patients at baseline, and again at the time of failure in those who had virological failure.²⁰ Virological failure resulting in study drug discontinuation was defined as: failure to achieve plasma HCV RNA concentrations less than 25 IU/mL during the 3 weeks of therapy; confirmed HCV RNA concentrations of 25 IU/mL or more at two consecutive measurements at any point in patients with on-treatment HCV RNA concentrations less than 25 IU/mL; or a confirmed increase in HCV RNA at two consecutive measurements of greater than 1 log₁₀ IU/mL above the nadir at any timepoint during treatment. Liver stiffness was measured with FibroScan (Echosens, Paris, France) according to the manufacturer's instructions.

Vital signs and symptom-directed physical examinations and adverse events graded according to the NIAID Division of AIDS toxicity table (version 1.0) were recorded at baseline, day 2, day 4, day 7, week 2, and week 3 during treatment, and then at weeks 4 and 12 after the end of treatment. Blood was taken for haematology and chemistry at screening, baseline, day 2, day 4, day 7, week 2, and week 3 during treatment, and then at weeks 4 and 12 after the end of treatment. 12-lead electrocardiograms were done at screening, baseline, week 2, and week 3 during treatment, and then at weeks 4 and 12 after the end of treatment.

Plasma samples from patients infected with HCV were stored in aliquots at -80°C until further processing. Viral RNA was extracted and the genome sequenced using an Applied Biosystems 3730x1 DNA analyser as previously described.²¹ Data were analysed with the ABL-DeepChek-HCV 1.4 software (ABL SA, Luxembourg, Luxembourg).

To confirm compliance, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to detect and quantify the anti-HCV drug concentrations in plasma collected from each patient. Briefly, blood samples were collected from 25 patients at 8 h, 24 h, and 48 h after initiating treatment. 50 μL of plasma was extracted with 3 mL of ethyl acetate containing abacavir (as an internal standard). The supernatant was collected, air dried, then reconstituted in 2 mmol/L ammonium acetate solution with 75% methanol. Five target compounds were simultaneously monitored and quantified by LC-MS/MS in the multiple reaction monitoring mode, including sofosbuvir (m/z 530.2/243.2), daclatasvir (m/z 739.5/339.3), ledipasvir (m/z 889.4/637.3), asunaprevir (m/z 748.4/648.4), and simeprevir (m/z 750.4/315.2). A Dionex Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) coupled with an AB SCIEX API5000 triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA) was used for analysis.

Outcomes

The primary efficacy endpoint was the proportion of individuals achieving SVR12 as defined by HCV RNA below the lower limit of quantification (25 IU/mL). The main safety endpoint was the frequency and severity of adverse events. Secondary endpoints were: the proportion of patients with undetectable HCV viral load at specified timepoints during treatment (day 2, day 4, day 7, week 2, and week 3) and after treatment (week 4 and week 12); the kinetics of circulating HCV RNA from baseline; the proportion of patients with adverse events; discontinuation rates related to adverse events; safety laboratory changes; and the occurrence of HCV resistance mutations.

Statistical analysis

A minimum of 14 patients were needed to power this proof-of-concept clinical study and therefore guide the decision for comparative late-stage clinical studies. Considering the feasibility and cost of the study, and accounting for dropouts, a sample size of 18 patients with six in each treatment group was selected to meet power calculation requirements. Assuming at least a 1% increase in SVR12 rate in each group from the literature,¹³ with a sample size of six in each group, the power to detect such an increase ranged from 78% to 99% at the α level of 0.05, depending on the magnitude of the increase.

Baseline characteristics between groups were compared using the Kruskal-Wallis test for continuous outcomes and Fisher's exact tests for binary outcomes. The difference in HCV viral load decline between groups was compared using the Kruskal-Wallis test and corrected by Dunn's test for multiple comparison between groups. HCV RNA concentrations and liver stiffness before treatment, at the end of treatment, and 12 weeks after treatment were compared using Wilcoxon signed-rank test for each group. Statistical analysis was based on the intention-to-treat principle. Since this was a phase 2a proof-of-concept study, the small sample size did not allow the analysis of predictive factors for uRVR using a multivariate model.

Statistical analyses were done with Stata (release 13; StataCorp). Viral kinetic modelling was done with a multiscale model^{22,23} for all 26 participants (appendix p 12).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April 5, 2015, and April 15, 2015, 27 patients with chronic hepatitis C were assessed for eligibility. One patient with cirrhosis (liver stiffness >12.5 kPa) was excluded and 26 were randomly assigned to the three treatment groups (figure 1). Six of 12 (50%, 95% CI 25–75) patients receiving sofosbuvir, ledipasvir, and asunaprevir, six of six (100%, 61–100) receiving sofosbuvir, daclatasvir, and simeprevir, and six of eight (75%, 41–95) receiving sofosbuvir, daclatasvir, and asunaprevir achieved plasma HCV RNA concentrations less than 500 IU/mL by day 2, and subsequently received triple direct-acting antiviral therapy for 3 weeks ($p=0.10$). Baseline characteristics of the patients who achieved a uRVR are shown in table 1. All participants were Chinese, six (33%) were male, seven (39%) had the *IL28* non-CC genotype, and eight (44%) had baseline HCV RNA less than 800 000 IU/mL. Four (22%) patients had stage 2 liver fibrosis. Patients in each of the 3-week treatment groups were well matched except for body-mass index. The eight patients who did not achieve uRVR were switched to sofosbuvir and ledipasvir by day 3 and followed up, but were not included in subsequent studies, and hence had a choice of duration of therapy of 8 weeks ($n=4$) or 12 weeks ($n=4$). Patients with uRVR had a significantly lower mean baseline HCV RNA than those without uRVR ($7.0 \log_{10}$ IU/mL [95% CI 6.8–7.2] vs $6.0 \log_{10}$ IU/mL [5.6–6.3], $p<0.0001$; appendix p 2). The eight patients without uRVR were not considered in the efficacy and safety analyses. However, none of them had grade 3–4 adverse events and all of these patients achieved SVR12.

At week 3, all 18 patients who achieved uRVR had undetectable plasma HCV RNA irrespective of the allocated direct-acting antiviral regimen. The median time to achieve plasma HCV RNA less than 25 IU/mL was shorter in patients receiving sofosbuvir, ledipasvir, and asunaprevir than in patients receiving sofosbuvir, daclatasvir, and asunaprevir (4 days [IQR 0] vs 14 days [7]; $p=0.01$). Plasma HCV RNA remained below the limit of quantification up to week 12 after completion of treatment (table 2). The median time to

achieve plasma HCV RNA less than 25 IU/mL was significantly shorter in patients who achieved uRVR (7 days [IQR 10]) than in those who did not achieve this threshold (17.5 days [10.5]; $p=0.003$; appendix p 3).

A post-hoc analysis of liver stiffness showed a significant decrease from baseline to 12 weeks post-treatment in patients receiving sofosbuvir, ledipasvir, and asunaprevir (6.1 kPa vs 4.9 kPa, $p=0.028$) and in patients receiving sofosbuvir, daclatasvir, and simeprevir (5.8 kPa vs 5.1 kPa, $p=0.046$), but not in patients receiving sofosbuvir, daclatasvir, and asunaprevir (5.5 kPa vs 5.4 kPa, $p=0.53$).

The effect of overall treatment on HCV clearance was estimated by taking into account the effectiveness of the regimen in inhibiting HCV RNA synthesis (e_a) and virion secretion or export (e_s), with $e=1$ being 100% effective and enhancing intracellular HCV RNA decay (by a factor κ). The model fit individual patient data well as shown in the appendix (p 9). HCV RNA kinetics exhibit a three-phase viral decline, with a rapid first phase, moderate decay during an intermediate phase, and then a slower third phase. No significant difference was recorded in the decline rate of the mean (figure 2) and median HCV load between the three treatment groups (appendix p 8). Based on 18 patients with uRVR, the estimated mean effectiveness of the treatments was $e_a=0.9962$ and $e_s=0.9987$ (appendix p 7). The mean estimated rate of viral clearance was 29.7 per day and the mean effectiveness of the direct-acting antiviral therapy in enhancing intracellular HCV RNA degradation, κ , was 1.49, consistent with previous estimates.²² Using data from all 26 treated patients, parameter values were not distributed differently among patients with uRVR and those without uRVR (data not shown). However, the estimated baseline viral load (V_0) was significantly lower in those with uRVR than in those without uRVR ($\log_{10} V_0$ 5.81 vs 7.23, $p<0.0001$; appendix p 11).

Resistance-associated variant analysis was done at baseline to elucidate the naturally occurring resistance profile. Since virus clearance was achieved rapidly in all patients during the treatment, it was difficult to do resistance-associated variant analysis at other timepoints. Sequencing of NS3/4A, NS5A, and NS5B was successful in all 26 baseline samples. Five naturally occurring protease inhibitor resistance-associated variants (V36L, T54S, S122R, I132V, or D168H/N/Y) were identified in NS3/4A in 23 (88%) of 26 baseline samples. The I132V variant was found in all 23 samples and among them, five had one additional mutation (two D168H/N/Y, one S122R, one T54S, and one V36L). Four NS5A variants associated with resistance to daclatasvir or ledipasvir (Q30R, L31F/V, H54N, or Y93H) were identified in 23 (88%) of 26 samples. The Q30R variant was found in all 23 samples and five had one or two additional mutations (appendix p 13). Other minor mutations are summarised in the appendix (p 13). None had the 282T mutation associated with sofosbuvir resistance.

Plasma drug concentrations provided a confirmation of drug delivery and patient compliance during the study (appendix pp 14, 15). Drugs taken by patients were consistent with their regimen, with only a few exceptions noted in which plasma drug concentrations were lower than the limit that could be quantified by HPLC-MS.

Of the 18 patients who completed treatment, the most common adverse events were headache and fatigue (table 3), and all adverse events were mild to moderate. Two patients had grade 3 laboratory abnormalities: one had anaemia due to menorrhagia related to uterine fibroids and another had transient hyperbilirubinaemia. The cause of the transient hyperbilirubinaemia is unknown. Previously, this adverse event was also reported in a clinical study of daclatasvir plus asunaprevir.²⁴ In our study, it was only recorded in one of six patients receiving sofosbuvir, ledipasvir, and asunaprevir. No grade 4 laboratory abnormalities were reported.

Discussion

In this exploratory cohort of Chinese patients with chronic genotype 1b HCV who had no cirrhosis and had a uRVR to triple direct-acting antiviral regimens, we show that these regimens are well tolerated and a 100% cure rate is achievable. This study reports the shortest treatment duration of pan-oral direct-acting antivirals used for patients with chronic HCV infection. The incidence of adverse events was low compared with other reported studies on pan-oral direct-acting antiviral agents,^{3–6} which might be related to the shorter duration of therapy. The patients are still being followed up and no relapse or adverse events have been recorded as of March, 2016.

Current recommendations for genotype 1b patients without cirrhosis include: 8–12-week therapy with ledipasvir and sofosbuvir; or 12-week therapy with paritaprevir with ritonavir plus ombitasvir and dasabuvir, or sofosbuvir plus simeprevir, or sofosbuvir plus daclatasvir, or grazoprevir (MK-5172) and elbasvir (MK-8742), yielding an SVR12 in more than 95% of recipients.^{1,2,25} A major factor restricting the wide availability of direct-acting antivirals to patients with HCV is the high cost of treatment, about US\$200–1200 per day, even when discounted.¹⁰ Several clinical trials were designed to shorten treatment duration to 6–8 weeks, with more than an 80–90% success rate.^{5,13,21} However, attempts to further shorten therapy to 4 weeks have so far failed. In the C-SWIFT genotype 1 study,²⁶ only three of the five patients with genotype 1b chronic hepatitis C without cirrhosis achieved an SVR at 4 weeks or 8 weeks after the end of therapy with a 4-week fixed-dose combination of grazoprevir (MK-5172), elbasvir (MK-8742), and sofosbuvir 400 mg once daily. Similarly, in the NIH/UMD Synergy trial²⁷ of patients with early F0–F2 fibrosis treated with ledipasvir and sofosbuvir plus GS-9451 and GS-9669, only five (20%) of 25 achieved SVR12, whereas ten (40%) of 25 in another cohort treated with ledipasvir and sofosbuvir plus GS-9451 achieved an SVR12.

Modelling of HCV RNA kinetics during anti-HCV drug therapy has predicted faster first phases in protocols using an NS5A inhibitor, such as daclatasvir²² or ledipasvir,²⁸ and faster second phases using a protease inhibitor.¹⁷ This finding together with the use of sofosbuvir,²⁹ should allow a shorter treatment duration. Our pilot study strongly suggests that combining the three different approved oral anti-HCV drug classes leads to a complete cure (SVR12) in 3 weeks in all patients with genotype 1b HCV without cirrhosis who had plasma HCV RNA reduced to less than 500 IU/mL within the first 2 days of therapy. No differences were observed between the three combinations of the drugs used in both day 2 response and SVR12.

Nevertheless, patients receiving sofosbuvir, ledipasvir, and asunaprevir had shorter time to antiviral efficacy than did patients receiving sofosbuvir, daclatasvir, and asunaprevir; the reason is unclear. The most apparent distinction between these two groups is the inclusion of ledipasvir (sofosbuvir, ledipasvir, and asunaprevir) versus daclatasvir (sofosbuvir, daclatasvir, and asunaprevir). Review of the scientific literature only found one related comparison, daclatasvir plus asunaprevir versus sofosbuvir and ledipasvir for hepatitis C genotype 1 in Japanese patients, but this was an indirect comparison.³⁰ No direct comparison of ledipasvir with daclatasvir has been done.

A substantial proportion of the enrolled individuals (18 [69%] of 26) achieved a very rapid and profound drop in plasma HCV RNA. We found that this rapid viral decline could be affected by the presence of baseline NS5A resistance-associated variants (appendix p 13). The reasons for the naturally occurring resistance-associated variants are still unknown. However, in our study, baseline resistance-associated variants did not affect SVR12 in patients, irrespective of whether they had a uRVR. Even for patients who did not have a uRVR, baseline resistance-associated variants did not affect SVR12 in patients treated for longer than 3 weeks (8 weeks or 12 weeks).

To our knowledge, this study is the first to use the principle of response-guided therapy with an all direct-acting antiviral regimen to shorten treatment duration, with a high proportion of patients achieving an SVR12. This concept, if validated in larger studies, could greatly reduce duration of therapy and subsequently compliance and cost, and improve the accessibility and affordability of direct-acting antiviral drugs, especially in middle-income and low-income countries. Future larger studies are currently being planned in Mongolia to shorten the duration of treatment with sofosbuvir, daclatasvir, and simeprevir to 3 weeks. Additionally, this should also reduce the emergence of resistance, development of side-effects, and help to curtail the use of counterfeit direct-acting antiviral drugs in some countries, which could be potentially harmful.

Our findings are not consistent with the concept that cure corresponds to an end of treatment viral load below a cure boundary of less than one viral particle in the extracellular body fluid (ie, 15 L), which corresponds to a concentration of about $10^{-4.22}$ IU/mL.^{13,14,31} The multiscale model predicted that none of the 18 treated patients would reach the cure boundary (appendix p 9). This finding prompts a reconsideration of whether reducing the amount of virus to less than one virion is really necessary to achieve a cure.

A weakness of our study is the small number of patients, and the results should be validated in larger studies. The small number of individuals in each group limits a comparison of different direct-acting antiviral regimens, and a direct comparison might be warranted in future studies. Furthermore, the patients were Chinese with genotype 1b HCV infection and without cirrhosis, of whom up to 85% had the *IL28B* CC genotype. *IL28B* CC genotype and genotype 1b HCV infection are predictive factors for an SVR, and some might argue that dual direct-acting antiviral therapies could achieve a good response in this population. Treatment with direct-acting antiviral drugs has been reported to increase the risk of recurrence of hepatocellular carcinoma in patients with previous HCV-related hepatocellular carcinoma.³² However, to our knowledge, this study is the first to report that SVR12 can be

achieved after 3 weeks of direct-acting antiviral therapies. Normally, interferon-based treatment can achieve SVR at 24 weeks in 44–79% of patients with HCV genotype 1 infection,³³ but still needs more than 48 weeks of treatment. Limited data support the use of dual direct-acting antiviral therapies to shorten treatment duration. Since nucleoside analogue inhibitors do not generate second-phase declines as fast as HCV protease inhibitors, the addition of a protease inhibitor to sofosbuvir and ledipasvir or daclatasvir could allow for a shorter treatment duration. This hypothesis is supported by our data. All patients are being followed up and long-term follow-up data will be reported later.

In our study, a high proportion of patients were young women with a low baseline HCV viral load. These characteristics could be associated with a favourable response to short duration pan-oral direct-acting antiviral therapy to less than 6 weeks.²⁷ Nevertheless, it has been estimated that there are at least 4 million people with such characteristics in China,³⁴ and we believe these findings could benefit more patients with chronic hepatitis C worldwide in the same setting. Application of this response-guided treatment approach to patients with different ethnic backgrounds, with different genotypes, and with cirrhosis should be addressed by larger clinical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before this study

We searched PubMed from Jan 1, 2010, to April 14, 2016, for articles published in English using a combination of the medical subject headings “HCV treatment” and “antiviral agent” as search terms. Two related clinical trials have been reported for hepatitis C virus (HCV) combination therapy studies (phase 2a). These trials have shown promising safety and efficacy using combination direct-acting antiviral drugs.

Added value of this study

Although our study is small, we showed high rates of sustained viral response at 12 weeks with use of the 3-week triple direct-acting antiviral therapy, which supports the possibility that this ultrashort regimen might be effective for some patients infected with HCV.

Implications of all the available evidence

Our study demonstrated that the duration of therapy of pan-oral direct-acting antiviral agents could be drastically shortened from the current recommended 12 weeks to only 3 weeks with triple direct-acting antiviral therapy, containing NS5B, NS3, and NS5A inhibitors, in non-cirrhotic Chinese patients infected with chronic HCV genotype 1b and who had an ultrarapid virological response. This could have great therapeutic, public health, and economic implications because genotype 1b rather than 1a is the predominant strain in Asian populations (estimated at about 50 million). Further large-scale studies with the use of a response-guided approach are needed in other populations with different genotypes and in cirrhotic patients

Figure 1. Trial profile

uRVR=ultrarapid virological response.

Figure 2. Decline in mean hepatitis C viral load

Solid lines are mean model trajectories calculated from predicted viral load (see appendix p 9). Dashed horizontal line indicates the assay lower limit of quantification.

Table 1

Baseline demographic and clinical characteristics of the patients who achieved an ultrarapid virological response

	Sofosbuvir, ledipasvir, and asunaprevir 3 weeks (n=6)	Sofosbuvir, daclatasvir, and simeprevir 3 weeks, (n=6)	Sofosbuvir, daclatasvir, and asunaprevir 3 weeks (n=6)
Age (years [mean, range])	41 (25–66)	40 (23–59)	31 (21–47)
Sex			
Male	2 (33%)	2 (33%)	2 (33%)
Female	4 (67%)	4 (67%)	4 (67%)
BMI (kg/m ² [mean, range])	23.7 (4.5)	21.1 (2.6)	19.2 (2.0)
HCV RNA (log ₁₀ IU/mL)			
Mean (SD)	6.3 (0.3)	5.7 (1.0)	5.9 (0.8)
Median (IQR)	6.2 (6.1–6.6)	5.6 (5.3–6.7)	5.9 (5.2–6.5)
HCV RNA 800 000 IU/mL	5 (83%)	2 (33%)	3 (50%)
Previous treatment			
Treatment experienced	3 (50%)	2 (33%)	1 (17%)
Treatment naive	3 (50%)	4 (67%)	5 (83%)
Previous response*			
Relapser	1 (33%)	2 (100%)	1 (100%)
Partial responder	1 (33%)	0	0
Null responder	1 (33%)	0	0
<i>IL28B</i> genotype			
CC	4 (67%)	4 (67%)	3 (50%)
CT	2 (33%)	2 (33%)	3 (50%)
<i>IFNL4</i> genotype			
TT/TT	4 (67%)	4 (67%)	5 (83%)
G/TT	2 (33%)	2 (33%)	1 (17%)
Liver stiffness measure (kPa)	6.1 (1.7)	5.8 (2.0)	5.5 (0.9)
Fibrosis stage (METAVIR) [†]			
F0–F1 (LSM 7.0 kPa)	4 (67%)	4 (67%)	6 (100%)
F2 (7.0 kPa<LSM 9.5 kPa)	2 (33%)	2 (33%)	0
F3 (9.5 kPa<LSM 12.5 kPa)	0	0	0

Data are mean (SD) or n (%), unless otherwise stated.

BMI=body-mass index. HCV=hepatitis C virus.

* Data indicate the response to the most recent previous regimen: a relapser is a patient who received at least 36 weeks of pegylated interferon and ribavirin with HCV DNA undetectable at the end of treatment, but detectable within 52 weeks of follow-up; a partial responder is a patient who

received at least 20 weeks of pegylated interferon and ribavirin for the treatment of HCV and achieved $\geq 2 \log_{10}$ IU/mL reduction in HCV RNA at week 12, but failed to achieve HCV RNA undetectable at the end of treatment; a null responder is a patient who received at least 12 weeks of pegylated interferon and ribavirin and failed to achieve a $\geq 2 \log_{10}$ IU/mL reduction in HCV RNA at week 12.

[†] Liver stiffness was determined by Fibroscan; no patient in this study had cirrhosis (fibrosis score >12.5 kPa).

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Table 2

Virological response of individuals who achieved an ultrarapid virological response at day 2 and received 3 weeks of treatment

	Sofosbuvir, ledipasvir, and asunaprevir (3 weeks)	Sofosbuvir, daclatasvir, and simprevir (3 weeks)	Sofosbuvir, daclatasvir, and asunaprevir (3 weeks)
During treatment period			
Day 2	1 (16.7% [3.0–56.3])	2 (33.3% [9.7–70.0])	0
Day 4	5 (83.3% [43.6–96.9])	2 (33.3% [9.7–70.0])	0
Day 7	6 (100.0% [61.0–100.0])	4 (66.7% [30.0–90.3])	2 (33.3% [9.7–70.0])
Week 2	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])	5 (83.3% [43.6–96.6])
Week 3	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])
During post-treatment period			
Week 4	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])
Week 12	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])	6 (100.0% [61.0–100.0])

The lower limit of quantification for plasma HCV RNA by Roche COBAS TaqMan test was 25 IU/mL.

Table 3

Adverse events and laboratory abnormalities during the treatment period

	Sofosbuvir, ledipasvir, and asunaprevir (n=6)	Sofosbuvir, daclatasvir, and simeprevir (n=6)	Sofosbuvir, daclatasvir, and asunaprevir (n=6)
Common adverse events			
Fatigue	1 (17%)	1 (17%)	2 (33%)
Nausea	1 (17%)	0	0
Headache	1 (17%)	1 (17%)	1 (17%)
Dizziness	0	1 (17%)	0
Insomnia	1 (17%)	0	1 (17%)
Abdominal pain	0	1 (17%)	1 (17%)
Constipation	1 (17%)	0	0
Diarrhoea	0	1 (17%)	1 (17%)
Dermatitis	0	1 (17%)	1 (17%)
Common cold	1 (17%)	0	1 (17%)
Laboratory abnormalities			
Any grade 3 laboratory abnormality during treatment	1 (17%)	1 (17%)	0
Decreased haemoglobin	0	1 (17%)	0
Raised total bilirubin	1 (17%)	0	0

There were no deaths or discontinuations due to adverse events. All adverse events and serious adverse events were recorded from the time the consent form was signed until 14 days after cessation of treatment. The relatedness (probable or possible) of the adverse event to the regimen was determined by the investigator; patients could have had more than one adverse event.