Safety and efficacy of sofosbuvir-velpatasvir to treat chronic hepatitis C virus infection in treatment-naive patients in Rwanda (SHARED-3): a single-arm trial

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Summary

Background Hepatitis C virus (HCV) genotype 4 is the predominant type of HCV found in sub-Saharan Africa. Various genotype 4 subtypes, such as 4r, frequently have resistance-associated substitutions that can increase rates of treatment failure with common direct-acting antiviral regimens. In-vitro studies suggest that the NS5A inhibitor velpatasvir is effective against viral isolates containing such resistance-associated substitutions, but its clinical efficacy against genotype 4 non-a/d subtypes in sub-Saharan Africa remains to be confirmed. We aimed to evaluate the safety and efficacy of sofosbuvir–velpatasvir among adults chronically infected with HCV and naive to direct-acting antiviral treatment in Rwanda, where genotype 4 non-a/d subtypes predominate.

Methods In this single-arm prospective trial, we enrolled adults (age ≥18 years) in Rwanda who had chronic HCV infection and a plasma HCV RNA titre of at least 1000 IU/mL. Patients were referred from hospitals with HCV treatment programmes throughout Rwanda and were sequentially enrolled and assessed for eligibility at a single study site. Individuals with decompensated liver disease or hepatitis B virus co-infection were excluded. Participants were given an oral fixed-dose combination tablet of sofosbuvir (400 mg) and velpatasvir (100 mg) once-daily for 12 weeks. The primary endpoint was the proportion of participants with a sustained virological response 12 weeks after completion of treatment (SVR12) in the intention-to-treat population. Viral sequencing of the NS5A and NS5B genes was done at baseline for all participants and end of follow-up (week 24) for participants who did not have SVR12. This study is registered with ClinicalTrials.gov (NCT03888729) and is completed.

Findings Between Sept 23, 2019, and Jan 10, 2020, 73 individuals were screened for eligibility, of whom 12 (16%) were excluded and 61 (84%) were enrolled. 40 (66%) participants were female, 21 (34%) were male, median age was 64 years (IQR 51–74), and median baseline HCV viral load was 5·7 $\log_{10}$ IU/mL (5·2–6·2). The genotypes identified among the participants were 4k (28 [46%] participants), 4r (11 [18%]), 4v (eight [13%]), 4q (five [8%]), 4l (three [5%]), 4b (one [2%]), 4c (one [2%]), and one undetermined genotype 4 subtype. Three isolates could not be sequenced and were of indeterminate genotype. Of the 55 HCV isolates that were successfully sequenced, all had at least two NS5A resistance-associated substitutions. 59 (97% [95% CI 89–99]) participants had SVR12. 18 (30%) participants had grade 3 adverse events (including 12 [20%] with hypertension), and none had grade 4 adverse events. Four (7%) participants had serious adverse events, including one asthma exacerbation, one abscess, one uterine myoma, and one pelvic fracture related to a motor vehicle accident. No serious adverse events were attributed to the study drug and no adverse event resulted in discontinuation of the study drug.

Interpretation A 12-week regimen of sofosbuvir–velpatasvir is safe and efficacious in treating chronic HCV genotype 4 infection in patients in Rwanda. This regimen could be an effective treatment option in regions known to have a high prevalence of HCV genotype 4 of diverse non-a/d subtypes.

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Introduction

Of the approximately 58 million individuals chronically infected with hepatitis C virus (HCV), 9 million live in sub-Saharan Africa.[1] HCV genotype 4 is the predominant genotype in sub-Saharan Africa and is estimated to make up 45% of HCV infections across low-income countries globally.[2] Within genotype 4, there is a high degree of phylogenetic diversity.[3] Several genotype 4 subtypes, particularly genotype 4r, have been associated with higher rates of resistance-associated substitutions that reduce susceptibility to widely used inhibitors of the HCV protein NS5A.[4–7] In individuals infected with genotype 4r HCV, pre-existing resistance-associated substitutions are typically present in NS5A in all relevant positions (Leu28Met/Val, Leu30Arg, Leu31Met, and Tyr93His), before and after NS5A inhibitor-based treatment failure.[8] HCV genotype 4r isolates harbour a significantly higher median number of NS5A resistance-

Research in context

Evidence before this study

Hepatitis C virus (HCV) genotype 4r has been shown to have frequent NS5A resistance-associated substitutions and is associated with a high rate of failure of commonly used treatment regimens such as sofosbuvir–ledipasvir and sofosbuvir–daclatasvir. We did a literature review in Google Scholar for studies published between Jan 1, 2010, and June 1, 2021, including any combination of the following MeSH search terms: "hepatitis C", "treatment", "genotype 4", and "sub-Saharan Africa". We focused our detailed review on reports including treatment outcomes for sofosbuvir–velpatasvir in populations with HCV genotype 4 non-a/d subtypes or from the sub-Saharan African region. We found five publications reporting treatment outcomes of patients, including those infected with HCV genotype 4 non-a/d subtypes, treated with sofosbuvir–velpatasvir; however, the number of patients reported in these studies was too small to inform clinical practice. The most recent HCV treatment guidelines of the European Association for the Study of the Liver specifically identify genotype 4r as a subtype requiring treatment to overcome inherent resistance to NS5A inhibitors; however, evidence regarding the efficacy of sofosbuvir–velpatasvir in this subtype was insufficient.

Added value of this study

The SHARED-3 trial prospectively assessed the safety and efficacy of sofosbuvir–velpatasvir in adults in Rwanda infected with HCV, predominantly genotype 4 non-a/d subtypes. Treatment with sofosbuvir–velpatasvir resulted in a sustained virological response at 12 weeks after completion of treatment (SVR12) for 59 (97%) of 61 participants, including ten (91%) of 11 participants with genotype 4r and 12 (92%) of 13 participants with three or more baseline NS5A resistance-associated substitutions. There were no serious adverse effects related to sofosbuvir–velpatasvir.

Implications of all the available evidence

Sofosbuvir–velpatasvir is effective in the treatment of infection with HCV genotype 4 non-a/d subtypes, including in patients with genotype 4r and patients with three or more baseline NS5A resistance-associated substitutions. By comparison, a previous study investigating sofosbuvir–ledipasvir treatment in a similar population found SVR12 in 27 (56%) of 48 participants infected with HCV genotype 4r and 33 (65%) of 51 participants with three or more baseline NS5A resistance-associated substitutions. These findings suggest sofosbuvir–velpatasvir might have superior efficacy compared to sofosbuvir–ledipasvir in the treatment of patients with these subtypes. Sofosbuvir–velpatasvir could be an effective first-line regimen for the treatment of individuals confirmed or suspected to be infected with treatment-resistant genotype 4 subtypes. It is crucial to improve access to sofosbuvir–velpatasvir in regions of sub-Saharan Africa where genotype 4r and other non-a/d subtype infections are highly prevalent.

associated substitutions at baseline (before NS5A inhibitor treatment) than isolates from more extensively studied genotype 4 subtypes, such as genotypes 4a or 4d.[8]

Previous studies have associated several of the genotype 4 subtypes endemic to sub-Saharan Africa with high rates of treatment failure with the commonly used NS5A-based direct-acting antiviral regimens sofosbuvir–ledipasvir and sofosbuvir–daclatasvir. In a previous prospective trial in Rwanda, 27 (56%) of 48 participants with genotype 4r had a sustained virological response 12 weeks after completion of treatment (SVR12) following treatment with sofosbuvir–ledipasvir, compared to 234 (93%) of 252 participants with other genotype 4 subtypes.[9] Two patients with genotype 4r and one with genotype 4b with multiple pretreatment resistance-associated substitutions did not reach SVR12 with sofosbuvir–ledipasvir in a prospective study in France.[10] In a retrospective analysis of treatment outcomes from a large and epidemiologically diverse treatment centre in South Africa, SVR12 was not observed in four of eight patients with genotype 4r.[11] Additionally, retrospective analyses of large databases in Europe have associated subtypes other than 4a or 4d with lack of response to treatment among immigrant populations treated with sofosbuvir–ledipasvir or sofosbuvir–daclatasvir.[4,8,12,13]

The combination of the direct-acting antivirals sofosbuvir and velpatasvir has shown high efficacy across genotypes 1–6 in robust clinical trials, as well as in large effectiveness studies in hepatitis treatment centres and national programmes.[14,15] However, clinical trials for sofosbuvir–velpatasvir have not included a substantial number of patients from sub-Saharan Africa with genotype 4 subtypes other than 4a or 4d, and clinical outcomes for patients treated with sofosbuvir–velpatasvir in sub-Saharan Africa have not been reported. In-vitro data have shown lower 50% effective concentration (EC_{50}) values for velpatasvir against common NS5A resistance-associated substitutions in genotype 4r, such as Leu30Arg and Tyr93His, than for ledipasvir or daclatasvir.[5,16,17] In this Article, we report the findings from the first prospective clinical trial of sofosbuvir–velpatasvir in an area of sub-Saharan Africa with endemic genotype 4 non-a/d subtypes, with frequent substitutions associated with resistance to commonly used NS5A-inhibitors at baseline, in patients who had not previously been treated with direct-acting antivirals.

Methods

Study design and participants

The Simplifying Hepatitis C Antiviral Treatment in Rwanda for Elsewhere in the Developing World

(SHARED-3) trial consists of two separate single-arm prospective studies. The first study, reported in this Article, evaluated the efficacy and safety of sofosbuvir–velpatasvir in adults with chronic HCV infection in Rwanda who had not previously undergone direct-acting antiviral treatment. The second study evaluated the efficacy and safety of sofosbuvir–velpatasvir–voxilaprevir in HCV-infected adults in Rwanda who had previously received direct-acting antiviral therapy without achieving SVR12, and is reported in an accompanying Article.[18]

Study participants were referred from hospitals with HCV treatment programmes throughout Rwanda after confirmation of active HCV infection by PCR detection of HCV RNA. Participants were sequentially enrolled and assessed for eligibility at a single study site (Rwanda Military Hospital, Kigali, Rwanda). Eligibility criteria were age 18 years or older and a plasma HCV RNA titre of at least 1000 IU/mL. Participants were also required to have a screening ultrasound excluding hepatocellular carcinoma, a haemoglobin concentration of 8·0 g/dL or higher, a platelet count of at least 40 000 per µL, liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and alkaline phosphatase) no more than ten times the upper limit of normal, and a calculated creatinine clearance of at least 30 mL/min, as estimated by the Cockcroft-Gault equation. Although a range of cutoffs for platelet count have been used as eligibility criteria for clinical trials of HCV drugs (typical range 40 000–100 000 per µL), a cutoff of 40 000 per µL was selected for this study as the most sensitive threshold to rule out decompensated cirrhosis and the most inclusive for study enrolment. Individuals with antiretroviral-treated HIV infection were eligible if they had a HIV RNA concentration of 200 copies per mL or less, and a CD4 count of at least 100 cells per µL. All participants were required to be able to provide informed written consent, able to comply with all study procedures, and of generally good health as determined by the study team. Exclusion criteria were a history of or current decompensated liver disease, active tuberculosis, other clinically significant illness (except HCV or HIV), active hepatitis B virus infection, active drug or alcohol abuse, pregnancy or current breastfeeding, and inability to provide blood samples per the study protocol.

This study was approved by the Rwanda National Ethics Committee (protocol number 0193/RNEC/2018; Kigali, Rwanda), Inshuti Mu Buzima Research Committee (Rwinkwavu, Rwanda), and the Partners Human Research Committee (protocol number 2018P002979; Boston, MA, USA). All participants provided written informed consent in their native language of Kinyarwanda.

Procedures

Evaluation for study eligibility included testing for plasma HCV RNA titre and genotype, HIV serology, HBsAg, right upper quadrant ultrasound, and standard clinical and laboratory assessments. Following initiation of study drug at study entry, visits occurred at weeks 4, 8, 12, and 24. All participants received a fixed-dose combination tablet containing 400 mg sofosbuvir and 100 mg velpatasvir, to be taken orally once daily for 12 weeks. The first dose was administered in the presence of a trained nurse or social worker, who provided adherence counselling and other information regarding the treatment. Plasma HCV RNA titre, complete blood count, and a comprehensive metabolic panel were obtained at 12 and 24 weeks.

Adverse events were assessed and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 2.1) by a study physician at weeks 4, 8, 12, and 24. Adherence was assessed by pill count at weeks 4, 8, and 12. Concomitant medications and potential drug–drug interactions were assessed at all scheduled study visits. Participants received telephone reminders and transport reimbursements for study visits. Missed visits were rescheduled by the study social worker. Criteria for premature treatment discontinuation were ALT elevation greater than 15 times the upper limit of normal; ALT or AST elevation greater than five times the screening or nadir value; ALT elevation greater than three times the screening value with total bilirubin greater than two times the upper limit of normal; grade 3 or 4 rash associated with constitutional symptoms; or any grade 4 event judged to be related to study drug.

Plasma HCV RNA was measured by COBAS AmpliPrep/COBAS TaqMan HCV quantitative test (Roche, Pleasanton, CA, USA) with a lower limit of quantification of 15 IU/mL. Liver fibrosis was assessed using the AST-to-platelet ratio index (APRI) and Fibrosis-4 index (FIB-4). An APRI score greater than 2·00 indicated the presence of cirrhosis (corresponding to METAVIR F4), and a FIB-4 index greater than 3·25 indicated significant fibrosis (corresponding to METAVIR of F2 or higher). PCR amplification of the HCV non-structural protein regions (NS3/4A, NS5A, and NS5B) was done on baseline plasma samples for all participants and endpoint plasma samples for participants without SVR12, with genotype-specific and subtype-specific primers based on genotype assignment from the HCV INNO-LiPA assay at DDL Diagnostic Laboratory (Rijswijk, Netherlands). Because of high sequence variability across genotype 4 subtypes, new subtype-specific primers were designed using public sequence information, when available. For unsuccessful amplification of the NS5A or NS5B genes, a partial NS5B sequence was amplified with genotype-independent primers. To assign HCV genotype and subtype, the NS3/4A, NS5A, and NS5B nucleotide and amino acid consensus sequences were compared with a set of reference sequences with known subtype using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (see appendix p 1 for full definitions of resistance-associated substitutions).[19]

See Online for appendix

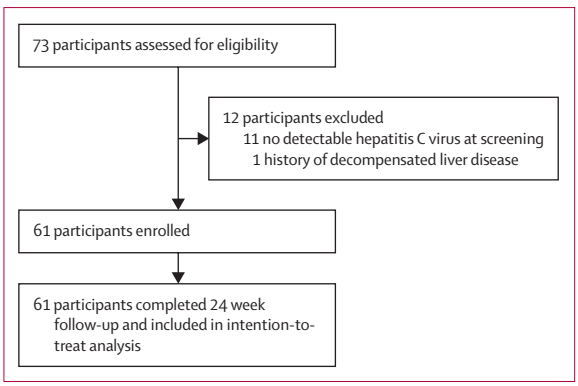

Figure 1: **Trial profile**

Outcomes

The primary efficacy outcome was the proportion of participants in the intention-to-treat population with SVR12, defined as an absence of quantifiable plasma HCV RNA at 12 weeks after completion of the course of study drug treatment. The primary safety outcome was the proportion of participants with grade 3 or 4 events or with premature study drug discontinuation due to an adverse event, assessed in all enrolled participants who received at least one dose of study drug. Prespecified secondary outcomes reported in this Article are the proportions of enrolled participants with each HCV genotype 4 subtype, the proportion of enrolled participants with SVR12 by genotype subtype, the proportion of enrolled participants with adherence of more than 90% of pills taken, and the proportion of enrolled participants with HIV co-infection who maintained HIV viral load suppression while on study drug, all assessed in the intention-to-treat population. The secondary outcome of the proportion of enrolled participants who showed significant changes in quality-of-life measurements from baseline to week 24 from initiation of treatment will be analysed and reported elsewhere.

Statistical analysis

The hypothesis of this study was that sofosbuvir–velpatasvir is safe and effective for the treatment of adults with chronic HCV infection in Rwanda without previous direct-acting antiviral treatment. A target sample size of 60 was chosen on the basis of feasibility. The proportion of enrolled participants who met the primary endpoint was calculated along with corresponding 95% CIs calculated using the Clopper-Pearson method. All analyses were done on the intention-to-treat population and testing was two-sided with a type I error rate of 5%. All statistical analyses were done using Stata (version 15.1).

This study was registered with ClinicalTrials.gov (NCT03888729).

Role of the funding source

The funder of the study provided the study drug; provided input on the study protocol, study design, data interpretation, and final manuscript; and conducted the analysis and interpretation of viral sequencing. The funder did not contribute to data collection.

Results

Between Sept 23, 2019, and Jan 10, 2020, 73 individuals were screened for eligibility for the study. 12 (16%) were excluded and 61 (84%) were enrolled on the basis of entry criteria (figure 1). The follow-up period for enrolled participants was completed on Aug 28, 2020. The enrolled participants consisted of 40 (66%) women and 21 (34%) men, with a median age of 64 years (IQR 51–74) and a median baseline HCV viral load of 5·7 $\log_{10}$ IU/mL (5·2–6·2; table 1). 36 (59%) participants reported primary education or less as their highest level of education, 35 (57%) were unemployed, and 44 (73%) reported an income equivalent to less than US$120 per month. One (2%) participant had HIV co-infection. Three (5%) participants had cirrhosis as defined by an APRI score greater than 2·00, and seven (11%) had advanced liver disease as defined by a FIB-4 score greater than 3·25.

Genotype subtype could be ascertained on baseline isolates for 58 (95%) participants, all of whom had genotype 4 HCV (table 1). The predominant subtypes were 4k (28 [46%] participants), 4r (11 [18%]), and 4v (eight [13%]), with the remainder comprising 4b, 4c, 4l, 4q, and one unknown genotype 4 subtype. Three isolates could not be sequenced and were of indeterminate genotype. We attempted to sequence baseline NS5A and NS5B genes of all participants. All isolates that were successfully sequenced (55 [90%]) had at least two NS5A resistance-associated substitutions, including Lys24Arg, Leu28Ile/Met/Thr/Val, Leu30Arg/Ser, Leu31Met/Val, Pro58Ser, and Tyr93His/Ser. 13 participants had three NS5A resistance-associated substitutions. Of 27 participants with available NS5B sequencing data, 15 (56%) had NS5B resistance-associated substitutions at baseline, including Glu237Gly, Phe289Leu, and Val321Ile (table 1). The frequencies of individual resistance-associated substitutions and combinations of resistance-associated substitution by subtype are presented in the appendix (pp 1–2).

SVR12 was observed in 59 (97% [95% CI 89–99]) of 61 participants. 60 (98%) participants had an HCV RNA titre below the lower limit of detection (15 IU/mL) at the end of treatment (week 12; table 2). The proportion of participants with SVR12 was 100% in all genotype subtypes except for 4k (27 [96%] of 28 participants) and 4r (ten [91%] of 11). SVR12 was observed in 41 (98%) of 42 participants with two baseline NS5A resistance-associated substitutions and in 12 (92%) of 13 with three or more baseline NS5A resistance-associated substitutions (table 2). 51 (84%) participants had pill counts indicating adherence of 100%, eight (13%) had adherence of 95% or greater and less than 100%, two (3%) had adherence of 90% or greater and less than 95%, and none had adherence below 90%.

	Participants (N=61)
Sociodemographic characteristics	
Age, years	64 (51–74)
Sex	
Female	40 (66%)
Male	21 (34%)
Education	
Primary education or less	36 (59%)
Greater than primary education	25 (41%)
Employment	
Unemployed	35 (57%)
Employed	26 (43%)
Monthly income, US$	
<120	44 (72%)
≥120	17 (28%)
Clinical characteristics	
BMI, kg/m²	24 (20–28)
Comorbidities	
Hypertension	33 (54%)
HIV co-infection	1 (2%)
Diabetes	9 (15%)
HCV RNA titre, $\log_{10}$ IU/mL	5·7 (5·2–6·2)
Albumin concentration, g/dL	
Median (IQR)	4·1 (3·9–4·4)
<3·5	4 (7%)
Platelet count, × 10^3/μL	
Median (IQR)	235 (179–282)
<90	2 (3%)
Aspartate aminotransferase concentration, IU/mL	35 (27–47)
Alanine aminotransferase concentration, IU/mL	35 (27–47)
Total bilirubin concentration, mg/dL	0·50 (0·39–0·74)
Aspartate aminotransferase-to-platelet ratio index	
≤1·0	54 (89%)
>1·0 to ≤2·0	4 (7%)
>2·0	3 (5%)
Fibrosis-4 index score >3·25	7 (11%)

(Table 1 continues in next column)

(Continued from previous column)

	Participants (N=61)
Virological characteristics	
HCV genotype by subtype	
4k	28 (46%)
4r	11 (18%)
4v	8 (13%)
4q	5 (8%)
4l	3 (5%)
4b	1 (2%)
4c	1 (2%)
4, undetermined subtype	1 (2%)
Unknown	3 (5%)
Number of HCV NS5A resistance-associated substitutions	
0	0
1	0
2	42 (69%)
≥3	13 (21%)
Unknown (no sequencing data)	6 (10%)
Number of HCV NS5B resistance-associated substitutions	
0	12 (20%)
≥1	15 (25%)
Unknown (no sequencing data)	34 (56%)

Data are median (IQR) or n (%). HCV=hepatitis C virus.

Table 1: **Baseline sociodemographic and clinical characteristics of study participants**

The one participant with HIV co-infection had SVR12 and maintained suppressed HIV viral load at the end of treatment.

One participant who did not have SVR12 was a 46-year-old woman with no comorbidities, a baseline APRI score of 0·73 (indicating no cirrhosis), and a baseline viral load of 6·0 $\log_{10}$ IU/mL. This participant was infected with HCV genotype 4r with three NS5A resistance-associated substitutions (Leu28Val, Leu30Arg, and Leu31Met) and one NS5B resistance-associated substitution (Val321Ile) at baseline. This participant had a detectable viral load at the end of treatment as well as at 12 weeks after the completion of treatment, and did not develop new resistance-associated substitutions after treatment. The second participant who did not have SVR12 was a 51-year-old man with diabetes, a baseline APRI score of 0·59 (indicating no cirrhosis), and a baseline viral load of 5·6 $\log_{10}$ IU/mL. This participant was infected with HCV genotype 4k with two NS5A resistance-associated substitutions (Leu30Arg and Leu31Met) at baseline, but baseline NS5B sequencing data were not available. No new NS5A resistance-associated substitutions emerged in this participant after treatment, and no NS5B resistance-associated substitutions were detected after treatment. This participant had viral load suppression at the end of treatment, but had a relapse at 12 weeks after the completion of therapy. Both of these participants had greater than 95% adherence by pill count at weeks 4, 8, and 12.

18 (30%) of 61 participants had a total of 21 grade 3 adverse events, and there were no grade 4 adverse events (table 3). Four grade 3 adverse events were categorised as serious (one asthma exacerbation, one abscess, one uterine myoma, and one pelvic fracture related to a motor vehicle accident) and resulted in hospitalisation, but were judged to be unrelated to study treatment. The most common grade 3 adverse event was hypertension (12 [20%] patients). There were no deaths. 59 (97%) participants had at least one grade 1 or 2 adverse event. The most common grade 1 or 2 adverse events were hypertension (24 [39%] participants), headache (18 [30%]), dizziness (12 [20%]), abdominal pain (11 [18%]), joint pain (ten [16%]), and upper respiratory tract infection

	Participants with SVR12
Overall	59/61 (97% [89–99])
By HCV genotype and subtype	
4b	1/1 (100%)
4c	1/1 (100%)
4k	27/28 (96%)
4l	3/3 (100%)
4q	5/5 (100%)
4r	10/11 (91%)
4v	8/8 (100%)
4, undetermined subtype	1/1 (100%)
Unknown genotype	3/3 (100%)
By number of HCV NS5A resistance-associated mutations	
0	NA
1	NA
2	41/42 (98%)
≥3	12/13 (92%)
Unknown	6/6 (100%)

Data are n/N (% [95% CI]) or n/N (%). SVR12=sustained virological response 12 weeks after completion of treatment. HCV=hepatitis C virus. NA=not applicable (no participants in category).

Table 2: **SVR12 by HCV genotype subtype and number of baseline HCV NS5A resistance-associated substitutions**

	Participants (N=61)
Discontinuation of study drug	0
Due to adverse events	0
Due to death	0
Due to loss to follow-up	0
Due to other disqualifying events	0
Serious adverse events	4 (7%)
Asthma exacerbation	1 (2%)
Myoma	1 (2%)
Motor vehicle accident	1 (2%)
Abscess	1 (2%)
Grade 3 adverse events*	18 (30%)
Hypertension	12 (20%)
Anaemia	2 (3%)
Asthma exacerbation	1 (2%)
Diabetes	1 (2%)
Headache	1 (2%)
Motor vehicle accident	1 (2%)
Myoma	1 (2%)
Abscess	1 (2%)
Urinary tract infection	1 (2%)
Grade 1 or 2 adverse events†	59 (97%)
Hypertension	24 (39%)
Headache	18 (30%)
Dizziness	12 (20%)
Abdominal pain	11 (18%)
Joint pain	10 (16%)
Upper respiratory infection	10 (16%)
Low back pain	9 (15%)
Fatigue	9 (15%)
Rash or pruritus	9 (15%)
Diabetes	7 (11%)
Laboratory abnormality (grade 3 or 4)	4 (7%)
Low haemoglobin concentration	3 (5%)
Low platelet count	1 (2%)

Data are n for number of events, or n (%) for number of participants with events. *Three participants had two grade 3 adverse events each; there were no grade 4 events. †Grade 1 or 2 adverse events that occurred in at least 10% of participants are listed.

Table 3: **Adverse events and laboratory abnormalities**

(ten [16%]). No adverse events resulted in premature treatment discontinuation. Four participants had grade 3 or 4 laboratory abnormalities during the study period, including three (5%) participants with low haemoglobin concentration and one (2%) participant with a low platelet count.

Discussion

This study indicates that sofosbuvir–velpatasvir is safe and efficacious for the treatment of chronic HCV infection in Rwanda in individuals infected with diverse HCV genotype 4 subtypes other than 4a and 4d, and with baseline HCV NS5A resistance-associated substitutions, as shown by the vast majority of participants having SVR12. These findings support the use of sofosbuvir–velpatasvir as first-line treatment for individuals from regions known to have a high prevalence of genotype 4 non-a/d subtypes or in individuals already determined to harbour these subtypes or multiple NS5A resistance-associated substitutions.

In its 2020 guidelines update, the European Association for the Study of the Liver (EASL) recommended that in geographical settings where HCV subtypes (such as genotype 4r) that are inherently resistant to NS5A inhibitors are present, or in migrants from those settings, HCV subtype should be ascertained by means of population or deep sequencing, if available and affordable, to avoid treatment failure.[20] These guidelines recommend that such individuals should be considered for treatment with a fixed-dose combination of sofosbuvir–velpatasvir–voxilaprevir, pending data with other pangenotypic regimens. Our data suggest that sofosbuvir–velpatasvir is an effective first-line treatment for individuals infected with HCV genotype 4r, and therefore might be appropriate if this subtype is known, based on population sequencing, or suspected, based on region of origin. For individuals infected with HCV 4q and 4r subtypes or with at least three NS5A resistance-associated substitutions, a greater proportion had SVR12 in this study than previously reported for patients treated with sofosbuvir–ledipasvir in the SHARED clinical trial in Rwanda (figure 2). However, direct comparison of treatment efficacy in these studies is limited by study design and sample size. Our data also support recent WHO guidelines recommending sofosbuvir–velpatasvir as a preferred first-line therapy in

low-income and middle-income countries (LMICs).[21] It is notable that the WHO guidelines also recommend sofosbuvir–daclatasvir as a pangenotypic regimen for use in LMICs, but does not provide specific guidance for those regions with a high prevalence of HCV subtypes that are less susceptible to treatment. The effectiveness of the sofosbuvir–daclatasvir treatment regimen in populations with a high proportion of HCV genotype 4 non-a/d subtypes or HCV genotype 4r has not been directly reported; however, the similar in-vitro activity of daclatasvir and ledipasvir against isolates with common NS5A resistance-associated substitutions could indicate a less than optimal response to daclatasvir-containing regimens.[16,17] Emergence of NS5A mutations in individuals for whom daclatasvir-based regimens have failed has also been noted.[12,22] More data regarding the effectiveness of sofosbuvir–daclatasvir in genotype 4 non-a/d subtypes such as genotype 4r are urgently needed.

Two participants treated with sofosbuvir–velpatasvir in our study did not have SVR12. One of these individuals was infected with HCV containing three NS5A resistance-associated substitutions (Leu28Val, Leu30Arg, and Leu31Met) along with the NS5B resistance-associated substitution Val321Ile, and there was no evidence of viral suppression at the end of treatment. HCV isolates from the other participant had Leu30Arg and Leu31Met substitutions in NS5A at baseline and end of follow-up. Neither participant had liver cirrhosis by non-invasive testing, comorbidities that would interfere with treatment efficacy, or reported use of medications known to have drug–drug interactions with sofosbuvir–velpatasvir. In-vitro studies have shown a low EC_{50} of velpatasvir to isolates with the NS5A substitutions identified in these participants.[5] Neither participant had developed new NS5A resistance-associated substitutions by the end of treatment. Together, these findings suggest inadequate drug levels, due to either low bioavailability or unrecognised poor adherence to treatment, as a possible cause for treatment failure.

Our study has several limitations. The extent of liver fibrosis was assessed using the non-invasive tests APRI and FIB-4. Although these markers are not as accurate as transient elastography or liver biopsy in the detection of cirrhosis, non-invasive tests are the recommended approach in resource-limited settings and APRI has been validated in sub-Saharan African settings.[23] Few participants with HIV co-infection participated in this study, which might limit generalisability to settings with a high HIV prevalence. However, sofosbuvir–velpatasvir has been shown to have high efficacy in individuals with HIV co-infection across genotypes.[24] The use of sofosbuvir–velpatasvir in populations with a high prevalence of HIV co-infection will require attention to avoid potential drug interactions with commonly used antiretroviral medications in this region, such as efavirenz. Additionally, our study was not powered to detect statistically significant differences in efficacy between genotype subtypes; however, the proportion of participants with SVR12 was uniformly high across subtypes. As a single-arm trial, the efficacy of the study drug as interpreted by the proportion of participants with SVR12 cannot be confirmed in relation to a control group. Furthermore, viral sequencing was not successful for genotype determination for three participants, and NS5A and NS5B resistance-associated substitutions were not successfully ascertained for all participants. Finally, the study setting provided more support for participant transport and communication than is likely in most real-world national treatment programmes in sub-Saharan Africa, which could have led to better treatment adherence. These simple and low-cost adherence support measures should be further evaluated and considered for HCV treatment programmes in resource-limited settings.[25]

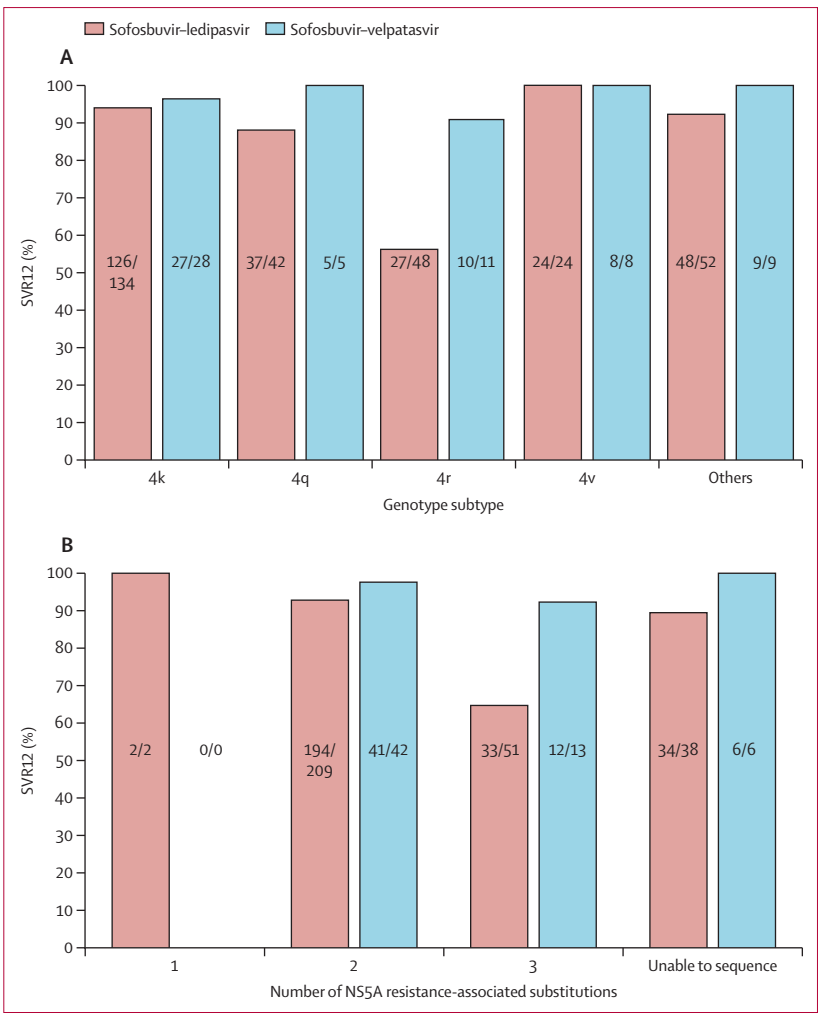

Figure 2: **Proportion of participants with SVR12 after sofosbuvir–velpatasvir treatment in the current study and sofosbuvir–ledipasvir treatment in the previous SHARED trial[9]**
(A) SVR12 by HCV genotype subtype. (B) SVR12 by number of baseline HCV NS5A resistance-associated substitutions. HCV=hepatitis C virus. SVR12=sustained virological response 12 weeks after completion of treatment

Our study suggests sofosbuvir–velpatasvir could be effective for optimising clinical outcomes for HCV treatment programmes in regions with substantial proportions of individuals with HCV genotype 4 subtypes with high numbers of baseline resistance-associated substitutions where genotype subtype determination or viral sequencing are not possible, affordable, or readily available. However, in most countries where these genotype 4 subtypes are endemic, first-line HCV treatment is largely based on availability and affordability of direct-acting antiviral regimens. Sofosbuvir–daclatasvir is currently the most commonly used regimen for first-line treatment in such settings. According to a recent analysis by the Clinton Health Access Initiative, the average freight-on-board cost in 2019 to supply a 12-week treatment course of a generic fixed-dose combination sofosbuvir–daclatasvir was $86.[26] The average cost of a 12-week course of sofosbuvir–velpatasvir decreased from $401 in 2018 to $225 in 2019 as a result of increased demand in LMICs and procurement of higher quantities from generic manufacturers in India. In some countries, the cost of generic sofosbuvir–velpatasvir exported from generic manufacturers in India in 2019 approximated that of sofosbuvir–daclatasvir.[26] These costs, however, do not reflect shipping, customs, and distributor-associated costs, which are highly variable and result in higher final prices to the purchaser in country.

Genotype 4 non-a/d subtypes with a high degree of baseline NS5A resistance-associated substitutions are prevalent across a wide geography of sub-Saharan Africa, including Burundi, Democratic Republic of the Congo, Eritrea, Ethiopia, Nigeria, Rwanda, Somalia, and Uganda.[3,4,6,8,13,27–29] In the context of ambitious targets for the elimination of HCV, national programmes in such countries will need to weigh a potentially higher cost of effective first-line treatment, such as sofosbuvir–velpatasvir, against the additional costs incurred by treatment failures associated with commonly used current regimens. These additional costs include the detection, follow-up, and treatment of individuals with poorly studied second-line treatment strategies, such as extended direct-acting antiviral regimens or the addition of ribavirin to standard NS5A-based regimens, or with the currently recommended regimen of sofosbuvir–velpatasvir–voxilaprevir, which remains unaffordable and unavailable in LMIC settings.[30] The cost of widespread treatment failure should also include losses in quality of life and economic productivity of substantial numbers of individuals, as well as the incremental increase in end-stage complications and mortality associated with failure or delay in achieving SVR12.[31] There is an urgent need for detailed investment cases comparing the cost and effectiveness of these treatment regimens on the basis of local HCV epidemiology including genotype subtypes and resistance-associated substitution prevalence to best inform national treatment programme selection and procurement of available HCV treatments. A rational approach to first-line treatment based on local prevalence of HCV resistance and further improvements in the affordability of best-in-class treatment will not only avert preventable morbidity and mortality, but also help ensure that LMICs with a high prevalence of difficult-to-treat HCV subtypes are not left behind in their progress towards HCV elimination.

Contributors

FK, FS, JK, SN, CMM, PMG, and NG developed the study protocol and the study design. FS, LM, JK, and AM collected data for the study. PMG and GC analysed the data and produced data tables, and NG produced figures. FK, FS, and NG conducted the literature review and wrote the first draft of the manuscript. All authors had full access to the data, participated in data interpretation, and provided critical feedback on the manuscript. FK and NG had final authority of the manuscript submission and all authors accepted responsibility for submission for publication. FK, GC, PMG, and NG were responsible for accessing and verifying the underlying data.

Declaration of interests

GC is an employee of and holds stock in Gilead Sciences. All other authors declare no competing interests.

Data sharing

The study protocol, study data, data dictionary, data collection instruments, and informed consent forms are available upon request from the corresponding author. De-identified individual participant data will be made available 9 months after the publication date and ending 36 months after the publication date. Forms and data can be accessed by written request to the corresponding author and the study sponsor, Partners In Health. The data will be made available following evaluation and approval of proposed use by the study sponsor and signed data access agreement with the study sponsor.

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