

Efficacy of ultra-short, response-guided sofosbuvir and daclatasvir therapy for hepatitis C in a single-arm mechanistic pilot study

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Abstract

Background: World Health Organization has called for research into predictive factors for selecting persons who could be successfully treated with shorter durations of direct-acting antiviral (DAA) therapy for hepatitis C. We evaluated early virological response as a means of shortening treatment and explored host, viral and pharmacokinetic contributors to treatment outcome.

Methods: Duration of sofosbuvir and daclatasvir (SOF/DCV) was determined according to day 2 (D2) virologic response for HCV genotype (gt) 1- or 6-infected adults in Vietnam with mild liver disease. Participants received 4- or 8-week treatment according to whether D2 HCV RNA was above or below 500 IU/ml (standard duration is 12 weeks). Primary endpoint was sustained virological response (SVR12). Those failing therapy were retreated with 12 weeks SOF/DCV. Host IFNL4 genotype and viral sequencing was performed at baseline, with repeat viral sequencing if virological rebound was observed. Levels of SOF, its inactive metabolite GS-331007 and DCV were measured on days 0 and 28.

Results: Of 52 adults enrolled, 34 received 4 weeks SOF/DCV, 17 got 8 weeks and 1 withdrew. SVR12 was achieved in 21/34 (62%) treated for 4 weeks, and 17/17 (100%) treated for 8 weeks. Overall, 38/51 (75%) were cured with first-line treatment (mean duration 37 days). Despite a high prevalence of putative NS5A-inhibitor resistance-associated substitutions (RASs), all first-line treatment failures cured after retreatment (13/13). We found no evidence treatment failure was associated with host IFNL4 genotype, viral subtype, baseline RAS, SOF or DCV levels.

Conclusions: Shortened SOF/DCV therapy, with retreatment if needed, reduces DAA use in patients with mild liver disease, while maintaining high cure rates. D2 virologic response alone does not adequately predict SVR12 with 4-week treatment.

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Editor's evaluation

Hepatitis C virus (HCV) infection continues to be a global public health problem with over 70 million infected. The current study provides a response to the WHO call for identifying patients with HCV who could be successfully treated with a shorter duration of direct-acting antiviral (DAA) therapy. It provides valuable knowledge to the ongoing research to shorten DAA therapy duration while maintaining high cure rates. Such efforts would impact both treatment access and achieving WHO elimination goals for HCV.

Introduction

Direct-acting antiviral (DAA) therapy for hepatitis C (HCV) offers high cure rates to those able to adhere to standard durations of treatment. In low- and middle-income countries, where treatment is limited to second-generation NS5A/NS5B-inhibitor combinations, standard treatment is at least 12 weeks. This duration presents a barrier to successful engagement in care for some populations (*Kracht et al., 2019; Petersen et al., 2016*), hampering the elimination of HCV as a public health threat. Novel treatment strategies are required for hard-to-reach individuals such as people who inject drugs and those of no fixed abode.

In Vietnam, DAA therapy remains prohibitively expensive for many of those infected. A standard 12-week course of sofosbuvir and daclatasvir (SOF/DCV) was priced at US\$2417–2472 in Ho Chi Minh City (HCMC) in 2019 (*Nguyen Thanh et al., 2019*). Despite the government subsidising 50% of drug costs since, the Ministry of Health estimates only 1000 individuals accessed DAA treatment through health insurance in 2019, and 2700 in 2020 (*Ministry of Health V, 2021*).

The World Health Organization has called for research into predictive factors for selecting persons who could be successfully treated with shorter durations of therapy (*World Health Organization, 2018*), which could expand access to treatment and reduce drug costs. Studies evaluating short-course therapy are challenging for infectious diseases where there are significant clinical risks of failure (e.g., TB and sepsis). However, HCV provides a model where treatment failures can be successfully retreated (*Cooke and Pett, 2021*) allowing exploration of mechanisms underlying successful therapy.

Shortened DAA therapy is associated with disappointing rates of cure, such that it could never be recommended routinely. A systematic review and meta-analysis into treatment optimisation for HCV with DAA therapy in individuals with favourable predictors of response, found that pooled sustained virological response (SVR) for regimens of ≤ 4 weeks duration was 63.1% (95% confidence interval [CI] 39.9–83.7), 6 weeks duration was 81.1% (75.1–86.6) and 8 weeks duration was 94.2% (92.3–95.9) (*Jones et al., 2019*). However improved rates of cure were seen with an increased number of individual-level factors known (or assumed) to be favourable, such as non-genotype 3 infection, lower body mass index (BMI), lower baseline viral load, mild liver disease, absence of prior treatment failure and a rapid virological response to treatment (*Jones et al., 2019*).

Rapid virological response offers a promising means of shortening treatment duration while maintaining high rates of cure. So-called response-guided therapy (RGT), whereby antiviral duration is shortened in individuals who rapidly suppress virus levels in blood after starting treatment, was routinely used in the era of interferon-based therapy, when an undetectable HCV RNA at 4 weeks

eLife digest Hepatitis C is a blood-borne virus that causes thousands of deaths from liver cirrhosis and liver cancer each year. Antiviral therapies can cure most cases of infection in 12 weeks. Unfortunately, treatment is expensive, and sticking with the regimen for 12 weeks can be difficult. It may be especially challenging for unhoused people or those who use injection drugs and who have high rates of hepatitis C infection.

Shorter durations of therapy may make it more accessible, especially for high-risk populations. But studies of shorter antiviral treatment durations have yet to produce high enough cure rates. Finding ways to identify patients who would benefit from shorter therapy is a key goal of the World Health Organization.

Potential characteristics that may predict a faster treatment response include low virus levels before initiating treatment, patient genetics, drug resistance mutations in the virus, and higher drug levels in the patient's blood during treatment. For example, previous research showed that a rapid decrease in virus levels in a patient's blood two days after starting antiviral therapy with three drugs predicted patient cures after three weeks of treatment.

To test if high cure rates could be achieved in just four weeks of treatment, Flower et al. enrolled 52 patients with hepatitis C in a study to receive the most widely accessible dual antiviral treatment (sofosbuvir and daclatasvir). Participants received four or eight weeks of treatment, depending on the amount of viral RNA in their blood after two days of treatment. The results indicate that a rapid decrease in virus levels in the blood does not adequately predict cure rates with four weeks of two-drug combination therapy. However, eight weeks may be highly effective, regardless of viral levels early in treatment.

Thirty-four individuals with low virus levels on the second day of treatment received four weeks of therapy, which cured 21 or 62% of them. All seventeen individuals with higher viral levels on day two were cured after eight weeks of treatment. Twelve weeks of retreatment was sufficient to cure the 13 individuals who did not achieve cure with four weeks of therapy. Even patients with drug resistance genes after the first round of therapy responded to a longer second round.

Flower et al. show that patient genetics, virus subtype, drug levels in the patient's blood, and viral drug resistance genes before therapy, were not associated with patient cures after four weeks of treatment. Given that retreatment is safe and effective, larger studies are now needed to determine whether eight weeks of therapy with sofosbuvir and daclatasvir may be enough to cure patients with mild liver disease. More studies are also necessary to identify patients that may benefit from shorter therapy durations. Finding ways to shorten antiviral therapy for hepatitis C could help make treatment more accessible and reduce therapy costs for both individuals and governments.

was used to determine a shorter course of pegylated interferon and ribavirin (*Fried et al., 2011*). Evidence supporting RGT with DAAs at earlier time points is emerging (*Cooke and Pett, 2021; Lau et al., 2016; Yakoot et al., 2017*), notably using day 2 (D2) viral load to determine treatment duration in genotype 1b infection. In this population, high cure rates were observed with just 3 weeks triple therapy (protease inhibitor [PI], NS5A inhibitor and NS5B inhibitor) (*Lau et al., 2016*). In a UK treatment shortening study, which used 4–8 weeks ombitasvir, paritaprevir, dasabuvir and ritonavir based on baseline viral load, all 10 individuals who became undetectable at D3 of treatment achieved first-line SVR12 regardless of treatment duration (*Cooke and Pett, 2021*). There is currently no data for RGT durations less than 8 weeks with SOF/DCV, which remains the lowest-priced and most widely available treatment option globally (*Clinton Health Access Initiative, 2021*).

Drug resistance in association with particular viral genotypes and subtypes is also known to influence treatment outcome (*Silva Filipe et al., 2017; Gupta et al., 2019*) and may predict who can be treated with shortened therapy. Vietnam has a high burden of genotype 6 HCV infection (around 35%) (*Irekeola et al., 2021*), which is rare outside South East Asia and under-represented in clinical trials. Genotype 6 is the most genetically diverse HCV lineage (*Hedskog et al., 2019*), raising concerns about the potential for emergence of resistant variants (*McPhee et al., 2019*).

The human *IFNL4* di-nucleotide polymorphism rs368234815 ($\Delta G/TT$) controls generation of the *IFNL4* protein and is also associated with impaired clearance of HCV (*Prokunina-Olsson et al., 2013*

and inferior responses to pegylated interferon-alpha/ribavirin therapy (Franco *et al.*, 2014) and SOF-based treatment (Ansari *et al.*, 2017; Ansari *et al.*, 2019). The impact of host *IFNL4* genotype in shortened DAA therapy is not well understood. It is also unknown how serum levels of SOF, its metabolite GS-331007, and DCV might impact treatment success with shortened therapy.

In this prospective single-arm mechanistic study in HCMC, individuals with genotypes 1 and 6 HCV infection and mild liver disease were treated with shortened course SOF/DCV. We tested the hypothesis that high rates of cure can be achieved with short-course DAAs when early on-treatment virological response is used to guide duration of therapy. We also compared host *IFNL4* genetic polymorphism, DAA drug levels, HCV subtypes and previously defined (in vitro) resistance-associated substitutions (RASs), in cures versus treatment failures to better understand the biological mechanisms determining treatment outcome.

Methods

Study population

Participants were recruited from the outpatient hepatitis clinic of the Hospital for Tropical Diseases (HTD) in HCMC, between February 2019 and June 2020. Eligible patients were ≥ 18 years and had chronic infection with HCV genotype 1 or 6 without evidence of liver fibrosis (defined as a FibroScan score ≤ 7.1 kPa, equivalent to F0-F1 disease) (Nitta *et al.*, 2009). In addition, participants were required to be HCV-treatment naïve, have a BMI ≥ 18 kg/m², a creatinine clearance ≥ 60 ml/min, with no evidence of HIV or Hepatitis B coinfection, or solid organ malignancy in the preceding 5 years. Full eligibility criteria are provided in the protocol available at <https://doi.org/10.1186/ISRCTN17100273>.

Patients referred to the trial were initially enrolled into an observational study which included FibroScan assessment and genotyping. Individuals in this cohort found to be potentially eligible for the trial were invited for further screening. All patients provided written informed consent.

Study design

All participants were treated with sofosbuvir 400 mg and daclatasvir 60 mg (Pharco Pharmaceuticals, Egypt) administered orally as two separate tablets, once daily. Individuals requiring dose adjustment for any reason were excluded.

Treatment duration was determined using hepatitis C viral load measured 2 days after treatment onset (D2). Participants with viral load < 500 IU/ml at D2 (after two doses of SOF/DCV) were treated with 4-week SOF/DCV. Those with HCV RNA ≥ 500 IU/ml received 8 weeks (Figure 1). The aforementioned study by Lau *et al.*, 2016 reported 100% SVR12 following 3-week triple therapy using this threshold. We chose a minimum 4-week duration based on our broader inclusion criteria and the use of dual-class therapy.

To determine viral kinetics on treatment (and on occasion of any failure), HCV viral load was measured at baseline (day 0) and at all subsequent follow-up visits on days 1, 2, 7 and then twice weekly until end-of-treatment (EOT) (Figure 1). Visits after EOT were scheduled twice weekly in the first month after completion of treatment, and then at 8 and 12 weeks after EOT.

The primary endpoint was sustained virological response (SVR12) defined as plasma HCV RNA less than the lower limit of quantification ($< \text{LLOQ}$) 12 weeks after the EOT without prior failure. Failure of first-line treatment was carefully defined to incorporate individuals who fully suppressed HCV RNA ($< \text{LLOQ}$) on therapy with late virological rebound, as well as those who never fully suppressed HCV

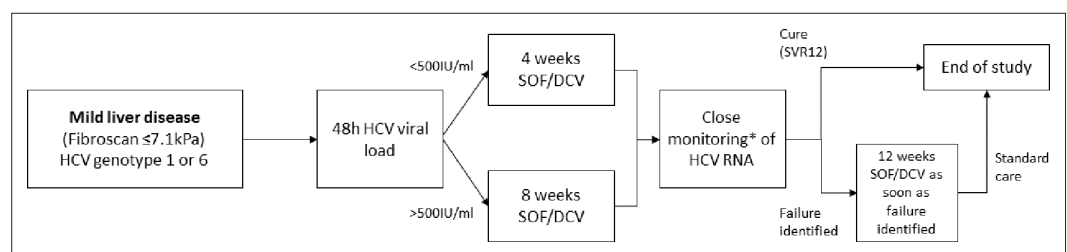


Figure 1. Study design. *HCV RNA on days 0, 1, 2, 7, 10, 14, 17, 21, 24, 28, (42, 56), EOT +3, EOT +7, EOT +10, EOT +14, EOT +17, EOT +21, EOT +24, EOT +28s, EOT +56, EOT +84.

viral load. In both cases, two consecutive viral loads >LLOQ, taken at least 1 week apart, were required to confirm failure, with the second >2000 IU/ml. Once failure was confirmed, participants commenced retreatment with standard duration SOF/DCV within 2 weeks (**Figure 1**).

Secondary endpoints were lack of initial virological response (<1 log₁₀ decrease in HCV viral load from baseline), serious adverse events (SAEs), grade 3/4 clinical adverse events (AEs), AEs of any grade leading to change in treatment (SOF, DCV or any other concomitant medication) and adverse reactions (ARs). Severity of all AEs and ARs were graded using the Common Toxicity Criteria for Adverse Events gradings (**National Institute of Health, 2017**).

Sample size justification

We set a target cure rate of ≥90%, and an unacceptably low cure rate of 70%. Assuming 90% power and one-sided $\alpha=0.05$, 37 participants were required to exclude the null hypothesis that cure was <90%. Assuming 5% loss to follow-up, and that, based on the study by **Lau et al., 2016**, 65% would suppress viral load <500 IU/ml by day 2 and receive 4 weeks (rather than 8 weeks) of therapy, the final target population was 60 participants, pooling genotypes 1 and 6.

Study assessments

At each visit, patients were assessed by a study doctor. AEs and ARs were recorded and graded according to a standardised scale (**National Institute of Health, 2017**) and medication adherence and use of healthcare facilities were recorded on case report forms.

HCV RNA was measured in the hospital using the available commercial platform. At start of study (for the first 41 participants enrolled), this was the Abbott Architect (LLOQ=12 IU/ml). This was subsequently replaced with the COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test, version 2.0 (Roche Molecular Systems, LLOQ=15 IU/ml). Standard laboratory tests—including full blood count, renal function and liver function tests—were performed in the hospital laboratory at baseline, EOT and EOT+12.

Virus sequencing

At screening, HCV genotype and subtype were determined using NS5B, Core and 5' UTR sequencing, according to the method described by **Le Ngoc et al., 2019**. To evaluate the impact of HCV subtypes and RASs on treatment outcome, whole-genome sequencing (WGS) was additionally performed on all enrolled participants' virus at baseline, and upon virological rebound and at start of retreatment in participants failing therapy. WGS of the HCV viral genome was attained using Illumina MiSeq platform as described previously (**Thomson et al., 2016; Smith et al., 2021b; Smith et al., 2021a; Manso et al., 2020**). The de novo assembly's nucleotide sequences were translated into amino acid and were aligned to H77 HCV reference (GenBank ID: NC_038882.1) and the NS5A and NS5B protein regions were extracted. We only looked for RAS that were present in at least 15% of the reads in the sample and had a read count of greater than 10.

We used the Public Health England (PHE) HCV Resistance Group's definition for RASs (**Bradshaw et al., 2019**). For genotype 1 we looked for RASs defined specifically for genotype 1 as they are well studied. For genotype 6 we looked for all RASs defined across all genotypes, as little work has been done on RASs in genotype 6.

For DCV, we looked for 24R, 28T, 30E/K/T, 31M/V, 32L, 58D and 93C/H/N/R/S/W in genotype 1 infection and additionally looked for 28S, 30R and 31F in genotype 6 infection. For SOF, we looked for 159F, 237G, 282T, 315H/N and 321A/I in genotype 1 infection and additionally looked for 289I in genotype 6 infection (**Ansari et al., 2017; Ansari et al., 2019**).

In addition to viral sequencing, we evaluated host genetic polymorphisms within the interferon lambda 4 (*IFNL4*) gene of all participants at baseline. Genotyping of *IFNL4* rs368234815 was performed on host DNA using the TaqMan SNP genotyping assay and primers described previously (**Prokunina-Olsson et al., 2013**) with Type-it Fast SNP Probe PCR Master Mix (Qiagen).

Pharmacokinetics and pharmacodynamics

To assess pharmacokinetics (PK) and pharmacodynamics (PD), the plasma drug levels of SOF, its inactive metabolite GS-331007, and DCV were measured at baseline, at day 14 and at EOT (day 28 or 56) in all participants. In addition, intensive drug level sampling was conducted in a subset of 40

participants, who were sequentially invited to join an ancillary PK study. In this subgroup, five samples were collected in each participant after the first dose of SOF/DCV and at day 28, according to one of two randomly assigned sampling schedules (A and B). In sampling schedule A, drug levels were measured at 0.5-, 2-, 4-, 6- and 24-hr post-dose; in sampling schedule B, drug levels were measured at 1-, 3-, 5-, 8- and 24-hr post-dose.

Drug quantification was performed using liquid chromatography-tandem mass spectrometer at Mahidol Oxford Tropical Medicine Research Unit, Bangkok. Two separate analytical assays were developed and validated to quantify SOF plus its metabolite GS-331007, and DCV, respectively. Full methodological details of the PK/PD analysis are provided in Appendix 1.

Statistical analysis

Primary and secondary outcomes

Analysis was performed under intention-to-treat (the per-protocol analysis, defined as including all participants taking 90–110% of prescribed treatment, was equivalent to the intention-to-treat analysis) with an additional post hoc analysis excluding those who were non-Gt1/6 from WGS. Where possible, proportions and 95% CIs were estimated from the marginal effects after logistic regression. Where no events were recorded and models would not converge, we used binomial exact 97.5% CIs. Absolute HCV VL was analysed using interval regression (incorporating censoring at the LLOQ) adjusting for baseline HCV VL. Differences between baseline means and medians in 4-week cures versus 4-week failures were analysed with unpaired t-tests and Wilcoxon rank-sum tests, respectively; differences in proportions were assessed using chi-squared tests or Fisher's exact tests as appropriate. Analyses were performed using Stata v16.1 (*StataCorp, 2019*).

Virus genomics

Fisher's exact test was used to test for association between presence and absence of each RAS and treatment outcome. To test for association between outcome and number of RAS, we used logistic regression.

Pharmacokinetics and pharmacodynamics

Intensive drug levels of SOF, its metabolite GS-331007, and DCV from the subset of 40 patients at days 0 and 28, together with any EOT samples at day 28, were analysed using non-compartmental analysis in PKanalix version 2020R1 (*Lixoft, 2022*). Two separate analyses were performed to characterise the pharmacokinetic properties of the study drugs.

In the first, naïve pooled analyses were performed separately on data from days 0 and 28 (not including EOT samples) to derive median pharmacokinetic parameters at each day. In these analyses, the median concentration at each protocol time was calculated. Individual concentration measurements below the LLOQ were set to LLOQ/2 when calculating the median values. It was assumed that the participants had no drug concentrations at time 0.

In the second analysis, data from days 0 and 28 were pooled for each individual. This resulted in a full pharmacokinetic profile for each subject, which was analysed with a non-compartmental approach. The mean value of drug concentrations was used if patients had two or more samples taken at the same time point. These derived individual drug exposures were used to evaluate the relationship between drug exposure and therapeutic outcome. It was assumed that the participants had no drug concentrations at time 0. In this analysis, the first measurement below LLOQ in a series of LLOQ samples was imputed as LLOQ/2 and the later measurements were ignored. In both approaches, SOF samples taken at ≥ 24 hr post-dose were excluded. SOF is a prodrug and has a very short half-life of less than 1 hr, which make concentrations at 24 hr after dose extremely unlikely (*de Kanter et al., 2014*). In addition, outcome variables and the relationship between outcome variables and drug exposure were evaluated. Additional detail of the PK/PD analysis is provided in Appendix 1.

Ethical approval

The trial was approved by the research ethics committees of The Hospital for Tropical Diseases (*Hospital for Tropical Diseases Ethics Committee, 2021*) (ref: CS/BND/18/25), *Vietnam Ministry of Health, 2022* (ref: 6172/QĐ-BYTnam MoH), Imperial College London (*Imperial College Research Ethics Committee, 2018*) (ref: 17IC4238), and Oxford University Tropical Research Ethics

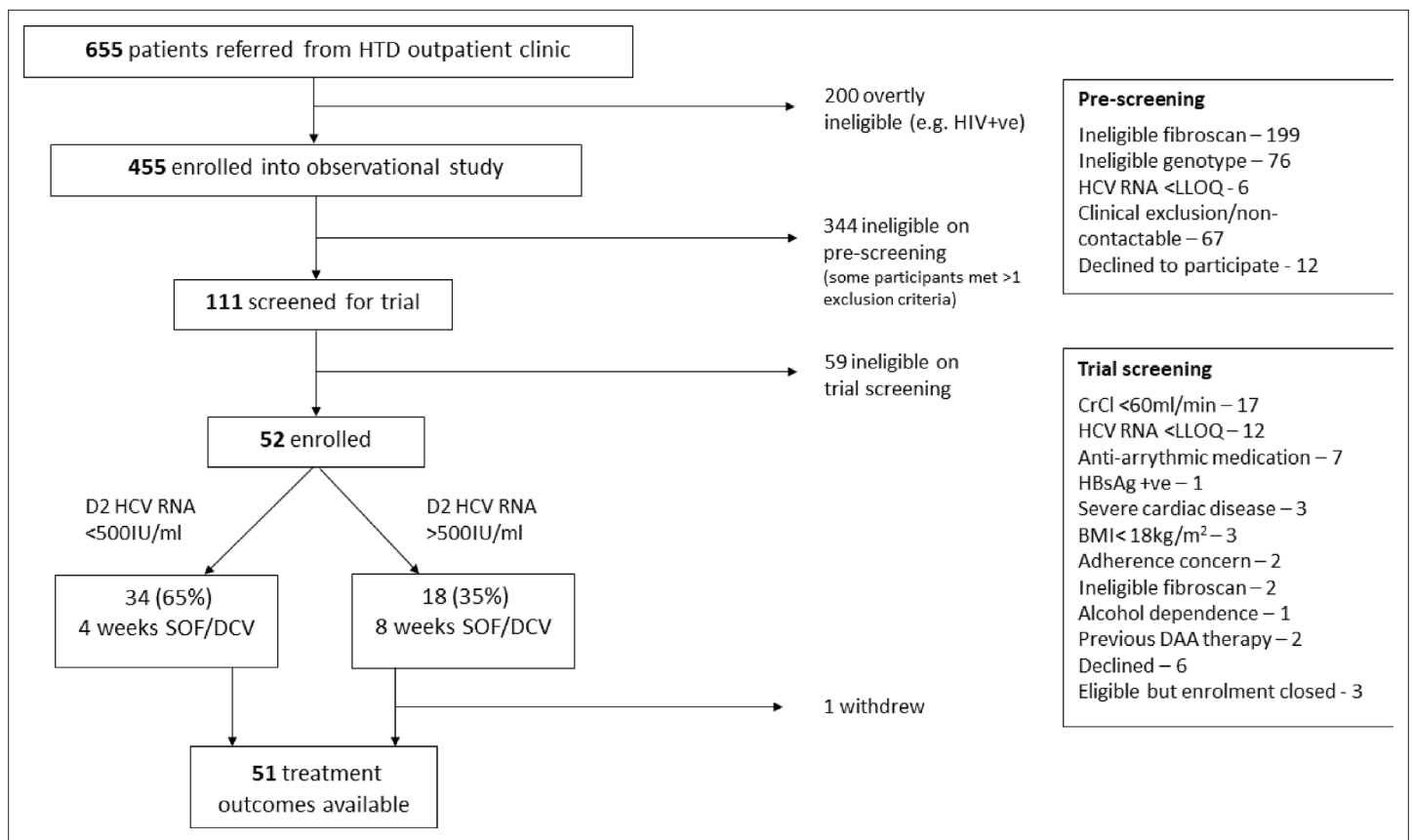


Figure 2. Screening and enrolment.

Committee (*Oxford Tropical Research Ethics Committee, 2018*) (ref: 43-17). The study's conduct and reporting is fully compliant with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (*World Medical Association, 2022*). The trial was registered at ISRCTN, registration number is ISRCTN17100273 (*ISRCTN registry, 2018*).

Results

Baseline characteristics

Of 455 patients screened, 52 were enrolled and 1 subsequently withdrew (*Figure 2*). Most exclusions were on account of either a FibroScan score of >7.1 kPa (with cirrhotic patients enrolled into a parallel study; *Flower et al., 2021*), or ineligible genotype.

22/51 were initially identified as genotype 1 infection and 30 as genotype 6. With the benefit of WGS data, it was confirmed that 22 (43%) had genotype 1 infection, 27 (53%) had genotype 6, 1 had genotype 2 and another had genotype 4 infection. The latter two individuals were included in the intention-to-treat analysis but excluded from a post hoc analysis of G1 and G6 infections only.

Recruitment was completed short of the initial target of 60 due to severe COVID-19-related restrictions in Vietnam from February 2020. These included clinic closures, travel restrictions and repurposing of the HTD as a COVID-19 treatment centre.

Baseline and clinical characteristics are described in *Table 1*. One participant, a male with genotype 1b infection who was cured with 4-week therapy, had an HCV viral load of 618 IU/ml on day 0 which may have been consistent with spontaneously resolving acute infection, but could equally reflect fluctuating viraemia. Baseline viral load was >10,000 IU/ml in all other participants, who were all assumed to have chronic infection.

Table 1. Baseline characteristics.

| | N/ median | %/range |
|---|-----------------|-------------------|
| Total participants | 52 | |
| Age in years | 49.5 | (25.0, 67.0) |
| Female | 29 | (56%) |
| Body-mass index in kg/m ² | 23.3 | (18.7, 30.6) |
| Genotype 1 | 22 | (43%) |
| 1a | 11 | |
| 1b | 12 (1 withdrew) | |
| Genotype 6 | 27 | (53%) |
| 6a | 12 | |
| 6e | 10 | |
| 6h | 2 | |
| 6l | 2 | |
| 6u | 1 | |
| Genotype 2(m) | 1 | |
| Genotype 4(k) | 1 | |
| Baseline HCV viral load in IU/ml | 1,932,775 | (618, 11,200,000) |
| HCV viral load – log ₁₀ IU/ml (range) | 6.3 | (2.8, 7.0) |
| Past medical history: | | |
| Illicit drug use | 4 | (8%) |
| Alcohol dependence (historic; current excluded) | 4 | (8%) |
| Diabetes | 2 | (4%) |
| Hypertension | 7 | (13%) |
| Ischaemic heart disease | 1 | (2%) |
| Tuberculosis | 2 | (4%) |
| Current smoker | 18 | (35%) |
| Previous spontaneous clearance of HCV with re-infection | 2 | (4%) |

9/21 cures and 1/12 treatment failures (one missed visit) had HCV RNA<LLOQ ($p=0.054$; **Table 3**), translating to 90% sensitivity (95% CI [56, 100]) for predicting cure with 4-week treatment. However, by day 10, 9/21 cures and 9/13 failures had HCV RNA<LLOQ ($p=1.00$), making a rapid virological response 50% [26, 74] sensitive in predicting cure with 4-week treatment. HCV RNA kinetics in all participants treated with 4-week SOF/DCV are shown in **Appendix 1—figure 2**, with cures (blue lines) distinguished from those experiencing treatment failure (red lines). Even though the numbers

Treatment duration, adherence and efficacy outcomes

By day 2, 34 participants (65%) had HCV viral load below the threshold of 500 IU/ml (**Figure 2; Table 2**), so received 4-week treatment. Eighteen participants were above the threshold at this time point, of which 1 withdrew after 9 days of treatment, meaning 17 completed 8-week therapy. Adherence was good, with 96% completing the full prescribed course of SOF/DCV (as assessed by self-reporting and physician pill count). Eighteen (35%) participants missed at least one visit because of COVID-19-related restrictions. Of the 51 participants with outcome data, 38 (75% [95% CI (63, 86)]) achieved SVR12 while 13 failed therapy and required retreatment. All treatment failures occurred in individuals who received 4-week therapy, translating to an SVR12 of 62% (21/34; 95% CI (44, 78)) in rapid responders who received 4-week therapy, and 100% (17/17; 97.5% CI (80, 100)) in slower responders who received 8-week SOF/DCV (**Figure 3; Table 2**).

Of the 13 participants who underwent retreatment, 100% were cured. The mean first-line SOF/DCV treatment duration was 37 days (standard deviation, SD 13.7), with a first-line cure rate of 75%. The mean (SD) total SOF/DCV duration (i.e., including 12-week retreatment where required), was 58 (34.2) days per patient, with a 100% cure rate. There was no evidence of differences in age, gender, BMI, IFNL4 genotype, transaminases or baseline HCV viral load between patients who achieved cure with 4-week treatment versus those who experienced treatment failure with 4-week treatment (**Table 3**).

Viral kinetics and timing of treatment failure

All participants had an initial virological response (i.e., ≥ 1 log₁₀ decrease in HCV viral load from baseline) (**Appendix 1—figures 1 and 2**). There was no evidence of association between time to complete virological suppression (HCV RNA<LLOQ) and treatment outcome (**Table 3; Appendix 1—figures 2 and 4**). In an exploratory analysis, we estimated first-line cure rates based on suppression below the LLOQ at other time points, which could be used for RGT. At day 7,

Table 2. Treatment outcome.

| | N/median | %/range |
|---|----------|-----------------------|
| Detectable HCV viral load (HCV VL) at day 2 | 50 | 96% |
| Abbott | 39/41 | 95% |
| COBAS | 11/11 | 100% |
| Median (IQR) HCV VL at day 2 in IU/ml | 269 | (104, 690) |
| Abbott | 217 | (101, 690) |
| COBAS | 459 | (209, 832) |
| Below threshold—for 4-week therapy | 34 | (65%) |
| Abbott | 31 | (66%) |
| COBAS | 3 | (60%) |
| Above threshold—for 8-week therapy | 18 | (35%) |
| Abbott | 16 | (34%) |
| COBAS | 2 | (40%) |
| Mean (SD) duration of first-line therapy received in days | 37 | (13.7) |
| Mean (SD) duration of all therapy received in days | 58 | (34.2) |
| Median weeks from enrolment to last visit (range) | 20 | (1, 42) |
| Primary outcome | | |
| Outcome available | 51 | |
| SVR12 by intention-to-treat analysis and per protocol analysis | 38 | (75% [95% CI 63, 86]) |
| SVR12 by sensitivity analysis (i) [missing results = failure] | 38 | (73% [95% CI 61, 85]) |
| SVR12 by post hoc analysis (ii) [G1 and G6 only] | 37 | (76% [95% CI 63, 88]) |
| Secondary endpoints | | |
| Lack of initial virological response | 0 | (0% [97.5% CI 0, 7]) |
| Serious adverse events | 0 | (0% [97.5% CI 0, 7]) |
| Grade 3/4 clinical adverse events | 0 | (0% [97.5% CI 0, 7]) |
| Non-serious adverse reactions | 18 | (35% [95% CI 22, 48]) |
| Adverse events or reactions leading to change in study medication | 0 | (0% [97.5% CI 0, 7]) |

Where not labelled, data presented as n (%; 97.5% confidence interval).

are small, this helps illustrate that early on-treatment response alone may be of limited value in determining cure with ultra-short therapy.

Since the two HCV assays used in our study have previously been shown to yield different HCV RNA results in the same individuals on therapy (*Maasoumy et al., 2016*), we conducted additional analyses of viral kinetics stratified by platform. We found no evidence of a difference between platforms in terms of proportion of participants with undetectable viral load at different time points (**Table 2**, **Table 3**), or in terms of first phase (days 0 to 2) or second phase (day 2 to first HCV RNA<LLOQ) viral decline on treatment (**Appendix 1—figure 3**). However, numbers were small meaning we may have lacked power to detect effects.

All treatment failures occurred during follow-up after EOT. Despite intensive twice weekly sampling from EOT to EOT +28d, the earliest virologic rebound occurred 3 weeks after completion of therapy (**Appendix 1—figure 5**). Pseudo-anonymised raw viral load data from this study is available in **Source data 1**.

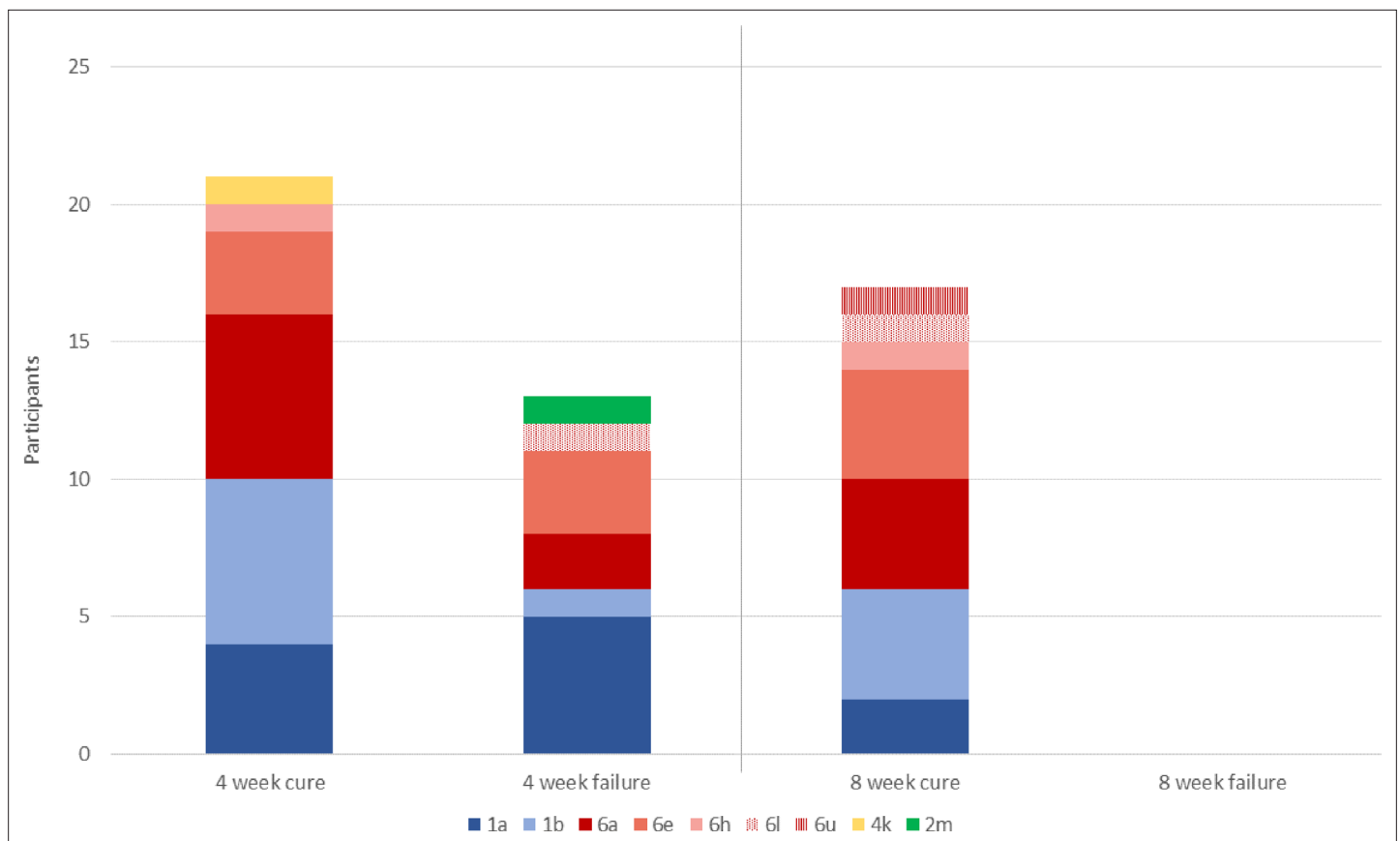


Figure 3. Primary outcome, with HCV subtypes (n=51). All 13 individuals who experience treatment failure with 4-week SOF/DCV were cured with 12-week SOF/DCV retreatment.

Viral genomics at baseline

WGS was attempted on all participants' virus at baseline, but consensus sequences could not be assembled in two individuals (who had low baseline viral load and were both cured with first-line therapy). This left 50 patients with baseline sequences, of which 49 had outcome data.

We found nine discrepancies between lab genotyping and sequencing-based genotyping. Five of these differences were at the level of subtypes for genotype 6 samples, highlighting difficulties inherent in classifying this rare and genetically diverse lineage using an amplicon approach for genotyping (lab genotyping). Two samples were called 6a/e using lab genotyping and WGS classified them as 6e. One sample was classified as 6e on lab genotyping, but WGS showed that it was a genotype 2m sample. WGS revealed another patient to have mixed infection with genotype 1a and genotype 6a; this had been classified by laboratory genotyping as a genotype 6a mono-infection. The individual with mixed infection received 4-week SOF/DCV but cure was not achieved, with relapse of the genotype 1a infection. They subsequently responded to 12-week retreatment.

We found no evidence of differences between genotypes or subtypes with regard to rates of treatment failure. Among genotype 1-infected individuals, 1/7 subtype 1b infections experienced treatment failure with 4-week therapy compared with 4/8 subtype 1a infections (including the mixed infection case) ($p=0.15$). Among genotype 6-infected individuals, 1/8 subtype 6a infections were not cured with 4-week SOF/DCV compared with 3/6 subtype 6e ($p=0.58$), 0/1 subtype 6h and 1/1 subtype 6l.

At baseline, the 159F SOF RAS was identified in one patient, and the 237G putative SOF RAS was identified in six patients (**Appendix 1—figures 6 and 7**). The DCV RAS 24R, 30R, 31M, 93H and 93S were detected at baseline (**Appendix 1—figures 8 and 9**).

In the assessment of SOF RAS (**Appendix 1—figure 6**), one patient who had 159F at baseline failed treatment, although this was a minority variant making up 20% of the sequencing reads (**Figure 4**).

Table 3. Comparison of baseline factors, drugs levels and virological response in individuals failed to achieve SVR12 with 4-week therapy versus those who cured with 4- or 8-week therapy.

| | 4-week cures (n=21) | 4-week failures (n=13) | p | 8-week cures (n=17) |
|---|------------------------|---------------------------|-------------------|---------------------|
| Host factors | | | | |
| Male (%) | 62% | 38% | 0.18 | 29% |
| Mean age | 45 | 48 | 0.23 | 55 |
| Mean BMI | 23 | 23 | 0.40 | 24 |
| Median ALT | 54 | 36 | 0.10 | 31 |
| Median AST | 34 | 28 | 0.44 | 33 |
| IFNL4 delG/TT and TT/TT genotypes (rs368234815) | 71% | 58% | 0.47 | 69% |
| Virus factors | | | | |
| Median D0 HCV VL | 916,000 | 2,139,258 | 0.20 | 4,982,889 |
| Abbott | 960,913 | 1,972,841 | 0.47 | 4,625,118 |
| COBAS | 916,000 | 5,260,000 | 0.40 | 4,605,000 |
| D2 VL<LLOQ | 2/21 (10%) | 0/13 (0%) | 0.51 | 0% |
| Abbott | 2/18 (11%) | 0/10 (0%) | 0.41 | 0/13 (0%) |
| COBAS | 0/3 (0%) | 0/3 (0%) | – | 0/5 (0%) |
| D7 VL<LLOQ | 9/21 (43%) | 1/12 (8%)* | 0.054 | 0% |
| Abbott | 8/18 (44%) | 1/9 (11%) | 0.09 | 0/13 (0%) |
| COBAS | 1/3 (33%) | 0/3 (0%) | 1.00 | 0/5 (0%) |
| D10 VL<LLOQ | 9/21 (43%) | 9/13 (69%) | 0.17 | 6% |
| Abbott | 8/17 (47%) | 8/10 (80%) | 0.12 | 1/10 (10%) |
| COBAS | 1/4 (25%) | 1/3 (33%) | 1.00 | 0/6 (0%) |
| D14 VL<LLOQ | 14/21 (68%) | 9/13 (69%) | 1.00 | 18% |
| Abbott | 11/16 (69%) | 6/9 (67%) | 1.00 | 1/11 (18%) |
| COBAS | 2/4 (50%) | 3/4 (75%) | 1.00 | 1/6 (17%) |
| HCV genotype 1 | 10/21 (48%) | 6/13 (46%) | 1.00 (vs Gt 6) | 6/17 (35%) |
| 1a | 4/21 (19%) | 5/13 (38%) | 0.15 (vs 1b) | 2/17 (12%) |
| 1b | 6/21 (24%) | 1/13 (8%) | | 4/17 (24%) |
| HCV genotype 6 | 10/21 (48%) | 6/13 (46%) | | 11/17 (65%) |
| 6a | 6/21 (29%) | 2/13 (15%) | 0.58 (vs. 6e) | 4/17 (24%) |
| 6e [†] | 3/21 (14%) | 3/13 (23%) | | 4/17 (24%) |
| Resistance-associated substitutions | | | | |
| Median (range) SOF-RAS | 0 (0–1) | 0 (0–2) | 0.76 | 0 (0–1) |
| Median (range) DCV-RAS | 2 (0–2) | 1 (0–2) | 0.17 | 2 (0–4) |
| Median (range) SOF- & DCV-RAS combined | 2 (0–3) | 2 (1–2) | 0.12 | 2 (0–4) |
| Drug exposure (n=37) § | n=15 | n=8 | | n=14 |

Table 3 continued on next page

Table 3 continued

| | 4-week cures (n=21) | 4-week failures (n=13) | p | 8-week cures (n=17) |
|---|----------------------|------------------------|-------|------------------------|
| Median AUC _{last} , SOF (h×ng/ml) [‡] | 2360 (1120–4550) | 2220 (937–3910) | 0.975 | 2120 (1430–2610) |
| Median AUC _{last} , GS-331007 (h×ng/ml) [‡] | 11,700 (8420–14,100) | 15,100 (9240–19,700) | 0.023 | 14,000 (10,200–17,400) |
| Median AUC _{last} , DCV (h×ng/mL) [‡] | 13,000 (6800–22,300) | 13,200 (6630–27,000) | 0.728 | 14,200 (9210–17,000) |

Results presented as median (5th–95th percentile).

*n=12, no HCV VL data for one participant’s day 7 visit.

[†]h, l and u subtypes excluded from the table/analysis due to small numbers (≤2).

[‡]AUC_{last} is the total exposure to the last time point (8 hr for SOF and 24 hr for GS-331007 and DCV).

[§]Complete d0 and d28 data only available for 37 participants.

237G was identified as a majority variant in two individuals where treatment failed but was also seen in four individuals who were cured (three received 4-week treatment; **Appendix 1—figure 6**).

The most prevalent DCV RAS was 31M, present in nine participants where treatment failed after 4-week first-line therapy (**Figure 4; Appendix 1—figures 8 and 9**). However, 31M was also found in 13 individuals cured with 4-week treatment, and 13 cured with 8 weeks. The next most prevalent RAS was 30R, present at baseline in three patients who had treatment failure, in five individuals cured with 4-week treatment and in four patients cured with 8-week treatment. 30R RAS was present in 11/12 6a genomes and 1/1 2m genomes but was absent in other subtypes. 31M RAS was present in 10/11 1a genomes and 12/12 6a genomes and was also found in other subtypes (**Appendix 1—figures 8 and 9**). Additionally, almost all of the subtype 6a samples carried both 30R and 31M RASs while other subtypes did not carry this combination (apart from the 2m sample).

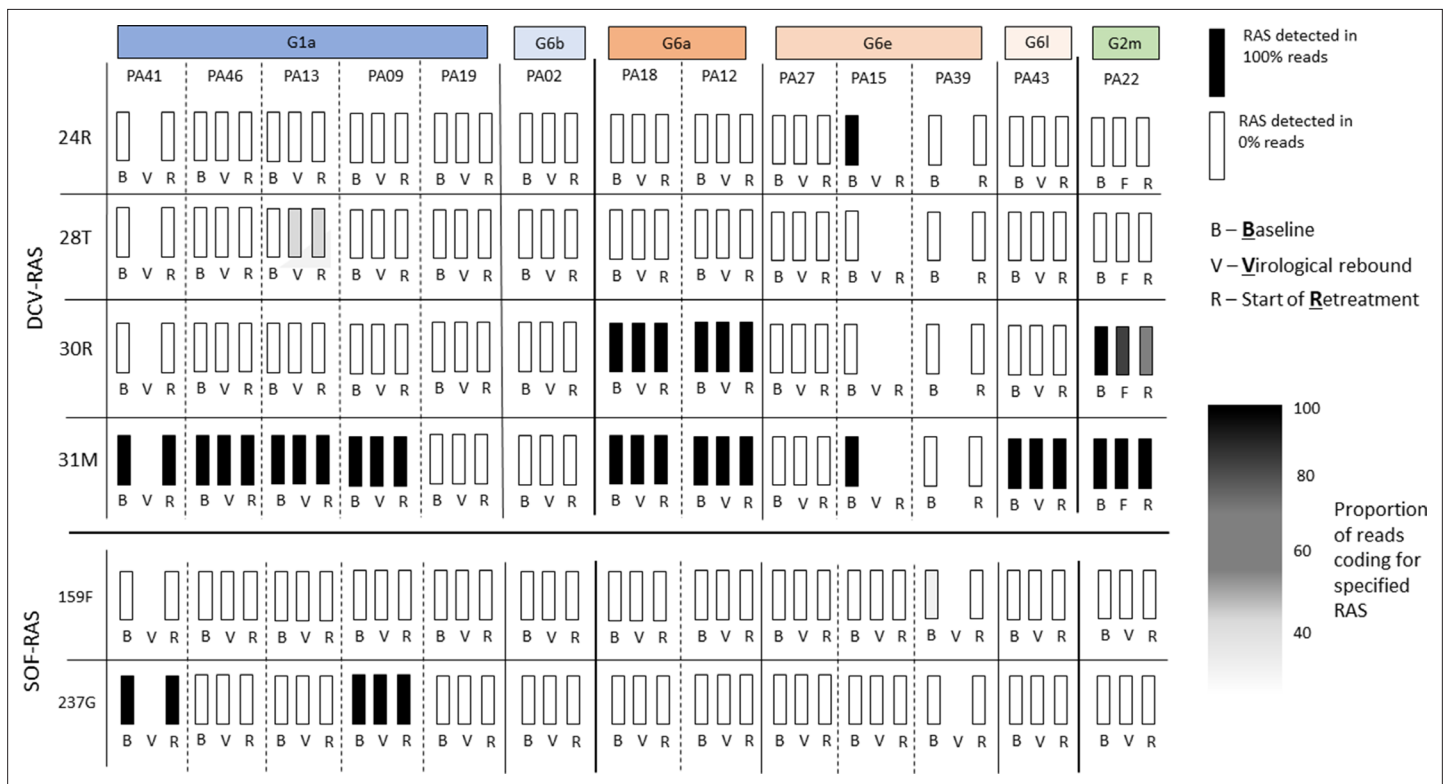


Figure 4. Sofosbuvir RAS and Daclatasvir RAS at baseline, treatment failure, and at start of retreatment in all participants who failed first-line treatment.

Viral genomics in participants failing first-line therapy

Among 13 individuals who experienced treatment failure, we compared the emerging viral genome with baseline virus (**Figure 4**). Full genome sequences could not be assembled for three participants at time of virological relapse; however, we were able to generate whole viral genomes using samples from the start of retreatment for two of these individuals. No new genomes were identified at treatment failure (ruling out any new infections). No new SOF RAS were identified on virologic rebound. DCV 28T RAS (not present at baseline) was identified in one participant failing therapy (**Figure 4; Appendix 1—figure 11**) as a minority variant at time of virological rebound and start of retreatment (at 30% and 25% of reads, respectively). Given 100% of retreated individuals achieved SVR12 with standard duration of therapy, we found no evidence to suggest this emerging RAS was clinically significant. There was no evidence of differences in the number of combined SOF- and DCV-RAS at baseline in those who failed 4-week therapy (median 2, range 0–3) versus those who cured with 4 weeks (median 2, range 1–2) ($p=0.12$), or in those with a slower initial virological response, who received 8 weeks (median 2, range 0–4).

Pharmacokinetics and pharmacodynamics

Pharmacokinetic parameters derived from the naïve pooled analysis (based on 40 patients on day 0 and 37 patients on day 28) are presented in **Appendix 1—table 1**. Exposure after the individual analysis as well as outcome measurements are presented in **Appendix 1—table 2**. In the individual analysis and the linear regression between outcome measurements and drug exposure, three patients were excluded as they did not have dense samples collected at day 28 ($n=37$). In the analysis of outcome variables, data from all 40 patients were used. No significant relationship between outcome variables and drug exposure was found using linear regression (**Appendix 1—table 3**).

In the subset of 37 patients who underwent dense PK analysis at days 0 and 28, 23 patients received 4-week SOF/DCV and 14 patients received 8-week therapy. There was no significant difference between total drug exposure (AUC_{last}) for SOF and DCV in 4-week cures ($n=15$) versus 4-week failures ($n=8$) (**Table 3**). However, GS-331007 exposures were significantly higher in the patient group with treatment failures ($p=0.023$).

Safety

SOF/DCV was well-tolerated, and no participants discontinued treatment due to drug side effects. Eighteen participants (35%; 95% CI 22%, 48%) reported at least one non-serious AR. The most common of these were insomnia, gastritis and dizziness, which are all consistent with undesirable effects described in the summary of product characteristics of SOF/DCV (**EMA, 2014**). There were no SAEs or grade 3 or 4 AEs.

Discussion

In this mechanistic study in individuals with genotype 1 or 6 HCV infection and mild liver disease, treated with 4- or 8-week SOF/DCV depending on HCV viral load 2 days after starting treatment, first-line cure rate was 75% [95% CI (63, 86)], with a mean of 37 days treatment. This saved 47 days of DAA therapy per participant compared with a standard 12-week course, but cure rate fell below our target of $\geq 90\%$. For the secondary endpoint—SVR12 after combined first-line therapy or retreatment—cure was 100%, with mean treatment duration of 58 days, saving 26 days DAAs per participant.

Effect of shortening therapy

Inferior rates of cure are well described when DAA therapy is shortened below 8 weeks without use of early on-treatment virological response, falling below 50% with ≤ 4 weeks therapy without stratification (**Jones et al., 2019; Emmanuel et al., 2017; Cooke and Pett, 2021; Sulkowski et al., 2017**). A few small studies have reported high rates of cure with shortened therapy based early virological response (**Lau et al., 2016; Etzion et al., 2020; El-Shabrawi et al., 2018**;). The only previous RGT study to use less than 6-week treatment, by Lau et al., found a cure rate of 100% with just 3-week DAA therapy in 18 individuals whose HCV viral load was suppressed below 500 IU/ml after 2 days of therapy. This was the same threshold and time point used in our study. One important difference was in the treatment regimen, which included a protease inhibitor (simeprevir or asunaprevir). Although

NS5A- (DCV) and NS5B- (SOF) inhibitors rapidly eliminate HCV from the blood, second-phase decline in viral load appears to be enhanced by addition of a protease inhibitor (*Perelson and Guedj, 2015*). This may be crucial in sustaining high rates of cure with shortened therapy. Viral kinetics in our participants were broadly similar to those observed in patients treated with DCV-containing regimens in the study by Lau et al., with a rapid first-phase viral decline leading to an approximate 4 log₁₀ IU/ml decline in HCV RNA in the first 48 hr of treatment. However, a detailed comparison of viral kinetics is limited by differences in sampling schedule, baseline viral loads and the HCV PCR platforms used. Another key difference relates to infecting genotypes—all participants in the Lau study had genotype 1b infection, compared with just 23% (n=12) in ours. Genotype 1b is associated with favourable outcomes with some DAAs compared with other genotypes (*Ferenci et al., 2014; Kohli et al., 2015*;). Although real-world 1b outcomes with standard duration SOF/DCV appear similar to other non-3 genotypes (*Charatcharoenwittaya et al., 2020*;), subtype may be more important when treatment is shortened.

Role for RGT with SOF/DCV

Cure rates with this strategy are too low for it to be recommended routinely. With standard duration therapy, SVR12 is known not to be impacted by time to first undetectable HCV RNA (*Kowdley et al., 2016*) or by the presence of detectable virus at the EOT (*Pal et al., 2020*). This also appears to be true of shortened treatment: in one individual who experienced treatment failure, HCV viral load was already <LLOQ by day 7; in five of the 4-week cures, HCV VL was only suppressed to <LLOQ virus for the first time at EOT (*Appendix 1—figure 4*). Comparison of 4-week cures and 4-week treatment failures indicates that an HCV RNA<LLOQ by day 7 may be a useful discriminator of 4-week treatment outcome (p=0.054). However, day 10, HCV RNA<LLOQ was not predictive of response to shortened treatment. Day 7 viral load thresholds for shortening DAA therapy are currently being evaluated as part of a large ongoing randomised controlled trial in Vietnam (*McCabe et al., 2020*;).

A case for 8-week SOF/DCV therapy

Given the high rates of cure observed with 8-week SOF/DCV in participants with a slow initial virological response (17/17), there is a case for reducing SOF/DCV therapy from 12 to 8 weeks in individuals with mild liver disease. Prior evidence for caution regarding 8-week SOF/DCV comes predominantly from a small 2015 study in HIV-coinfected individuals (*Wyles et al., 2015*), in which 7/10 treatment failures in the 8-week arm received half-dose daclatasvir (30 mg) because participants were taking concomitant darunavir–ritonavir. This dose adjustment was subsequently deemed unnecessary once drug-interaction data emerged, such that this study is likely to underestimate the efficacy of 8-week SOF/DCV. More recent studies corroborate our finding of >90% cure with 8 weeks NS5A/NS5A inhibitor combination (*Yakoot et al., 2017; El-Shabrawi et al., 2018; Boyle et al., 2020*). Larger trials are warranted to evaluate 8-week SOF/DCV therapy for patients with mild liver disease (irrespective of speed of virological response). This could save significant costs, particularly in countries where pricing is determined per pill rather than per treatment course, such as Vietnam, and the USA (*Emmanuel et al., 2017; Clinton Health Access Initiative, 2021*).

Impact of RASs and retreatment concerns

To our knowledge, this study is the largest assessment of G6 RAS in vivo with SOF/DCV therapy. We hypothesised that a high number of putative RAS at baseline may be associated with higher rates of failure with shortened treatment. However, we found no evidence that number or type of SOF- or DCV-RAS was different at baseline in 4-week cures compared with 4-week treatment failures (*Table 3, Appendix 1—figures 6 and 8*), although numbers were small. Additionally, the excellent retreatment outcomes observed (13/13) are reassuring, particularly for low-resource settings where protease inhibitor-based retreatment options are limited. Only one novel RAS was detected after first-line treatment failure, and the individual concerned achieved SVR with standard duration retreatment, suggesting this was not clinically relevant.

Impact of drug levels

This was the first assessment of the impact of DAA drug levels on efficacy of shortened therapy. The inactive SOF metabolite GS-331007 is the main circulating metabolite of SOF prior to undergoing

renal excretion, and it is frequently used to describe SOF's pharmacokinetics (*Smolders et al., 2019*). We hypothesised that accumulation and slow elimination of GS-331007 and DCV in vivo might protect against the re-emergence of HCV viraemia. However, we found no evidence of a difference in AUC_{last} between 4-week cures and 4-week treatment failures for SOF or DCV. Total exposure to GS-331007 was higher in treatment failures (15,100 [9240–19,700] vs. 11,700 [8420–14,100] [$p=0.023$]). This was a surprising result, given that SOF and GS-331007 AUCs are near dose proportional over the dose range of 200–1200 mg (*Smolders et al., 2019*), and higher day 10 concentrations of GS-331007 have been associated with improved rates of cure with SOF/ribavirin treatment (*Ahmed et al., 2019*). Further PK studies are warranted to better understand if SOF metabolism impacts treatment outcomes.

Limitations

Our study has important limitations. First, it was powered to determine the overall cure rate with 4- and 8-week treatment, rather than outcomes with each duration. It is possible that we would have seen patients failing 8-week therapy with a larger sample, and our cure estimates may therefore be imprecise. Second, the participating cohort did not include any individuals with HIV, hepatitis B co-infection or renal impairment and only four participants reported a history of injecting drug use, of which none were currently injecting. These populations are known to have an altered immunological response and constitute an important part of the HCV epidemic. Third, in order to identify the timing of failure, the protocol required a visit schedule with many more visits than is standard of care, which many patients would not be able to follow. Consequently, adherence was very high, which may not reflect real-world practice.

Another potential limitation relates to our use of two different HCV RNA platforms which have previously been shown to give discrepant results in the same individuals (*Dahari et al., 2016*). The Abbott Architect has a lower LLOQ than the COBAS AmpliPrep/COBAS TaqMan and may detect HCV RNA for longer on therapy than the COBAS (*Maasoumy et al., 2016*), though we found no evidence of difference in viral decline by platform. With regard to the PK analysis our non-compartmental analysis of drug levels may not adequately account for drug accumulation of sofosbuvir's metabolite GS-331007 and DCV between days 0 and 28, which was observed (see Appendix 1 for more detail).

In summary, our findings indicate that shortened SOF/DCV therapy cures a significant proportion of patients with mild liver disease without compromising retreatment with the same drug combination in those who fail first-line therapy. This study adds to a growing case for shortening SOF/DCV therapy in individuals with mild liver disease from 12 to 8 weeks, offering retreatment with 12-week SOF/DCV when required. There was no evidence that relatively high numbers of putative RASs at baseline were associated with treatment outcomes, suggesting routine sequencing at baseline or prior to retreatment remains unnecessary. We also found no evidence that drug levels affect virological response or influence treatment outcome. Further work is required to understand which factors predict cure with ultra-short DAA treatment.

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Additional information

Competing interests

Leanne McCabe: has received a grant from the Medical Research Council UK to the MRC Clinical Trials Unit [MC_UU_00004/03]. The author has no other competing interests to declare. Sarah L Pett: has received grants from Gilead Sciences, ViiV Healthcare, Janssen-Cilag, Academy of Medical Sciences, EDCTP, NIHR, NIH, and is a member of the TIPAL (Treating people with idiopathic pulmonary fibrosis with the addition of lansoprazole trial, ISRCTN13526307) DSMB. The author has no other competing interests to declare. Graham S Cooke: is a board member of MHRA. The author has no other competing interests to declare. The other authors declare that no competing interests exist.

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Author contributions

Barnaby Flower, Data curation, Formal analysis, Supervision, Validation, Investigation, Visualization, Writing – original draft, Project administration, Writing – review and editing; Le Manh Hung, Conceptualization, Supervision, Project administration, Writing – review and editing; Leanne McCabe, Software, Formal analysis, Investigation, Methodology, Writing – original draft, Project administration, Writing – review and editing; M Azim Ansari, Resources, Data curation, Software, Formal analysis, Validation, Investigation, Methodology, Writing – original draft; Chau Le Ngoc, Formal analysis, Validation, Methodology, Project administration; Thu Vo Thi, Hang Vu Thi Kim, Investigation, Project administration, Writing – review and editing; Phuong Nguyen Thi Ngoc, Investigation, Methodology, Project administration; Le Thanh Phuong, Resources, Supervision, Project administration, Writing – review and editing; Vo Minh Quang, Tran Nguyen Bao, Rogier van Doorn, Resources, Supervision, Project administration; Thuan Dang Trong, Supervision, Project administration, Writing – review and editing; Thao Le Thi, Jennifer Ilo Van Nuil, Supervision, Project administration; Cherry Kingsley, Resources, Project administration, Writing – review and editing; David Smith, Resources, Methodology; Richard M Hoglund, Resources, Software, Validation, Investigation, Methodology, Writing – original draft, Writing – review and editing; Joel Tarning, Resources, Formal analysis, Validation, Methodology; Evelyne Kestelyn, Supervision, Validation, Project administration; Sarah L Pett, Supervision, Visualization, Methodology, Writing – review and editing; Hugo Turner, Conceptualization, Supervision, Writing – review and editing; Guy E Thwaites, Conceptualization, Resources, Supervision, Funding acquisition, Project administration, Writing – review and editing; Eleanor Barnes, Conceptualization, Resources, Data curation, Formal analysis, Supervision, Investigation, Visualization, Methodology, Writing – review and editing; Motiur Rahman, Resources, Supervision, Methodology, Project administration; Ann Sarah Walker, Conceptualization, Data curation, Formal analysis, Supervision, Funding acquisition, Validation, Investigation, Visualization, Methodology, Project administration, Writing – review and editing; Jeremy N Day, Conceptualization, Data curation, Formal analysis, Supervision, Validation, Investigation, Methodology, Project administration, Writing – review and editing; Nguyen VV Chau, Resources, Supervision, Funding acquisition; Graham S Cooke, Conceptualization, Data curation, Supervision, Funding acquisition, Validation, Investigation, Visualization, Methodology, Project administration, Writing – review and editing

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Patients referred to the trial were initially enrolled into an observational study which included fibroscan assessment and genotyping. Individuals in this cohort found to be potentially eligible for the trial were invited for further screening. All patients provided written informed consent. The trial was approved by the research ethics committees of The Hospital for Tropical Diseases (ref: CS/BND/18/25), Vietnam Ministry of Health (ref: 6172/QĐ-BYTnam MoH), Imperial College London (ref: 17IC4238), and Oxford University Tropical Research Ethics Committee (ref: 43-17). The study's conduct and reporting is fully compliant with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. The trial was registered at ISRCTN, registration number is ISRCTN1710027336.

Decision letter and Author responseDecision letter <https://doi.org/10.7554/eLife.81801.sa1>Author response <https://doi.org/10.7554/eLife.81801.sa2>**Additional files****Supplementary files**

- MDAR checklist
- Source data 1. Pseudo anonymised HCV RNA data for every participant.

Data availability

The study protocol and processed study data have been uploaded to the ISRCTN registry (ISRCTN17100273; <https://doi.org/10.1186/ISRCTN17100273>). The data are available under unrestricted access. The raw, pseudo-anonymised viral load data is available in Source Data File 1. The virus sequencing dataset has been uploaded to Dryad (<https://datadryad.org>) and is available here: <https://doi.org/10.5061/dryad.x0k6djhnj>. All data generated in this study is provided in the main text, appendix 1 and Source Data File 1.

The following datasets were generated:

| Author(s) | Year | Dataset title | Dataset URL | Database and Identifier |
|---|------|---|---|---|
| Cooke GS, Day JN, Walker AS | 2022 | A pilot study to assess shortened therapy for hepatitis C infected adults in Vietnam | https://doi.org/10.1186/ISRCTN17100273 | ICRCTN, 10.1186/ISRCTN17100273 |
| Flower B, Hung L, McCabe L, Ansari M, Ngoc C, Thi T, Thi Kim H, Thi Ngoc P, Phuong L, Quang V, Trong T, Thi T, Bao T, Kingsley C, Smith D, Hoglund R, Tarning J, Kestelyn E, Pett S, van Doorn R, Nuil J, Thwaites G, Barnes E, Rahman M, Walker A, Day J, Vinh Chau N, Cooke G | 2022 | Ultra-short response-guided Hepatitis C treatment with sofosbuvir and daclatasvir: the SEARCH study HCV sequence data | https://doi.org/10.5061/dryad.x0k6djhnj | Dryad Digital Repository, 10.5061/dryad.x0k6djhnj |

References

- Ahmed B, Munir B, Ghaffar A, Yameen M, Jalal F, Farooq MU, Shehzadi S, Ashraf S, Liaqat A. 2019. Pharmacokinetics profile of serum and cellular sofosbuvir along with its concentration effect analysis in HCV patients receiving sofosbuvir and ribavirin. *Pakistan Journal of Pharmaceutical Sciences* **32**:1269–1274 PMID: [31303600](https://pubmed.ncbi.nlm.nih.gov/31303600/).
- Ansari MA, Pedergrana V, L C Ip C, Magri A, Von Delft A, Bonsall D, Chaturvedi N, Bartha I, Smith D, Nicholson G, McVean G, Trebes A, Piazza P, Fellay J, Cooke G, Foster GR, Hudson E, McLauchlan J, Simmonds P, Bowden R, et al. 2017. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. *Nature Genetics* **49**:666–673. DOI: <https://doi.org/10.1038/ng.3835>, PMID: [28394351](https://pubmed.ncbi.nlm.nih.gov/28394351/)
- Ansari MA, Aranday-Cortes E, Ip CL, da Silva Filipe A, Lau SH, Bamford C, Bonsall D, Trebes A, Piazza P, Sreenu V, Cowton VM, Hudson E, Bowden R, Patel AH, Foster GR, Irving WL, Agarwal K, Thomson EC, Simmonds P, Klenerman P, et al. 2019. Interferon lambda 4 impacts the genetic diversity of hepatitis C virus. *eLife* **8**:e42463. DOI: <https://doi.org/10.7554/eLife.42463>, PMID: [31478835](https://pubmed.ncbi.nlm.nih.gov/31478835/)
- Boyle A, Marra F, Peters E, Datta S, Ritchie T, Priest M, Heydtmann M, Barclay ST. 2020. Eight weeks of sofosbuvir/velpatasvir for genotype 3 hepatitis C in previously untreated patients with significant (F2/3) fibrosis. *Journal of Viral Hepatitis* **27**:371–375. DOI: <https://doi.org/10.1111/jvh.13239>, PMID: [31756019](https://pubmed.ncbi.nlm.nih.gov/31756019/)
- Bradshaw D, Mbisa JL, Geretti AM, Healy BJ, Cooke GS, Foster GR, Thomson EC, McLauchlan J, Agarwal K, Sabin C, Mutimer D, Moss P, Irving WL, Barnes E, Hepatitis C Trust, UK. 2019. Consensus recommendations for resistance testing in the management of chronic hepatitis C virus infection: public health england HCV resistance group. *The Journal of Infection* **79**:503–512. DOI: <https://doi.org/10.1016/j.jinf.2019.10.007>, PMID: [31629015](https://pubmed.ncbi.nlm.nih.gov/31629015/)
- Charatcharoenwittaya P, Wongpaitoon V, Komolmit P, Sukeepaisarnjaroen W, Tangkijvanich P, Piratvisuth T, Sanpajit T, Sutthivana C, Bunchorntavakul C, Sobhonslidsuk A, Chonprasertsuk S, Siripattanamongkol C, Sethasine S, Tanwandee T, THASL Collaborating Group for the Study of the Use of Direct-acting Antivirals for Chronic Hepatitis C. 2020. Real-world effectiveness and safety of sofosbuvir and nonstructural protein 5A inhibitors for chronic hepatitis C genotype 1, 2, 3, 4, or 6: a multicentre cohort study. *BMC Gastroenterology* **20**:47. DOI: <https://doi.org/10.1186/s12876-020-01196-0>, PMID: [32138687](https://pubmed.ncbi.nlm.nih.gov/32138687/)
- Clinton Health Access Initiative. 2021. HCV Market Intelligence Report 2021 and Preliminary HBV Market Insights. Hepatitis C Market. <https://www.clintonhealthaccess.org/news/hepatitis-c-report-2021/>
- Cooke GS, Pett SML. 2021. Strategic treatment optimization for HCV (STOPHCV1): a randomised controlled trial of ultrashort duration therapy for chronic hepatitis C. *Wellcome Open Res* **6**:93. DOI: <https://doi.org/10.12688/wellcomeopenres.16594.2>
- Dahari H, Halfon P, Cotler SJ. 2016. Resurrection of response-guided therapy for sofosbuvir combination therapies. *Journal of Hepatology* **65**:462–464. DOI: <https://doi.org/10.1016/j.jhep.2016.05.028>, PMID: [27242315](https://pubmed.ncbi.nlm.nih.gov/27242315/)
- de Kanter CTMM, Drenth JPH, Arends JE, Reesink HW, van der Valk M, de Knegt RJ, Burger DM. 2014. Viral hepatitis C therapy: pharmacokinetic and pharmacodynamic considerations. *Clinical Pharmacokinetics* **53**:409–427. DOI: <https://doi.org/10.1007/s40262-014-0142-5>, PMID: [24723109](https://pubmed.ncbi.nlm.nih.gov/24723109/)
- El-Shabrawi MH, Abdo AM, El-Khayat HR, Yakoot M. 2018. Shortened 8 weeks course of dual sofosbuvir/daclatasvir therapy in adolescent patients, with chronic hepatitis C infection. *Journal of Pediatric Gastroenterology and Nutrition* **66**:425–427. DOI: <https://doi.org/10.1097/MPG.0000000000001838>, PMID: [29135824](https://pubmed.ncbi.nlm.nih.gov/29135824/)
- EMA. 2014. Daklinza (SOF/DCV) summary of product characteristics. *European Medicines Agency* **1**:e1.
- Emmanuel B, Wilson EM, O'Brien TR, Kottlil S, Lau G. 2017. Shortening the duration of therapy for chronic hepatitis C infection. *The Lancet. Gastroenterology & Hepatology* **2**:832–836. DOI: [https://doi.org/10.1016/S2468-1253\(17\)30053-5](https://doi.org/10.1016/S2468-1253(17)30053-5), PMID: [28802815](https://pubmed.ncbi.nlm.nih.gov/28802815/)
- Etzion O, Dahari H, Yardeni D, Issachar A, Nevo-Shor A, Cohen-Naftaly M, Ashur Y, Uprichard SL, Arbib OS, Munteanu D, Braun M, Cotler SJ, Abufreha N, Keren-Naus A, Shemer-Avni Y, Mor O, Murad J, Novack V, Shlomai A. 2020. Response guided therapy for reducing duration of direct acting antivirals in chronic hepatitis C infected patients: a pilot study. *Scientific Reports* **10**:17820. DOI: <https://doi.org/10.1038/s41598-020-74568-x>, PMID: [33082372](https://pubmed.ncbi.nlm.nih.gov/33082372/)
- Ferenci P, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, Tam E, Marinho RT, Tsai N, Nyberg A, Box TD, Younes Z, Enayati P, Green S, Baruch Y, Bhandari BR, Caruntu FA, Sepe T, Chulanov V, Janczewska E, et al. 2014. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *The New England Journal of Medicine* **370**:1983–1992. DOI: <https://doi.org/10.1056/NEJMoa1402338>, PMID: [24795200](https://pubmed.ncbi.nlm.nih.gov/24795200/)
- Flower B, McCabe L, Le Ngoc C, Le Manh H, Le Thanh P, Dang Trong T, Vo Thi T, Vu Thi Kim H, Nguyen Tat T, Phan Thi Hong D, Nguyen Thi Chau A, Dinh Thi T, Tran Thi Tuyet N, Tarning J, Kingsley C, Kestelyn E, Pett SL, Thwaites G, Nguyen Van VC, Smith D, et al. 2021. High cure rates for hepatitis C virus genotype 6 in advanced liver fibrosis with 12 weeks sofosbuvir and daclatasvir: the vietnam SEARCH study. *Open Forum Infectious Diseases* **8**:ofab267. DOI: <https://doi.org/10.1093/ofid/ofab267>, PMID: [34337093](https://pubmed.ncbi.nlm.nih.gov/34337093/)
- Franco S, Aparicio E, Parera M, Clotet B, Tural C, Martinez MA. 2014. IFNL4 ss469415590 variant is a better predictor than rs12979860 of pegylated interferon-alpha/ribavirin therapy failure in hepatitis C virus/HIV-1 coinfecting patients. *AIDS* **28**:e52. DOI: <https://doi.org/10.1097/QAD.0000000000000052>, PMID: [24072198](https://pubmed.ncbi.nlm.nih.gov/24072198/)

- Fried MW, Hadziyannis SJ, Shiffman ML, Messinger D, Zeuzem S. 2011. Rapid virological response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. *Journal of Hepatology* **55**:69–75. DOI: <https://doi.org/10.1016/j.jhep.2010.10.032>, PMID: 21145856
- Gupta N, Mbituyumuremyi A, Kabahizi J, Ntaganda F, Muvunyi CM, Shumbusho F, Musabeyezu E, Mukabatsinda C, Ntirenganya C, Van Nuil JI, Kateera F, Camus G, Damascene MJ, Nsanzimana S, Mukherjee J, Grant PM. 2019. Treatment of chronic hepatitis C virus infection in rwanda with ledipasvir-sofosbuvir (SHARED): a single-arm trial. *The Lancet. Gastroenterology & Hepatology* **4**:119–126. DOI: [https://doi.org/10.1016/S2468-1253\(18\)30382-0](https://doi.org/10.1016/S2468-1253(18)30382-0), PMID: 30552056
- Hedskog C, Parhy B, Chang S, Zeuzem S, Moreno C, Shafraan SD, Borgia SM, Asselah T, Alric L, Abergel A, Chen J-J, Collier J, Kapoor D, Hyland RH, Simmonds P, Mo H, Svarovskaia ES. 2019. Identification of 19 novel hepatitis C virus subtypes-further expanding HCV classification. *Open Forum Infectious Diseases* **6**:fz076. DOI: <https://doi.org/10.1093/ofid/ofz076>, PMID: 30949527
- Hospital for Tropical Diseases Ethics Committee. 2021. Tropical Disease. Vietnam: NHS Foundation Trust. <https://bv.bnhietdoi@tphcm.gov.vn>
- Imperial College Research Ethics Committee. 2018. Research Ethics. London, UK: Imperial College. <https://researchethicscommittee@imperial.ac.uk>
- Irekeola AA, Malek NA, Wada Y, Mustafa N, Muhamad NI, Shueb RH. 2021. Prevalence of HCV genotypes and subtypes in southeast asia: A systematic review and meta-analysis. *PLOS ONE* **16**:e0251673. DOI: <https://doi.org/10.1371/journal.pone.0251673>
- ISRCTN registry. 2018. A pilot study to assess shortened therapy for hepatitis C infected adults in Vietnam. ISRCTN. <https://www.isrctn.com/ISRCTN17100273>
- Jones CR, Flower BF, Barber E, Simmons B, Cooke GS. 2019. Treatment optimisation for hepatitis C in the era of combination direct-acting antiviral therapy: A systematic review and meta-analysis. *Wellcome Open Res* **4**:e1. DOI: <https://doi.org/10.12688/wellcomeopenres.15411.1>
- Kohli A, Osinusi A, Sims Z. 2015. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet* **385**:1107–1113. DOI: [https://doi.org/10.1016/S0140-6736\(14\)61228-9](https://doi.org/10.1016/S0140-6736(14)61228-9)
- Kowdley KV, Nelson DR, Lalezari JP, Box T, Gitlin N, Poleyndard G, Rabinovitz M, Ravendhran N, Sheikh AM, Siddique A, Bhore R, Noviello S, Rana K. 2016. On-treatment HCV RNA as a predictor of sustained virological response in HCV genotype 3-infected patients treated with daclatasvir and sofosbuvir. *Liver International* **36**:1611–1618. DOI: <https://doi.org/10.1111/liv.13165>, PMID: 27188960
- Kracht PAM, Gee EA, Poel A. 2019. Introducing hepatitis C virus healthcare pathways in addiction care in the netherlands with a breakthrough project: a mixed method study. *Harm Reduct J* **16**:45. DOI: <https://doi.org/10.1186/s12954-019-0316-4>
- Lau G, Benhamou Y, Chen G, Li J, Shao Q, Ji D, Li F, Li B, Liu J, Hou J, Sun J, Wang C, Chen J, Wu V, Wong A, Wong CLP, Tsang STY, Wang Y, Bassit L, Tao S, et al. 2016. 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. *The Lancet. Gastroenterology & Hepatology* **1**:97–104. DOI: [https://doi.org/10.1016/S2468-1253\(16\)30015-2](https://doi.org/10.1016/S2468-1253(16)30015-2), PMID: 27917405
- Le Ngoc C, Tran Thi Thanh T, Tran Thi Lan P. 2019. Differential prevalence and geographic distribution of hepatitis C virus genotypes in acute and chronic hepatitis C patients in vietnam. *PLOS ONE* **14**:e0212734. DOI: <https://doi.org/10.1371/journal.pone.0212734>
- Lixoft SAS. 2022. PKanalix 2020R1 single page user guide. v.2021. PKanalix. <https://pkanalix.lixoft.com/pkanalix-2020r1-single-page-user-guide/>
- Maasoumy B, Vermehren J, Welker M-W, Bremer B, Perner D, Höner Zu Siederdisen C, Deterding K, Lehmann P, Cloherty G, Reinhardt B, Pawlotsky J-M, Manns MP, Zeuzem S, Cornberg M, Wedemeyer H, Sarrazin C. 2016. Clinical value of on-treatment HCV RNA levels during different sofosbuvir-based antiviral regimens. *Journal of Hepatology* **65**:473–482. DOI: <https://doi.org/10.1016/j.jhep.2016.04.006>, PMID: 27085252
- Manso CF, Bibby DF, Lythgow K, Mohamed H, Myers R, Williams D, Piorkowska R, Chan YT, Bowden R, Ansari MA, Ip CLC, Barnes E, Bradshaw D, Mbisa JL. 2020. Technical validation of a hepatitis C virus whole genome sequencing assay for detection of genotype and antiviral resistance in the clinical pathway. *Frontiers in Microbiology* **11**:576572. DOI: <https://doi.org/10.3389/fmicb.2020.576572>, PMID: 33162957
- McCabe L, White IR, Chau NVV. 2020. The design and statistical aspects of VIETNARMS: a strategic post-licensing trial of multiple oral direct-acting antiviral hepatitis C treatment strategies in vietnam. *Trials* **21**:413. DOI: <https://doi.org/10.1186/s13063-020-04350-x>
- McPhee F, Ueland J, Vellucci V, Bowden S, Sievert W, Zhou N. 2019. Impact of preexisting hepatitis C virus genotype 6 NS3, NS5A, and NS5B polymorphisms on the *in vitro* potency of direct-acting antiviral agents. *Antimicrobial Agents and Chemotherapy* **63**:1–12. DOI: <https://doi.org/10.1128/AAC.02205-18>, PMID: 30718256
- Ministry of Health V. 2021. National Plan for Prevention and Control of Viral Hepatitis. <https://emoh.moh.gov.vn/publish/attach/getfile/408692> [Accessed January 11, 2021].
- National Institute of Health. 2017. Common terminology criteria for adverse events. v5.0. CTCAE. https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf
- Nguyen Thanh D, Tran Thi Thanh T, Nghiem My N, Anh Nguyen H, Le Thanh P, Vo Minh Q, Rahman M, Le Manh H, The Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. 2019. Baseline characteristics and treatment cost of hepatitis C at hospital for tropical diseases, ho chi minh city, vietnam in direct-acting antiviral

- treatment era. *Journal of Gastroenterology and Hepatology Research* **8**:2962–2971. DOI: <https://doi.org/10.17554/j.issn.2224-3992.2019.08.857>
- Nitta Y**, Kawabe N, Hashimoto S, Harata M, Komura N, Kobayashi K, Arima Y, Shimazaki H, Nakano T, Murao M, Ichino N, Osakabe K, Aoki H, Hosoe Y, Sugiyama H, Nishikawa T, Yoshioka K. 2009. Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C. *Hepatology Research* **39**:675–684. DOI: <https://doi.org/10.1111/j.1872-034X.2009.00500.x>, PMID: 19261000
- Oxford Tropical Research Ethics Committee**. 2018. Constitution, standing orders and membership for OxTREC. University Offices, Wellington Square, Oxford: University of Oxford Research Services. <https://oxtrec@admin.ox.ac.uk>
- Pal V**, Ancha N, Mann J, Modi AA. 2020. Effect of low positive end of treatment viral load with direct-acting antiviral therapy on sustained virologic response. *Canadian Journal of Gastroenterology & Hepatology* **2020**:8815829. DOI: <https://doi.org/10.1155/2020/8815829>, PMID: 32802821
- Perelson AS**, Guedj J. 2015. Modelling hepatitis C therapy--predicting effects of treatment. *Nature Reviews. Gastroenterology & Hepatology* **12**:437–445. DOI: <https://doi.org/10.1038/nrgastro.2015.97>, PMID: 26122475
- Petersen T**, Townsend K, Gordon LA, Sidharthan S, Silk R, Nelson A, Gross C, Calderón M, Proschan M, Osinusi A, Polis MA, Masur H, Kottitil S, Kohli A. 2016. High adherence to all-oral directly acting antiviral HCV therapy among an inner-city patient population in a phase 2a study. *Hepatology International* **10**:310–319. DOI: <https://doi.org/10.1007/s12072-015-9680-7>, PMID: 26612014
- Prokunina-Olsson L**, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, Hergott D, Porter-Gill P, Mumy A, Kohaar I, Chen S, Brand N, Tarway M, Liu L, Sheikh F, Astemborski J, Bonkovsky HL, Edlin BR, Howell CD, Morgan TR, et al. 2013. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nature Genetics* **45**:164–171. DOI: <https://doi.org/10.1038/ng.2521>, PMID: 23291588
- Silva Filipe A**, Sreenu V, Hughes J. 2017. Response to DAA therapy in the NHS england early access programme for rare HCV subtypes from low and middle income countries. *J Hepatol* **67**:1348–1350. DOI: <https://doi.org/10.1016/j.jhep.2017.06.035>
- Smith DA**, Bradshaw D, Mbisa JL, Manso CF, Bibby DF, Singer JB, Thomson EC, da Silva Filipe A, Aranday-Cortes E, Ansari MA, Brown A, Hudson E, Benselin J, Healy B, Troke P, McLauchlan J, Barnes E, Irving WL. 2021a. Real world SOF/VEL/VOX retreatment outcomes and viral resistance analysis for HCV patients with prior failure to DAA therapy. *Journal of Viral Hepatitis* **28**:1256–1264. DOI: <https://doi.org/10.1111/jvh.13549>, PMID: 34003556
- Smith DA**, Fernandez-Antunez C, Magri A, Bowden R, Chaturvedi N, Fellay J, McLauchlan J, Foster GR, Irving WL, Simmonds P, Pedergnana V, Ramirez S, Bukh J, Barnes E, Ansari MA, STOP-HCV Consortium. 2021b. Viral genome wide association study identifies novel hepatitis C virus polymorphisms associated with sofosbuvir treatment failure. *Nature Communications* **12**:6105. DOI: <https://doi.org/10.1038/s41467-021-25649-6>, PMID: 34671027
- Smolders EJ**, Jansen AME, Horst PGJ, Rockstroh J, Back DJ, Burger DM. 2019. Viral hepatitis C therapy: pharmacokinetic and pharmacodynamic considerations: A 2019 update. *Clin Pharmacokinet* **58**:1237–1263. DOI: <https://doi.org/10.1007/s40262-019-00774-0>
- StataCorp**. 2019. Stata statistical software. v.16.1. College Station, TX. StataCorp LLC.
- Sulkowski MS**, Flamm S, Kayali Z, Lawitz EJ, Kwo P, McPhee F, Torbeyns A, Hughes EA, Swenson ES, Yin PD, Linaberry M. 2017. Short-duration treatment for chronic hepatitis C virus with daclatasvir, asunaprevir, beclabuvir and sofosbuvir (fourward study). *Liver International* **37**:836–842. DOI: <https://doi.org/10.1111/liv.13335>, PMID: 27943563
- Thomson E**, Ip CLC, Badhan A. 2016. Comparison of next-generation sequencing technologies for comprehensive assessment of full-length hepatitis C viral genomes. *J Clin Microbiol* **54**:2470–2484. DOI: <https://doi.org/10.1128/JCM.00330-16>
- Vietnam Ministry of Health**. 2022. Ethical Evaluation Committee in Biomedical Research of Ministry of Health. Ha Noi. <https://admin@iecmoh.vn>
- World Health Organization**. 2018. Guidelines for the Care and Treatment of Persons Diagnosed with Chronic Hepatitis C Virus Infection. <https://www.who.int/publications/i/item/9789241550345> [Accessed July 1, 2018].
- World Medical Association**. 2022. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects> [Accessed September 6, 2022].
- Wyles DL**, Ruane PJ, Sulkowski MS, Dieterich D, Luetkemeyer A, Morgan TR, Sherman KE, Dretler R, Fishbein D, Gathe JC, Henn S, Hinestrosa F, Huynh C, McDonald C, Mills A, Overton ET, Ramgopal M, Rashbaum B, Ray G, Scarsella A, et al. 2015. Daclatasvir plus sofosbuvir for HCV in patients coinfecting with HIV-1. *The New England Journal of Medicine* **373**:714–725. DOI: <https://doi.org/10.1056/NEJMoa1503153>, PMID: 26196502
- Yakoot M**, Abdo AM, Abdel-Rehim S, Helmy S. 2017. Response tailored protocol versus the fixed 12weeks course of dual sofosbuvir/daclatasvir treatment in egyptian patients with chronic hepatitis C genotype-4 infection: A randomized, open-label, non-inferiority trial. *EBioMedicine* **21**:182–187. DOI: <https://doi.org/10.1016/j.ebiom.2017.05.011>, PMID: 28647541

Appendix 1

Efficacy of ultra-short, response-guided sofosbuvir and daclatasvir therapy for Hepatitis C: a single arm pilot mechanistic study

Pharmacokinetic and Pharmacodynamic (PK/PD) process

Sofosbuvir and GS-331007 were extracted from 100 μ L of plasma using phospholipid removal in the 96-well plate format (Phree, 8E-S133-TGB, Phenomenex), followed by separation on a Gemini, 50 mm \times 2.0 mm I.D. 5 μ m, column (00B-4435-B0, Phenomenex). Quantification was performed using selected reaction monitoring for the transitions m/z 530.2 \rightarrow 243.2 (sofosbuvir), 536.2 \rightarrow 243.1 (isotope-labelled internal standard for sofosbuvir), 261.3 \rightarrow 113.1 (GS-331007), and 265.3 \rightarrow 113.1 (isotope-labelled internal standard for GS-331007).

The lower limit of quantification (LLOQ) was set to 1.95 ng/mL for sofosbuvir and 20.5 ng/mL for GS-331007. A total of 9 quality control samples (3 \times low, 3 \times mid and 3 \times high concentration) were analysed for each analyte within each batch of clinical samples (96-well plate), resulting in an accuracy of 2.81–2.93% RSE for sofosbuvir and 2.19–3.50% RSE for GS-331007.

DCV was extracted from 100 μ L of plasma using supported liquid extraction in the 96-well plate format (ISOLUTE SLE +96 well plate, 820–0200 P01, Biotage), followed by separation on a Gemini, 50 mm \times 2.0 mm I.D. 5 μ m, column (00B-4435-B0, Phenomenex). Quantification was performed using selected reaction monitoring for the transitions m/z 739.5 \rightarrow 339.3 (DCV) and 747.5 \rightarrow 339.3 (isotope-labelled internal standard for DCV). The LLOQ was set to 1.64 ng/mL for DCV. A total of 9 quality control samples (3 \times low, 3 \times mid and 3 \times high concentration) were analysed within each batch of clinical samples (96-well plate), resulting in an accuracy of 2.46–2.62% RSE for DCV.

PK/PD analysis

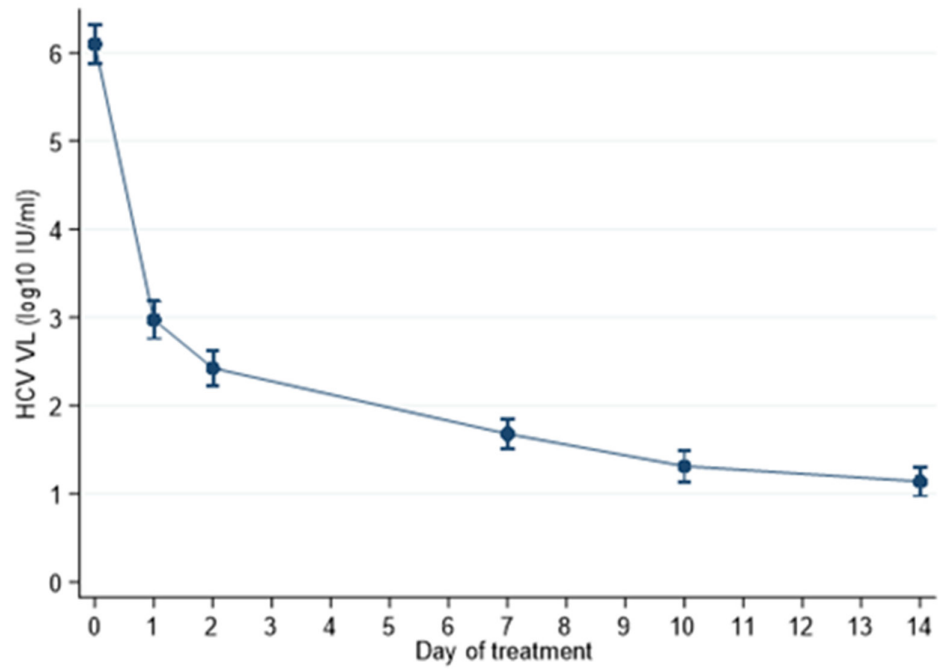
Maximum concentration (C_{\max}) and time to reach maximum concentration (t_{\max}) were derived directly from the observed drug concentrations. The software's automatic slope calculator was used to derive the elimination rate (λ) (adjusted R2 value with uniform weighting) and terminal elimination half-life ($t_{1/2}$). The drug exposure measured as area under the concentration-time curve (AUC) was calculated for each drug using the trapezoidal method. Linear interpolation was used for ascending concentrations and log-linear interpolation for descending concentrations. For the individual analysis, the linear method was used for all measurements due to accumulation between the day 0 and day 28 measurements. Both the AUC to the last time point (AUC_{last} , 8 hours for SOF and 24 hours for GS-331007 and DCV) and AUC to infinity (AUC_{inf}) were calculated.

A non-compartmental pharmacodynamic analysis were conducted using viral load data from enrolment to day 14 to calculate area under the viral load – time curve and terminal elimination half-life of the viral clearance curve, using the same methodology as explained above. In addition, the relative reduction in viral load between enrolment and day 1, and between enrolment and day 7 were calculated. Ordinary linear regression of drug exposure (AUC_{last}) from the individual pharmacokinetic analysis and the outcome measurements were performed in GraphPad Prism 9.3.1 (GraphPad Software, San Diego, California USA).

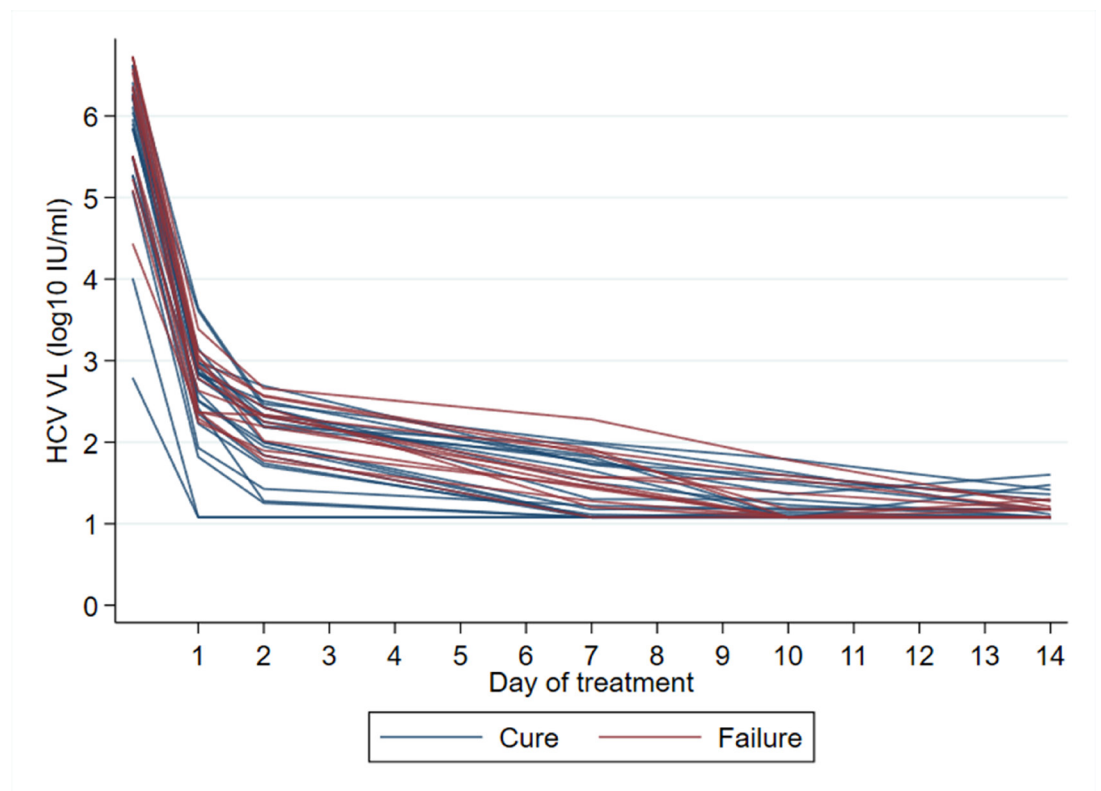
Limitations of PK/PD analysis

Two different sampling schedules were used to collect pharmacokinetic samples on day 0 and day 28, resulting in an overlapping sampling profile overall. Therefore, data collected within an individual on a specific day was not dense enough to justify a non-compartment analysis. However, if the data on day 0 and 28 were combined, it resulted in a complete pharmacokinetic profile for the individual. Two separate pharmacokinetic analyses were carried out. A naïve-pooled analysis was performed separately conducted on day 0 and 28 data. This resulted in a summary of exposure (AUC and C_{\max}) and half-life of the drugs, but it would not be possible to link these measurements to treatment outcome due to the different sampling strategies. Therefore, a second analysis was performed in which the data from day 0 and 28 were pooled for each individual. This resulted in complete pharmacokinetic profiles for each patient, and the pharmacokinetic-pharmacodynamic analysed demonstrated no significant relationship between drug exposure and treatment outcome. Drug accumulation was observed for the sofosbuvir metabolite GS-331007 and DCV between day 0 and 28. However, the individual analysis should still generate median exposure values for each patient, which can be linked with treatment outcome. Another drawback is that no pre-dose samples were collected. Therefore, the non-

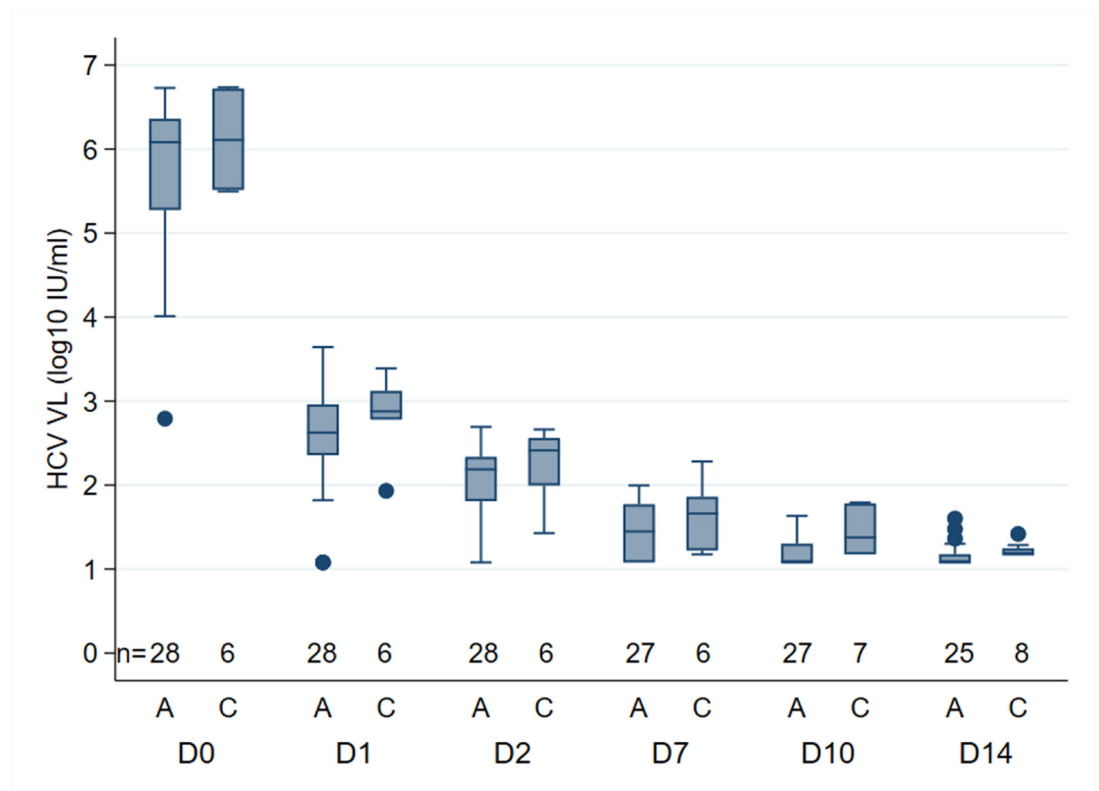
compartmental analysis will assume no concentration at day 0 on day 28 for GS-331007 and DCV, even though drug accumulation was observed.



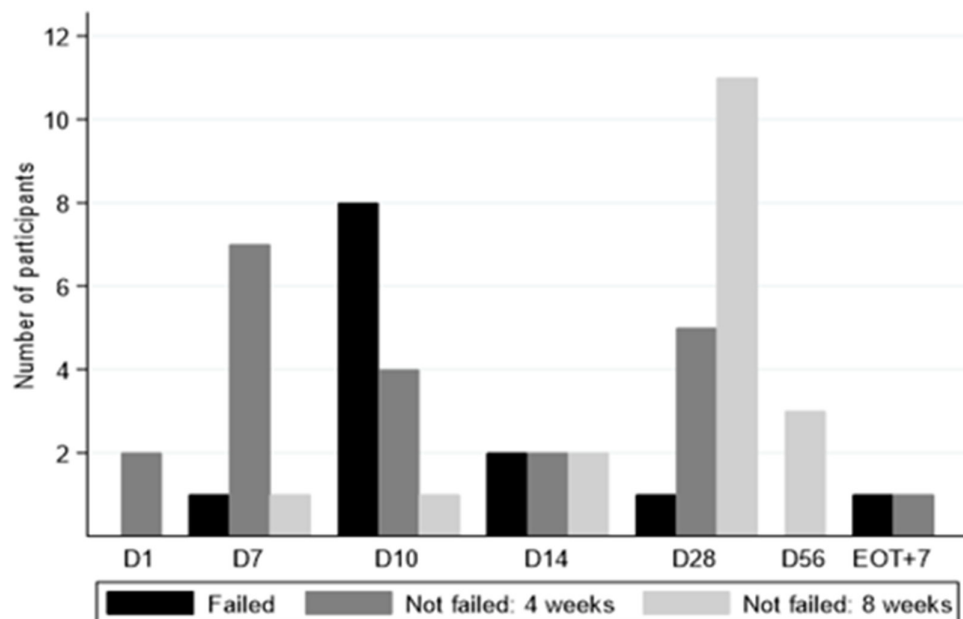
Appendix 1—figure 1. Mean (95% CI) HCV RNA by visit day.



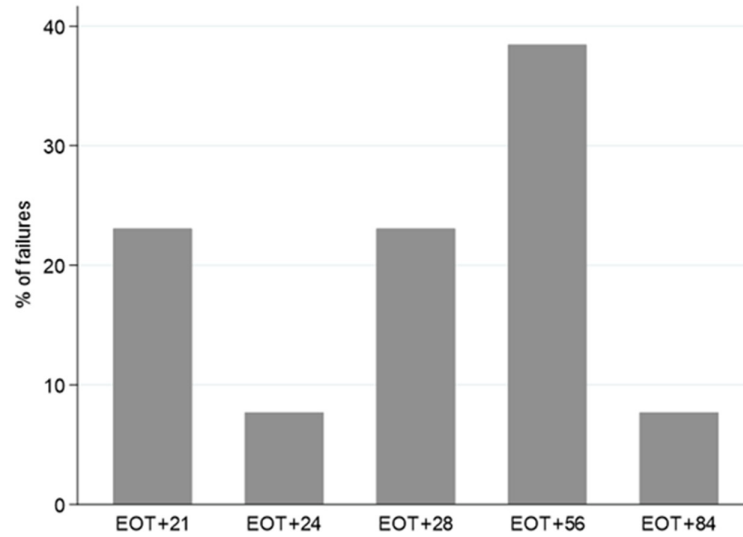
Appendix 1—figure 2. HCV RNA kinetics in participants treated with 4 weeks SOF/DCV.



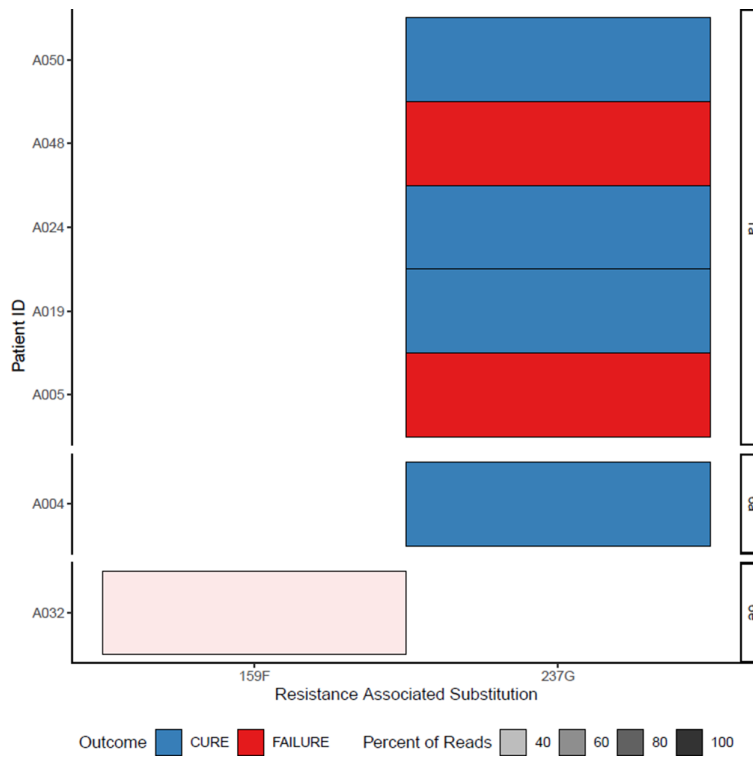
Appendix 1—figure 3. Median HCV RNA (log₁₀), by PCR assay, at different time points in participants treated with 4 weeks SOF/DCV A = Abbott Architect (LLOQ = 12 IU/ml). C=COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test, version 2.0 (Roche Molecular Systems, LLOQ = 15 IU/ml)



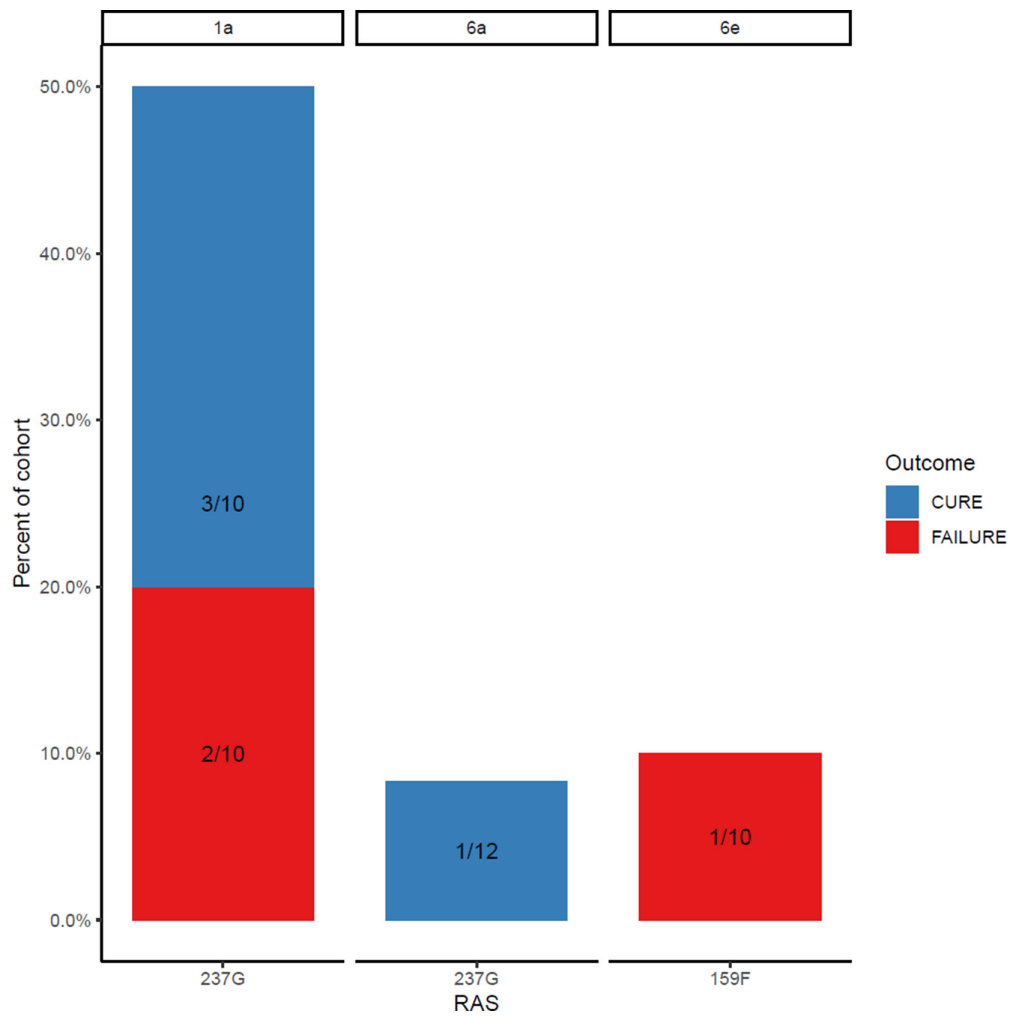
Appendix 1—figure 4. Time to viral suppression <LLOQ and eventual treatment outcome. *No treatment failures in 8 week arm. D28 is the EOT visit for those who received 4 weeks. D56 visit is the EOT visit for those who received 8 weeks.



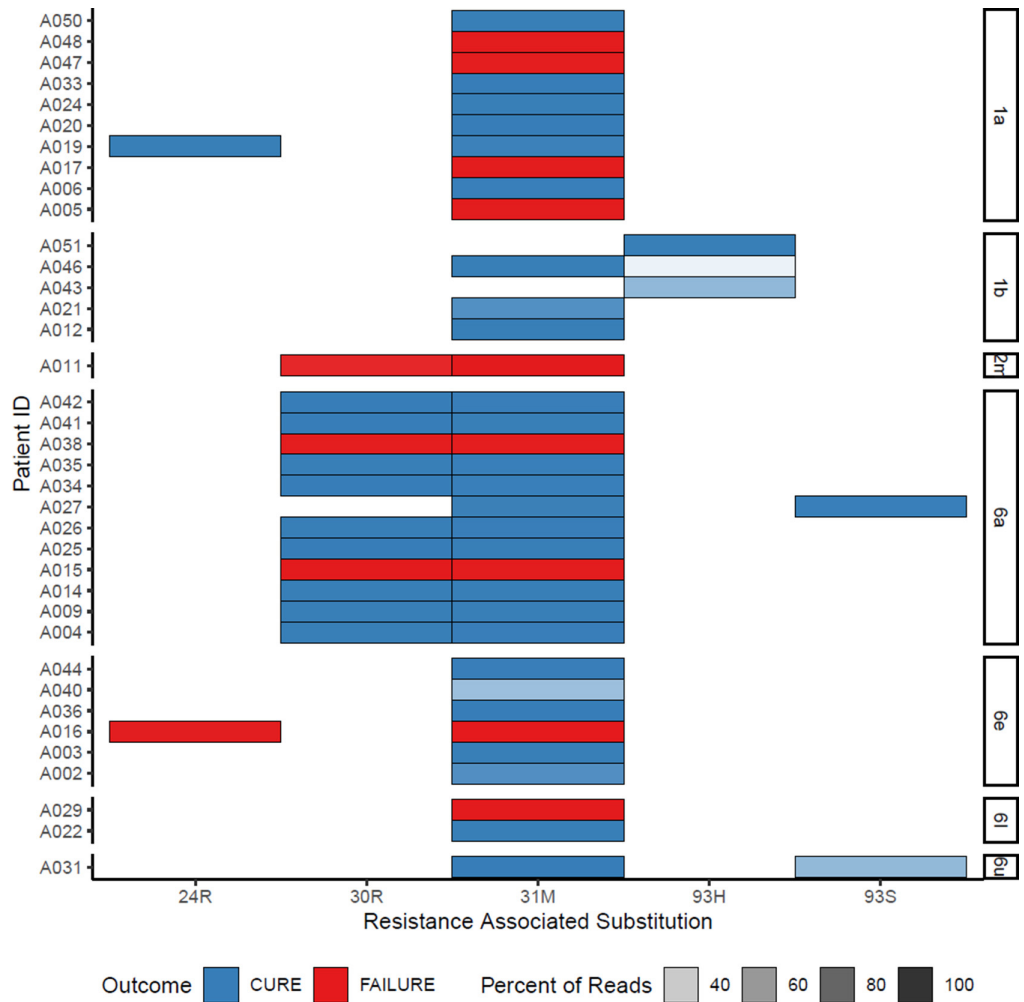
Appendix 1—figure 5. Timing of treatment failure (confirmed HCV VL >2000 IU/mL) (n=13). *Note twice weekly sampling in first 4 weeks after EOT, monthly thereafter.



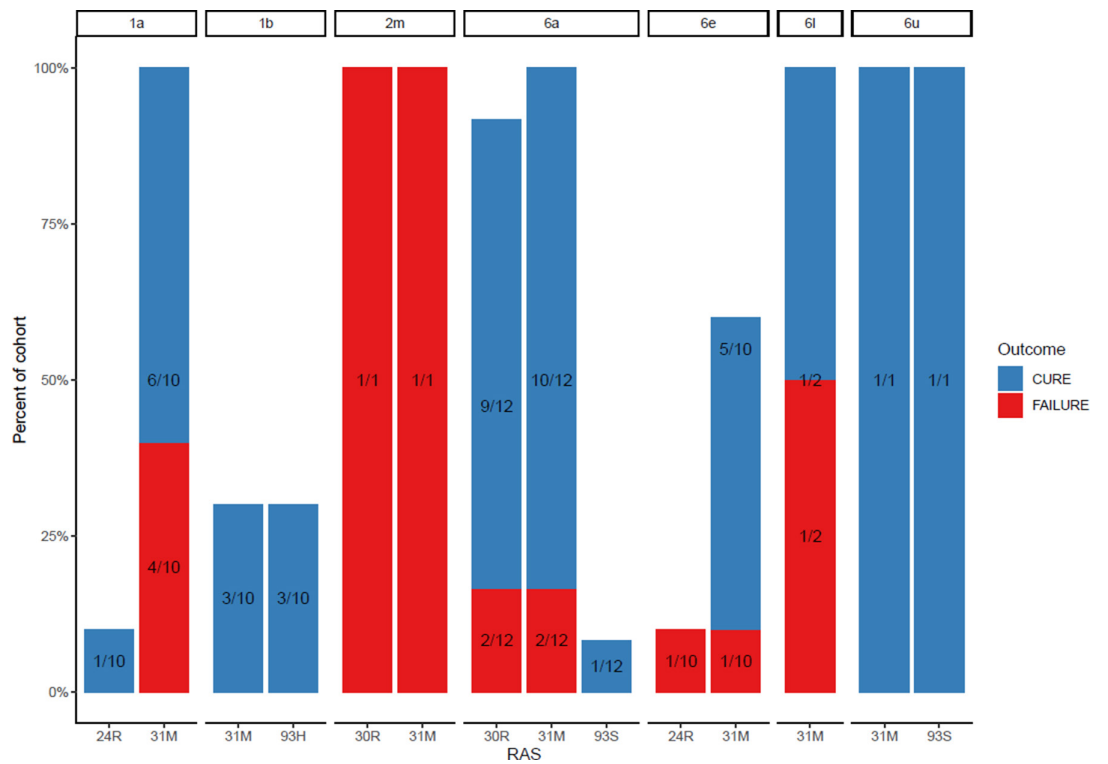
Appendix 1—figure 6. All sofosbuvir resistance-associated substitutions at baseline (with treatment outcome).



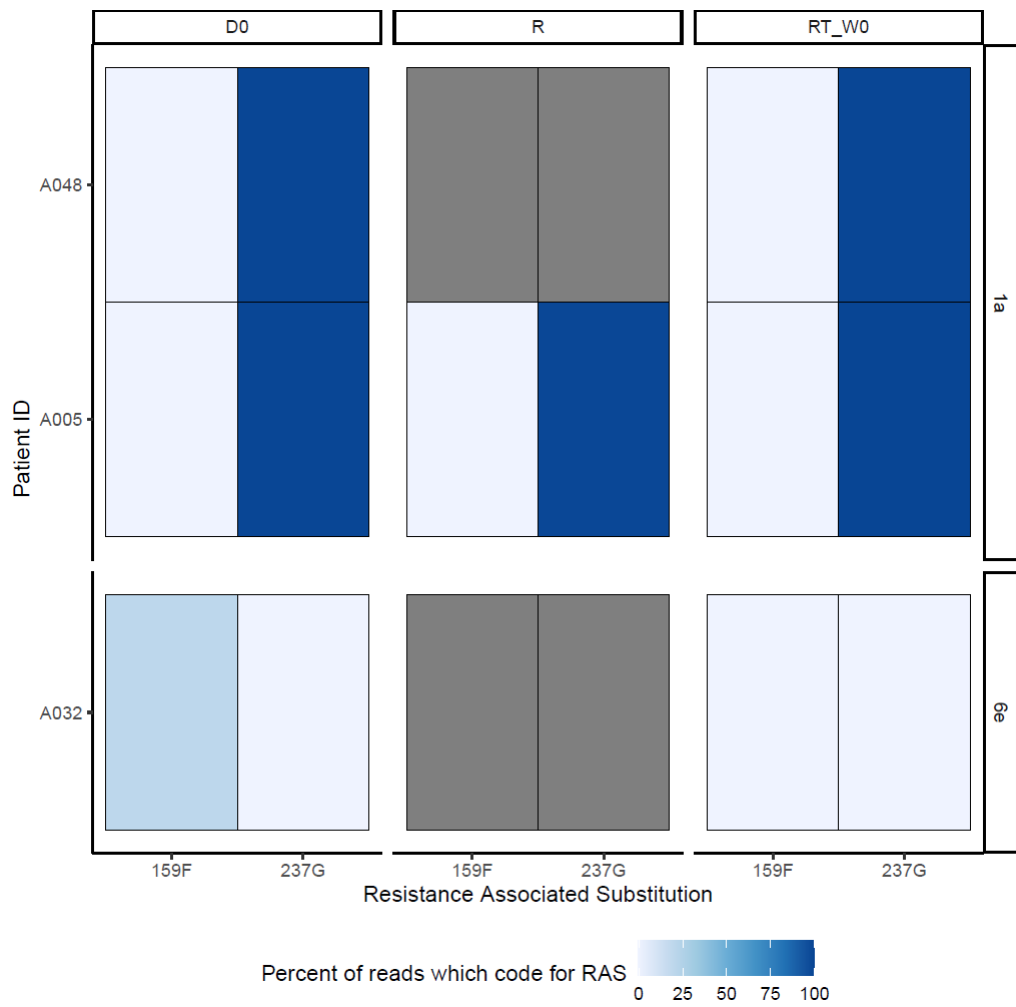
Appendix 1—figure 7. Proportion of each subtype with sofosbuvir resistance-associated substitutions at baseline (with treatment outcome).



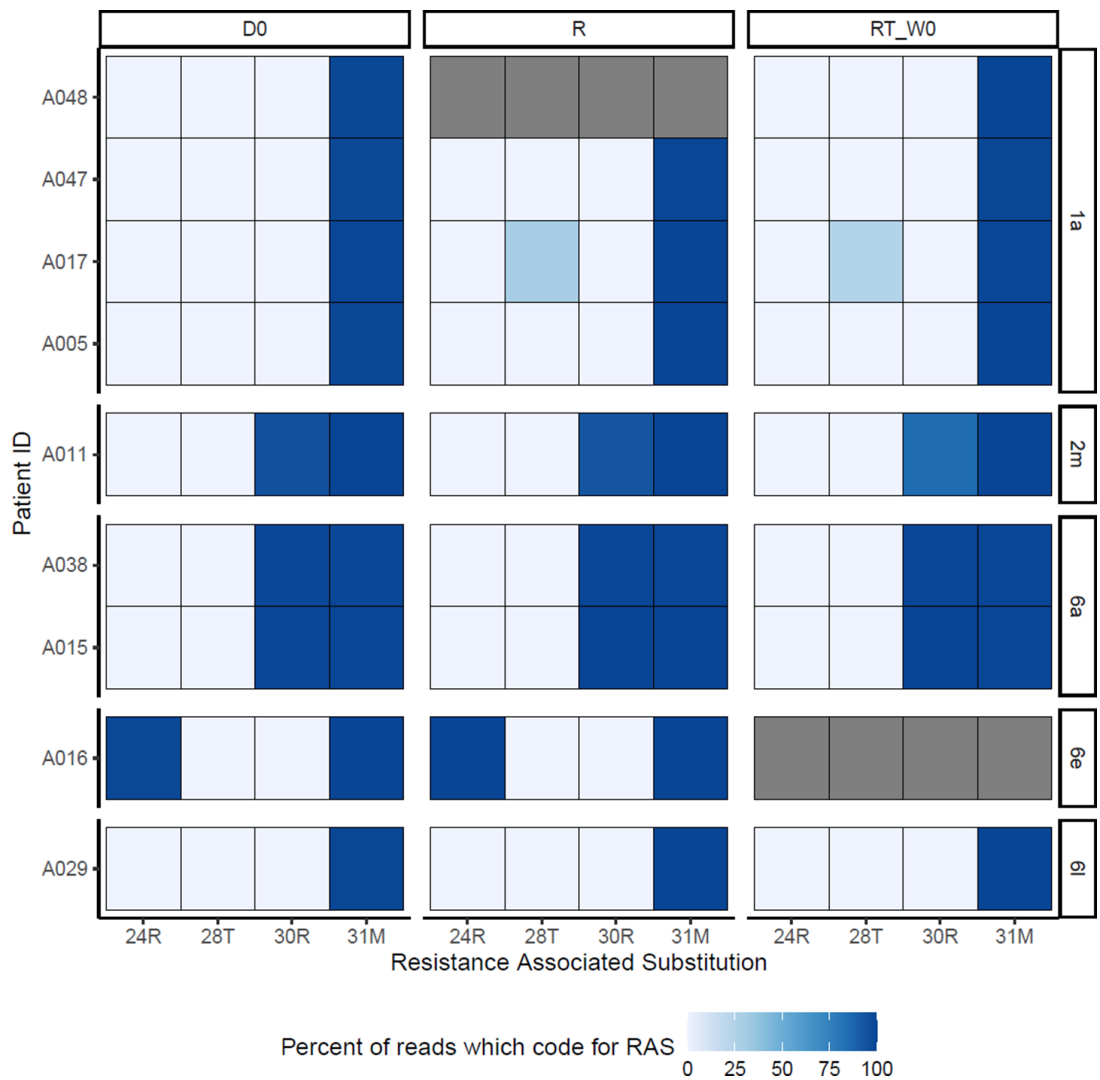
Appendix 1—figure 8. All daclatasvir resistance-associated substitutions at baseline (with treatment outcome).



Appendix 1—figure 9. Proportion of each subtype with daclatasvir resistance-associated substitutions at baseline (with treatment outcome).



Appendix 1—figure 10. SOF-RAS at baseline (D0), time of virological rebound (R) and start of retreatment (RT_W0) Grey boxes represent missing data.



Appendix 1—figure 11. DCV-RAS at baseline (D0), time of virological rebound (R) and start of retreatment (RT_W0) Grey boxes represent missing data.

Appendix 1—table 1. Pharmacokinetic parameters from the naïve-pooled analysis.

| | Sofosbuvir | | GS-331007 | | Daclatasvir | |
|-------------------------|------------|--------|-----------|--------|-------------|--------|
| | Day 0 | Day 28 | Day 0 | Day 28 | Day 0 | Day 28 |
| C_{max} (ng/mL) | 1,320 | 1,070 | 988 | 1,230 | 1,170 | 1,110 |
| t_{max} (h) | 1.00 | 1.00 | 3.00 | 4.00 | 3.00 | 3.00 |
| $t_{1/2}$ (h) | 0.670 | 0.650 | 9.20 | 12.4 | 7.31 | 8.18 |
| AUC_{last} (h×ng/mL)* | 1,550 | 1,600 | 10,500 | 14,600 | 11,400 | 12,400 |
| AUC_{inf} (h×ng/mL)* | 1,550 | 1,600 | 12,700 | 20,400 | 12,800 | 14,400 |

C_{max} is the maximum observed concentration, t_{max} is the time to reach the maximum concentration, $t_{1/2}$ is the terminal elimination half-life (calculated using the 3–6 last concentration measurements, depending on drug and day), AUC_{last} is the total exposure to the last time point (8 hours for SOF and 24 hours for GS-331007 and DCV), AUC_{inf} is the total exposure extrapolated to infinity.

*Extrapolation based on the last observed concentration measurement.

Appendix 1—table 2. Pharmacokinetic exposure from the individual analysis and pharmacodynamic parameters.

| Pharmacokinetics | | | |
|-------------------------------|----------------------------|---------------------|----------------------|
| | Sofosbuvir | GS-331007 | Daclatasvir |
| AUC _{last} (h×ng/mL) | 1,140 (598-2,150) | 3,430 (2,200-4,720) | 9,770 (5,080-16,200) |
| Pharmacodynamics | | | |
| AUC (days ×IU/mL) | 252,000 (19,200-1,370,000) | | |
| t _{1/2} (days) | 2.25 (0.986–5.22) | | |
| %ReductionEnrolment-Day1 | 99.9 (99.0–100) | | |
| %ReductionEnrolment-Day7 | 100 (100–100) | | |

Data is presented as median (5th–95th percentile). AUC_{last} is the total exposure to the last time point (8 hours for SOF and 24 hours for GS-331007 and DCV). AUC₁₄ is the area under the viral load-time curve from enrolment (day 0) to day 14, t_{1/2} is the terminal viral half-life (estimated using at least three measurements), %Reduction_{Enrolment-Day1} is the reduction in viral load from enrolment to day 1, %Reduction_{Enrolment-Day7} is the reduction in viral load from enrolment to day 7.

The half-life could not be determined for one participant due to only one sample above the lower limit of quantification.

Appendix 1—table 3. Pharmacokinetic-pharmacodynamic linear regression analysis.

| Viral outcome measurement vs. | Sofosbuvir AUC _{last} | | GS-331007 AUC _{last} | | Daclatasvir AUC _{last} | |
|---|--|-------|---|-------|--|---------|
| | Slope (95% CI) | p | Slope (95% CI) | p | Slope (95% CI) | p-value |
| Area under the viral load-time curve | -157 (-423–109) | 0.239 | 16.2 (-74.4–107) | 0.719 | -14.2 (-67.1–38.6) | 0.589 |
| Viral elimination half-life | 1.55×10 ⁻⁴ (-8.70×10 ⁻⁴ - 5.60×10 ⁻⁴) | 0.662 | -3.64×10 ⁻⁵ (-2.74×10 ⁻⁴ - 2.01×10 ⁻⁴) | 0.757 | 2.17×10 ⁻⁵ (-1.16×10 ⁻⁴ - 1.60×10 ⁻⁴) | 0.751 |
| Relative reduction in viral load at day 1 | 1.31×10 ⁻⁶ (-4.54×10 ⁻⁶ - 7.16×10 ⁻⁶) | 0.652 | 2.67×10 ⁻⁸ (-1.94×10 ⁻⁶ - 1.99×10 ⁻⁶) | 0.978 | 2.81×10 ⁻⁷ (-8.62×10 ⁻⁷ - 1.42×10 ⁻⁶) | 0.621 |
| Relative reduction in viral load at day 7 | 2.53×10 ⁻⁷ (-2.81×10 ⁻⁷ - 7.86×10 ⁻⁷) | 0.343 | 5.09×10 ⁻⁸ (-1.29×10 ⁻⁷ - 2.31×10 ⁻⁷) | 0.569 | 1.44×10 ⁻⁸ (-9.11×10 ⁻⁹ - 1.20×10 ⁻⁷) | 0.783 |

95%CI is the 95% confidence interval around the slope.