Safety and efficacy of sofosbuvir–velpatasvir–voxilaprevir for re-treatment of chronic hepatitis C virus infection in patients with previous direct-acting antiviral treatment failure in Rwanda (SHARED-3): a single-arm trial

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Summary

Background Hepatitis C virus (HCV) genotype 4 non-a/d subtypes, which frequently have NS5A resistance-associated substitutions, are highly prevalent in sub-Saharan Africa. These subtypes, particularly genotype 4r, have been associated with higher rates of failure of treatment regimens containing the NS5A inhibitors ledipasvir or daclatasvir, which are the most accessible direct-acting antivirals in low-income countries. Clinical evidence regarding the efficacy of re-treatment options for these subtypes is limited. We aimed to evaluate the safety and efficacy of sofosbuvir–velpatasvir–voxilaprevir for the treatment of adults in Rwanda with chronic HCV infection, predominantly of genotype 4, and a history of direct-acting antiviral treatment failure.

Methods In this single-arm prospective trial, we enrolled adults (aged ≥18 years) with a HCV RNA titre of at least 1000 IU/mL, and a documented history of direct-acting antiviral failure. Patients were assessed for eligibility at a single study site after referral from hospitals with HCV treatment programmes throughout Rwanda, and participants for whom sofosbuvir–ledipasvir treatment had failed in the previous SHARED trial were also included. Participants with decompensated liver disease or hepatitis B virus co-infection were excluded. Participants were treated once daily with an oral fixed-dose combination tablet containing sofosbuvir (400 mg), velpatasvir (100 mg), and voxilaprevir (100 mg) 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 NS3, NS5A, and NS5B genes was done at baseline in all participants and at end of follow-up (week 24) in participants with treatment failure. The study is registered with ClinicalTrials.gov (NCT03888729) and is completed.

Findings Between Sept 23, 2019, and Jan 10, 2020, 49 individuals were screened and 40 participants were enrolled. 20 (50%) were female, 20 (50%) were male, median age was 63 years (IQR 56–68), and median HCV viral load was 6·2 $\log_{10}$ IU/mL (5·8–6·5) at baseline. The genotype subtypes identified were 4r (18 [45%] participants), 4k (six [15%]), 4b (five [13%]), 4q (four [10%]), 4l (two [5%]), 4a (one [3%]), 4m (one [3%]), and 3h (one [3%]). One (3%) genotype 4 isolate could not be subtyped, and one (3%) isolate was of unknown genotype. All successfully sequenced isolates (33 [83%]) had at least two NS5A resistance-associated substitutions and 25 (63%) had three or more. 39 (98% [95% CI 87–100]) participants had SVR12. Seven (18%) participants had a total of ten grade 3, 4, or 5 adverse events, including three (8%) cases of hypertension, and one (3%) case each of cataract, diabetes, gastrointestinal bleeding, joint pain, low back pain, vaginal cancer, and sudden death. Four of these events were categorised as serious adverse events resulting in hospitalisation. The one sudden death occurred at home from an unknown cause 4 weeks after the completion of treatment. No serious adverse event was determined to be related to the study drug or resulted in treatment discontinuation.

Interpretation A 12 week course of sofosbuvir–velpatasvir–voxilaprevir is safe and efficacious for the re-treatment of individuals infected with HCV genotype 4 non-a/d subtypes with frequent baseline NS5A resistance-associated substitutions, following failure of previous direct-acting antiviral treatment. Improved affordability and access to sofosbuvir–velpatasvir–voxilaprevir in regions with these subtypes is crucial.

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Introduction

Approximately 45 000 (16%) of the estimated 290 000 annual deaths due to chronic hepatitis C virus (HCV) infection worldwide occur in sub-Saharan Africa, and the vast majority of infections in this region are undiagnosed and untreated.[1] However, an increasing number of national hepatitis C treatment policies, plans, and programmes have been established or are in

Research in context

Evidence before this study

People infected with hepatitis C virus (HCV) genotype 4r have a disproportionately high rate of treatment failure with NS5A inhibitor-based direct-acting antiviral regimens because of the high prevalence of resistance-associated substitutions. The optimal treatment regimen for individuals with these subtypes following failure of earlier direct-acting antiviral regimens has not been well established. We did a literature review in Google Scholar for literature published in English between Jan 1, 2010, to June 1, 2021 that reported re-treatment outcomes for any individuals infected with HCV genotype 4r or other genotype 4 non-a/d subtypes. We used the MeSH search terms "hepatitis C," "treatment failure", "genotype 4r", and "sub-Saharan Africa". We found three publications reporting re-treatment outcomes for a total of 19 individuals with HCV genotype 4 non-a/d subtypes. Five of these individuals were treated with sofosbuvir–velpatasvir–voxilaprevir, of whom four were reported to have a sustained virological response 12 weeks after completion of treatment (SVR12). All three studies were done in Europe and none were dedicated prospective trials for people infected with HCV genotype 4 non-a/d subtypes.

Added value of this study

This study prospectively evaluated the safety and efficacy of sofosbuvir–velpatasvir–voxilaprevir for the re-treatment of 40 adults with infected HCV genotype 4 non-a/d subtypes in a country with a high rate of endemic genotype 4r infection. HCV NS5A and NS5B resistance-associated substitutions were prevalent among these participants before treatment initiation. 39 (98%) participants had SVR12, and there were no serious adverse events related to the study drug.

Implications of all the available evidence

Sofosbuvir–velpatasvir–voxilaprevir is efficacious in the retreatment of individuals with HCV genotype 4 and other genotype 4 non-a/d subtypes with frequent NS5A and NS5B resistance-associated substitution and previous treatment failure. Given the high proportion of these resistant subtypes in countries of eastern and central Africa and the increasing number of individuals receiving first-generation NS5A-inhibitor-based direct-acting antiviral regimens as first-line treatment, there will be an increasing need for effective and proven retreatment options. There is an urgent need for improved availability and affordability of sofosbuvir–velpatasvir–voxilaprevir in these settings and further research is required to assess the effectiveness of alternative regimens.

development in the region, partially due to a substantial and steady decrease in the price of generically manufactured first-line direct-acting antivirals.[2,3] Numerous reports have been published on HCV treatment programmes and outcomes in sub-Saharan Africa, including in Cameroon, Ethiopia, South Africa, and Togo.[4–7] As a notable leader in the public health response to HCV in the region, Rwanda has established a 5-year national HCV elimination programme and has treated over 51000 patients as of 2021.[8–10]

The most prevalent HCV genotype in much of sub-Saharan Africa is genotype 4, which is estimated to comprise 60–97% of infections in the central subregion and 60–93% of infections in the eastern subregion.[11] Genotype 4 displays a remarkable degree of phylogenetic diversity in this region.[12,13] Several genotype 4 subtypes have shown a high number of resistance-associated substitutions that confer inherent resistance to several first-generation NS5A inhibitors. These subtypes—described as non-4a/d, rare, or hard-to-treat genotype 4 subtypes—have been reported across a wide geographical area of sub-Saharan Africa.[14,15] Genotype 4r, in particular, is associated with increased failure rates with NS5A inhibitor-based treatment regimens; in the earlier SHARED study,[16] a prospective trial investigating a 12-week course of sofosbuvir–ledipasvir in patients with chronic HCV infection in Rwanda, only 27 (56%) of 48 participants infected with HCV genotype 4r had a sustained virological response 12 weeks after completion of treatment (SVR12). Individuals with genotype 4r infection in Rwanda are more likely to have a history of hospitalisation or surgery and higher baseline HCV viral load than those infected with other subtypes.[17] Retrospective studies from Europe have shown higher failure rates in African migrants infected with HCV genotype 4 non-a/d subtypes after treatment with ledipasvir-based and daclatasvir-based regimens.[14,15,18] An analysis of the European Resistance Database found that, although genotype 4r comprised only seven (5%) of 129 of total treatment-naive patients with genotype 4, it comprised 17 (26%) of 66 patients with genotype 4 and previous treatment failure.[19] Additionally, HCV genotype 4r isolates typically had pre-existing NS5A resistance-associated substitutions at three key positions (Leu28Met/Val, Leu30Arg, Leu31Met), and the frequency of NS5A resistance-associated substitutions was significantly higher in genotype 4r than in other more common genotype 4 subtypes.[19] Comparable findings were reported among individuals of sub-Saharan African origin in the UK, where four (44%) of nine individuals infected with HCV genotype 4r treated with NS5A or NS5B inhibitor-based direct-acting antiviral regimens had SVR12.[15]

Data regarding optimal re-treatment regimens in individuals infected with genotype 4r or other genotype 4 non-a/d subtypes are largely lacking. Because of

restricted treatment options in resource-limited settings, the most common approach to re-treatment typically includes extension of available NS5A-based regimens to 16–24 weeks, with or without the addition of ribavirin.[20] Real-world data on this treatment approach for individuals infected with hard-to-treat genotype 4 subtypes have not been reported, although the additional costs of an extended regimen and adverse events associated with ribavirin-containing regimens are well established.[21] Re-treatment outcomes have been reported for several fixed-dose direct-acting antiviral combinations, including sofosbuvir–velpatasvir–voxilaprevir, glecaprevir–pibrentasvir, and glecaprevir–elbasvir in a small number of patients.[15,18] Based on these limited experiences and a moderate quality of data, the European Association for the Study of the Liver recommendation (graded as 2 [weak] on the Grading of Recommendations Assessment, Development and Evaluation system) is that individuals with HCV genotype 4r infection and a history of failed direct-acting antiviral treatment be treated with sofosbuvir–velpatasvir–voxilaprevir for 12 weeks.[22] The American Association for the Study of Liver Diseases recommends sofosbuvir–velpatasvir–voxilaprevir for treatment of individuals infected with HCV genotype 4 and with previous direct-acting antiviral failure, but it does not specifically provide guidance for those infected with genotype 4 non-a/d subtypes.[23]

Given this knowledge gap, additional data on re-treatment for patients infected with genotype 4 non-a/d subtypes is urgently needed.[24,25] Here we report the results of a prospective study of the safety and efficacy of sofosbuvir–velpatasvir–voxilaprevir in individuals with various genotype 4 subtypes endemic to the sub-Saharan Africa region and in whom previous NS5A inhibitor-based direct-acting antiviral treatment had failed.

Methods

Study design and participants

This Article describes the second of two single-arm studies conducted as part of the Simplifying Hepatitis C Antiviral Treatment in Rwanda for Elsewhere in the Developing World (SHARED-3) trial. This study evaluated the efficacy and safety of sofosbuvir–velpatasvir–voxilaprevir in HCV-infected adults with a history of direct-acting antiviral treatment failure in Rwanda. The results of the first study, which evaluated the efficacy and safety of sofosbuvir–velpatasvir in adults with chronic HCV infection in Rwanda without previous direct-acting antiviral treatment, are reported in an accompanying Article.[26]

Participants were referred by treating clinicians from hospitals with HCV treatment programmes throughout Rwanda, and participants who had had sofosbuvir–ledipasvir treatment failure in the previous SHARED trial were also included.[16] Eligibility assessment was done at a single study site (Rwanda Military Hospital, Kigali, Rwanda). Eligibility criteria were age 18 years or older, a HCV RNA titre of at least 1000 IU/mL, and a documented history of direct-acting antiviral failure, defined as a quantifiable HCV viral load more than 12 weeks after completion of treatment without interruption. Participants were also required to have a screening ultrasound that excluded 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). 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

All study procedures were delivered by a local team of non-specialist clinicians (two general practitioners, two nurses, and one social worker) and supervised by two Rwandan internists with specialised training in HCV management. At screening, we tested for plasma HCV RNA concentration and genotype, HIV antibodies, HBsAg, a right upper quadrant ultrasound, and standard clinical and laboratory assessments. On-study visits occurred at entry and weeks 4, 8, 12, and 24. All participants received a fixed-dose combination tablet containing 400 mg sofosbuvir, 100 mg velpatasvir, and 100 mg voxilaprevir, 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 also provided counselling regarding the importance of adherence and monitoring for potential side-effects. Plasma HCV RNA titre, complete blood count, and a comprehensive metabolic panel were obtained at weeks 12 and 24.

Clinical and laboratory adverse events were assigned grades 1 to 5 according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse

See Online for appendix

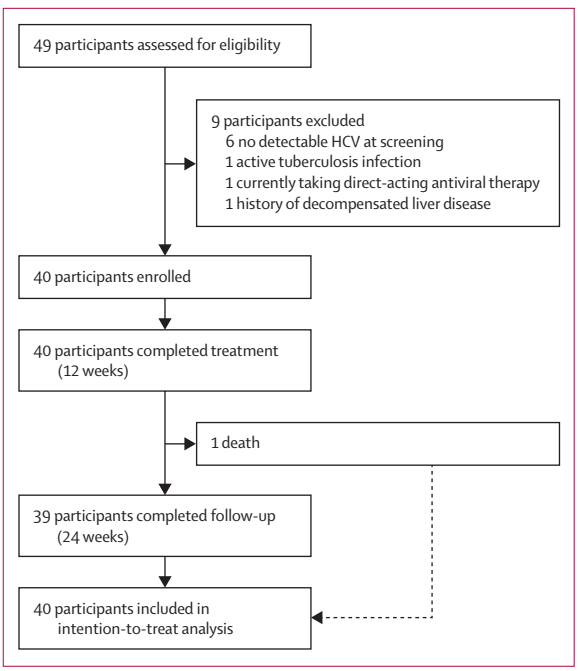

Figure: **Trial profile**

Events (version 2.1) by a study physician at weeks 4, 8, 12, and 24. Elevated total bilirubin was categorised as grade 3 (2·6 to <5·0 times ULN) or grade 4 (≥5·0 times ULN), elevated creatinine was categorised as grade 3 (>1·8 to <3·5 times ULN, or 1·5 to <2·0 times baseline) or grade 4 (≥3·5 times ULN or ≥2·0 times baseline), and low sodium was categorised as grade 3 (121 to <125 mEq/L) or grade 4 (≤120 mEq/L). Serious adverse events were defined as life-threatening events. Adherence was determined 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 discontinuation of the study drug included a grade 3 or 4 rash associated with constitutional symptoms, or any grade 4 event determined to be related to the study drug.

Plasma HCV RNA was measured at weeks 12 and 24 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) score and Fibrosis-4 (FIB-4) index. An APRI score greater than 2·00 was considered to indicate 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 conducted on baseline plasma samples for all participants and on week 24 plasma samples for participants without SVR12, with genotype-specific and subtype-specific primers based on genotype assignment from the HCV INNO-LiPA assay (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. Next-generation deep sequencing was done on the amplicons. In the case of unsuccessful NS5B amplification with the specific primers, a partial NS5B sequence was amplified with genotype-independent primers and sequenced using population sequencing or deep sequencing. To assign more accurate 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 resistance-associated substitution definitions).[27]

Outcomes

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

Statistical analysis

The hypothesis of this study was that sofosbuvir–velpatasvir–voxilaprevir would be safe and effective for the treatment of adults with chronic HCV infection who had previously had failure of direct-acting antiviral treatment. The target sample size of 40 was based on feasibility. The proportion of enrolled participants who met the primary endpoint was calculated along with corresponding 95% CIs determined 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%; a p value less than 0·05 was considered

	Participants (N=40)
Sociodemographic characteristics	
Age, years	63 (56–68)
Sex	
Female	20 (50%)
Male	20 (50%)
Education	
Primary education or less	21 (53%)
Greater than primary education	19 (48%)
Employment	
Unemployed	20 (50%)
Employed	20 (50%)
Monthly income, US$	
<120	26 (65%)
≥120	14 (35%)
Clinical characteristics	
Previous HCV treatment regimens*	
Sofosbuvir–ledipasvir	21 (53%)
Sofosbuvir–ledipasvir plus ribavirin	1 (3%)
Sofosbuvir–ledipasvir and sofosbuvir–daclatasvir	8 (20%)
Sofosbuvir–ledipasvir and sofosbuvir–ledipasvir plus ribavirin	8 (20%)
Sofosbuvir–daclatasvir and sofosbuvir–ledipasvir plus ribavirin	1 (3%)
Sofosbuvir–velpatasvir and sofosbuvir–ledipasvir plus ribavirin	1 (3%)
BMI, kg/m²	26 (22–28)
Comorbidities	
Hypertension	11 (28%)
HIV co-infection	7 (18%)
Diabetes	6 (15%)
HCV RNA titre, $\log_{10}$ IU/mL	6·2 (5·8–6·5)
Albumin concentration, g/dL	
Median (IQR)	4·1 (3·8–4·3)
<3·5	3 (8%)
Platelet count, $\times 10^3$/μL	179 (130–250)
Aspartate aminotransferase concentration, IU/mL	43 (32–90)
Alanine aminotransferase concentration, IU/mL	44 (34–72)
Total bilirubin concentration, mg/dL	0·6 (0·4–0·9)
Aspartate aminotransferase-to-platelet ratio index	
≤1	25 (63%)
>1·0 to ≤2·0	6 (15%)
>2·0	9 (23%)
Fibrosis-4 index score >3·25	15 (38%)

(Table 1 continues in next column)

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	Participants (N=40)
Virological characteristics	
HCV genotype by subtype	
4r	18 (45%)
4k	6 (15%)
4b	5 (13%)
4q	4 (10%)
4l	2 (5%)
4a	1 (3%)
4m	1 (3%)
4, undetermined subtype	1 (3%)
3h	1 (3%)
Unknown	1 (3%)
Number of HCV NS3 resistance-associated substitutions	
0	26 (65%)
≥1	0
Unknown (no sequencing data)	14 (35%)
Number of HCV NS5A resistance-associated substitutions	
0	0
1	0
2†	8 (20%)
≥3‡	25 (63%)
Unknown (no sequencing data)	7 (18%)
Number of HCV NS5B resistance-associated substitutions	
0	14 (35%)
≥1	16 (40%)
Unknown (no sequencing data)	10 (25%)

Data are median (IQR) or n (%). HCV=hepatitis C virus. *Where multiple regimens are listed, each regimen was taken sequentially and not in combination; categories are mutually exclusive. †Leu30His/Arg and Leu31Met/Val. ‡Leu28Met/Thr/Val, Leu30His/Arg, and Leu31Met/Val; Leu30Arg/Ser, Leu31Ile/Met/Val, and Tyr93Cys/His/Ser/Trp; Leu28Ile/Met/Thr/Val, Leu30His/Arg, Leu31Met/Val, and Tyr93His/Ser; Leu30Arg/Ser, Leu31Met, Pro58Leu/Ser, and Tyr93His/Ser; or Leu28Met, Leu30Arg, Leu31Val, and Pro58Ser.

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

to indicate statistical significance. 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 analysis and interpretation of viral sequencing. The funder did not contribute to data collection.

Results

Between Sept 23, 2019, and Jan 10, 2020, 49 individuals were screened for eligibility for the study and nine (18%) were excluded (figure). 40 participants were enrolled on the basis of entry criteria, and the follow-up period was completed on Aug 28, 2020. Of the enrolled participants, 20 (50%) were women and 20 (50%) were men. Median age was 63 years (IQR 56–68), and median HCV viral load was 6·2 $\log_{10}$ IU/mL (5·8–6·5) at baseline (table 1). Seven (18%) participants had HIV co-infection, with a baseline median CD4 cell count of 575 cells per μL (541–652); all had an undetectable HIV viral load at baseline. At baseline, 11 (28%) participants had a history of hypertension and

	Participants with SVR12
Overall	39/40 (98% [87–100])
By HCV genotype and subtype	
4r	18/18 (100%)
4k	6/6 (100%)
4b	5/5 (100%)
4q	3/4 (75%)
4l	2/2 (100%)
4a	1/1 (100%)
4m	1/1 (100%)
4, undetermined subtype	1/1 (100%)
3h	1/1 (100%)
Unknown	1/1 (100%)
By number of NS3 resistance-associated substitutions	
0	25/26 (96%)
≥1	NA
Unknown	14/14 (100%)
By number of NS5A resistance-associated substitutions	
0	NA
1	NA
2	8/8 (100%)
≥3	24/25 (96%)
Unknown	7/7 (100%)
By number of NS5B resistance-associated substitutions	
0	14/14 (100%)
≥1	15/16 (94%)
Unknown	10/10 (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 HCV resistance-associated substitutions**

	Participants (N=40)
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 (10%)
Diabetes	1 (3%)
Gastrointestinal bleeding	1 (3%)
Vaginal cancer	1 (3%)
Sudden death	1 (3%)
Grade 3–5 adverse events*	7 (18%)
Grade 3	
Hypertension	3 (8%)
Cataract	1 (3%)
Diabetes	1 (3%)
Lower back pain	1 (3%)
Non-specific joint pain	1 (3%)
Grade 4	
Gastrointestinal bleeding	1 (3%)
Vaginal cancer	1 (3%)
Grade 5	
Sudden death	1 (3%)
Grade 1 or 2 adverse events†	34 (85%)
Hypertension	16 (40%)
Abdominal pain	9 (23%)
Headache	7 (18%)
Lower back pain	5 (13%)
Joint pain	5 (13%)
Diabetes	5 (13%)
Nausea or vomiting	5 (13%)
Fatigue	5 (13%)
Rash	5 (13%)
Cough	4 (10%)
Dizziness	4 (10%)
Laboratory abnormality (grade 3 or 4)	
Elevated total bilirubin concentration	1 (3%)
Elevated creatinine concentration	1 (3%)
Low sodium concentration	1 (3%)

Data are n for number of events, or n (%) for number of participants with events. *Three participants had two grade 3–5 adverse events each; four grade 3–5 adverse events were also categorised as serious adverse events. †Grade 1 or 2 adverse events occurring in at least 10% of participants are listed.

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

six (15%) had a history of diabetes. 37 (93%) participants had previously had treatment failure following a full course with sofosbuvir–ledipasvir, 11 (28%) with sofosbuvir–ledipasvir plus ribavirin, nine (23%) with sofosbuvir–daclatasvir, and one (3%) with sofosbuvir–velpatasvir; 18 (45%) had had more than one failed regimen. Nine (23%) participants had an APRI score greater than 2·00 and 15 (38%) had a FIB-4 score greater than 3·25. In the nine participants with an APRI score greater than 2·0, the median Child-Pugh score was 6 (IQR 5–6).

Based on viral sequencing data, one (3%) participant was infected with HCV genotype 3h and the remaining 38 (95%) with available sequencing data were infected with HCV genotype 4 (table 1). The most frequent subtypes were 4r (18 [45%] participants), 4k (six [15%]), 4b (five [13%]), and 4q (four [10%]). Other subtypes identified were 4a, 4l, and 4m. One (3%) isolate of genotype 4 could not be subtyped, and one (3%) could not be sequenced and was categorised as unknown genotype. All participants with available NS5A sequencing data (n=33) were infected with HCV with at least two NS5A resistance-associated substitutions (including Leu28Ile/Met/Thr/Val, Leu30His/Arg/Ser, Leu31Ile/Met/Val, Pro58Leu/Ser, and Tyr93Cys/His/Ser/Trp; table 1, appendix p 1), and 25 (63%) had three or more NS5A resistance-associated substitutions. 30 participants had available sequencing data for NS5B, including 16 (40%) who had at least one resistance-associated substitution (including Glu237Asp/Gly, Phe289Leu, Val321Ile, and Ser282Thr; table 1, appendix p 1). None of the patients with available sequencing data (n=26) showed NS3 resistance-associated substitutions at baseline (table 1).

SVR12 was observed in 39 (98% [95% CI 87–100]) participants. A breakdown of SVR12 by genotype subtype and number of resistance-associated mutations is provided in table 2. 39 participants had undetectable HCV RNA titres at the end of treatment (week 12). By pill count, 37 (93%) participants had an adherence of 100%, and three (8%) had adherence between 95% and 100%.

The single participant who did not have SVR12 had an HCV RNA titre of 4·5 $\log_{10}$ IU/mL before treatment and 4·4 $\log_{10}$ IU/mL at the end of treatment. The participant had a reported medical history that included hypertension, asthma, chronic obstructive pulmonary disease, and a stable lung mass noted on previous imaging. He did not have cirrhosis according to non-invasive test results (APRI score 0·39, FIB-4 score 1·76) and had laboratory examination results within normal ranges upon study entry, including liver function tests, serum albumin concentration, bilirubin concentration, creatinine concentration, and platelet count. The pill count indicated that the participant took 100% of the prescribed doses, and the study team did not identify any barriers to adherence. The participant did not report any adverse events throughout the study and was not taking any medications that interacted with the study medication. He had previously been treated with a 12 week course of sofosbuvir–ledipasvir and a 12 week course of sofosbuvir–daclatasvir. The participant was infected with HCV genotype 4q. At baseline, the participant's isolate had no resistance-associated substitutions in NS3, three (Leu30Ser, Leu31Val, Tyr93Trp) in NS5A, and two (Ser282Thr and Phe289Leu) in NS5B. At the end of treatment (week 12), the participant's viral isolate showed that three resistance-associated substitutions had developed in NS3 (Thr54Thr/Ser, Ala156Cys/Ser/Thr, and Asp168Asp/Glu), while the Ser282Thr resistance-associated substitution in NS5B had been lost and reverted to the Ser282 wild type. All three NS5A resistance-associated substitutions and Phe289Leu in NS5B were maintained from baseline to the end of treatment. The participant died suddenly at home 4 weeks after the completion of treatment (included in adverse events).

Overall, seven (18%) participants had a total of ten grade 3, 4, or 5 adverse events (table 3), none of which were judged to be related to the study drug. The seven grade 3 adverse events were newly diagnosed or worsening hypertension (three [8%] participants), diabetes (one [3%]), lower back pain (one [3%]), non-specific joint pain (one [3%]), and visual disturbance associated with cataracts (one [3%]). The two grade 4 adverse events comprised a gastrointestinal bleed due to gastric ulceration and a new diagnosis of vaginal squamous cell carcinoma. Three participants had more than one grade 3–5 adverse event.

There were four serious adverse events, which included hospital admissions for three of the grade 3 or 4 adverse events in addition to one death (grade 5 adverse event; table 3).

The death occurred in a 67-year-old man 4 weeks after completing the course of study drug (week 16 of study participation). The death was witnessed by a family member and reported as a sudden event that occurred in the participant's home without preceding symptoms. No medical assessment or autopsy was done following the death.

The most common grade 1 or 2 adverse events (occurring in >10% of participants) were hypertension, abdominal pain, headache, lower back pain, joint pain, diabetes, nausea or vomiting, fatigue, and rash (table 3). No adverse event resulted in premature treatment discontinuation.

Discussion

Our study shows high efficacy of sofosbuvir–velpatasvir–voxilaprevir for re-treatment of patients infected with HCV of predominantly genotype 4 non-a/d subtypes with frequent NS5A resistance-associated substitutions, following failure of previous direct-acting antiviral treatments. To our knowledge, this is the first prospective re-treatment trial to be done in a region in which these genotype 4 subtypes are endemic, and the first prospective study for re-treatment of this patient population to date. The high prevalence at baseline of HCV NS5A and NS5B resistance-associated substitutions in participants in this study is likely to reflect the low threshold for acquisition of resistance-associated substitutions in HCV genotype 4 non-a/d subtypes following treatment failure with ledipasvir-based and daclatasvir-based direct-acting antiviral regimens.[18,28]

The efficacy of sofosbuvir–velpatasvir–voxilaprevir observed in this study is consistent with previous reports of successful SVR12 in a small number of individuals with the same types of baseline resistance-associated substitutions and previous NS5A inhibitor-based treatment failures.[15,18,19] In our cohort, sofosbuvir–velpatasvir–voxilaprevir was efficacious regardless of the number of baseline resistance-associated substitutions, including in patients with multiple resistance-associated substitutions in NS5A (including Leu28Ile/Met/Thr/Val, Leu30His/Arg/Ser, Leu31Met/Val, and Tyr93His/Ser) and NS5B (including Ser282Thr, Phe289Leu, and Val321Ile). Generally, re-treatment failure with sofosbuvir–velpatasvir–voxilaprevir is rare and has been more commonly reported in patients with HCV genotype 3 or 1a infections with cirrhosis, and in patients with history of liver transplantation or treatment failure with sofosbuvir–velpatasvir.[29,30] There is one previous report of re-treatment failure with sofosbuvir–velpatasvir–voxilaprevir in a patient infected with HCV genotype 4r who was originally from sub-Saharan Africa.[15] In our study, the participant who did not have SVR12 had a multiple baseline resistance-associated substitutions in NS5A (Leu30Ser, Leu31Val, and Tyr93Trp) and NS5B (Ser282Thr and Phe289Leu), which might have conferred resistance to the NS5A and NS5B inhibitor components of the study drug and resulted in the emergence of the NS3 resistance-

associated substitutions Thr54Thr/Ser, Ala156Cys/Ser/Thr, and Asp168Asp/Glu, while the Ser282Thr substitution in NS5B reverted to the Ser282 wild type.

It is notable that all but one of the participants had previously been treated with sofosbuvir–ledipasvir or sofosbuvir–daclatasvir. Only one participant had previous failure of a velpatasvir-based regimen and none had previously been treated with NS3 protease inhibitor-based regimens, both of which regimens have highly limited availability in Rwanda and the surrounding region. Few failures of velpatasvir-based regimens in patients infected with HCV genotype 4 have previously been reported in the literature. In-vitro studies have shown a lower half-maximal effective concentration for velpatasvir against the NS5A resistance-associated substitutions most commonly found in genotype 4r (such as Leu30Arg, Met31Leu, and Tyr93His), as compared with ledipasvir or daclatasvir.[31–33] These data suggest that a velpatasvir-based first-line treatment of genotype 4 non-a/d subtypes that frequently have baseline resistance-associated substitutions might result in fewer treatment failures. In the first of the two single-arm studies within SHARED-3, 97% of participants with genotype 4 non-a/d subtypes had SVR12 following treatment with sofosbuvir–velpatasvir as first-line treatment.[26]

In resource-constrained settings where highly resistant HCV genotype 4 subtypes are prevalent, re-treatment following failure of direct-acting antivirals often consists of an extended course of available NS5A inhibitor-based regimens, typically ledipasvir or daclatasvir, with or without the addition of ribavirin.[20,34] Such strategies have not been prospectively studied, and retrospective data are very scarce, with no evidence in genotype 4 subtypes with frequent baseline resistance-associated substitutions. Sofosbuvir–velpatasvir with ribavirin has been approved for the re-treatment of patients infected with HCV genotypes 1, 2, or 3 (including those with decompensated cirrhosis) following treatment failure, and might provide a locally available option for re-treatment of patients with genotype 4 non-a/d subtypes, but this approach has not been evaluated.[35] Although there have been some limited reports regarding successful outcomes using glecaprevir–pibrentasvir for re-treatment of patients infected with HCV genotype 4r following previous treatment failure, insufficient data are available to recommend this approach, and this drug combination is not accessible in sub-Saharan Africa.[18,22] Such regimens warrant further clinical study as viable and affordable options for re-treatment of chronic HCV in settings of where genotype 4 non-a/d subtypes are endemic.

There were several limitations to this study. First, non-invasive tests (APRI and FIB-4 scores) were used to assess the extent of liver fibrosis. These markers are less accurate than transient elastography or liver biopsy for detecting cirrhosis; however, in resource-limited settings, these non-invasive tests are the recommended approach, and the APRI score has been validated in sub-Saharan Africa settings.[36] Second, this study did not have sufficient power to detect statistically significant differences in efficacy between genotype subtypes, although all subtypes had similarly high proportions of participants with SVR12. Furthermore, one participant had an undetermined genotype due to unsuccessful viral sequencing, and amplification of coding regions of specific non-structural proteins was not successful in numerous participants and we were unable to determine the presence of resistance-associated substitutions for several participants. Additionally, participants enrolled in this study might have had greater motivation and care-seeking behaviour than the general population, which might limit the generalisability of treatment adherence rates measured in this study. Treatment adherence in this study was also likely to be higher than generally observed in real-world national treatment programmes in sub-Saharan Africa because participants received transport reimbursements and telephone reminders by study staff. However, all clinical interventions in our study were led and supervised by existing hospital staff, and these low-cost interventions are feasible within HCV treatment programmes in resource-limited settings.[37] Finally, as a single-arm trial, the efficacy of the study drug in terms of the proportion of participants with SVR12 cannot be compared against a control group.

Given the urgent and growing need for re-treatment of direct-acting antiviral treatment failures in sub-Saharan Africa, greater efforts are required to increase the affordability of and access to sofosbuvir–velpatasvir–voxilaprevir. Despite its inclusion in voluntary licensing agreements for 14 generic manufacturers with license to market generic products in 105 countries, sofosbuvir–velpatasvir–voxilaprevir has yet to be produced generically.[3] Sofosbuvir–velpatasvir–voxilaprevir manufactured by the originating company remains cost-prohibitive in regions where highly resistant HCV genotype 4 non-a/d subtypes are prevalent, thereby restricting access to this evidence-supported, potentially curative treatment. As resource-limited countries in sub-Saharan Africa continue to introduce and scale national HCV treatment programmes based on affordable first-line treatments (such as ledipasvir-based and daclatasvir-based regimens) in regions with HCV genotype 4-predominant epidemics, there will be an increasing burden of first-line treatment failures.[38] The morbidity and mortality, as well as the health system costs and productivity losses associated with unaddressed treatment failures, must be accurately and comprehensively estimated and considered in national plans and priorities.[39–41] To deliver on commitments to eliminate HCV as a public health threat in areas where highly resistant subtypes are endemic, it is urgent and crucial to improve the affordability and availability of effective, proven, and standardised therapies for patients with HCV with a history of direct-acting antiviral treatment failure.

Contributors

NG, FS, JK, SN, CMM, PMG, and FK developed the study protocol. LM, FS, JK, and AM collected data for the study. PMG and GC conducted the data analysis and produced tables, and NG produced the figures. NG and LM 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. NG, GC, PMG, and FK were responsible for accessing and verifying the underlying data. NG and FK had final authority over the submitted version and all authors accepted responsibility for submission for publication.

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 on 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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