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# Sofosbuvir and ribavirin for six weeks is not effective among people with recent HCV infection: The DARE-C II study

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**Summary:** Six weeks of sofosbuvir and ribavirin was safe and well tolerated, but efficacy was sub-optimal (SVR12 32%, 6/19). Further research is needed to determine whether more potent interferon-free direct-acting antiviral regimens will allow treatment duration to be shortened in recent HCV infection.

**Abstract**

While interferon-based therapy has excellent efficacy in acute and recent hepatitis C virus (HCV) infection, the side effect profile limits implementation. Sofosbuvir and ribavirin for 12–24 weeks is safe and well tolerated in chronic HCV with efficacy dependent on genotype and disease stage. The aim of this study was to assess the efficacy of sofosbuvir and ribavirin for six weeks in individuals with recent HCV infection. In this open-label study conducted in Australia and New Zealand, adults with recent HCV (duration of infection <12 months) received sofosbuvir 400mg daily and weight-based ribavirin (<75kg: 1000mg/day; ≥75kg: 1200mg/day) for six weeks. The primary efficacy endpoint was sustained virological response at post-treatment week 12 (SVR12). Nineteen participants commenced sofosbuvir and ribavirin (89% male, 74% HIV, 68% genotype 1a). Four (21%) reported a symptomatic HCV seroconversion illness, including 2 with jaundice. At baseline, median HCV RNA was 5.4 $\log_{10}$ IU/mL (IQR 4.4-6.8) and median estimated duration of infection was 37 weeks (IQR 27-41). At end-of-treatment, HCV RNA was non-quantifiable in 89% (n=17). SVR4 and SVR12 were 42% (n=8) and 32% (n=6), respectively. Treatment failure was due to non-response (n=2), post-treatment relapse (n=9), reinfection (n=1) and loss to follow up (n=1). The regimen was well tolerated with minimal haematological toxicity. SVR12 was related to baseline HCV RNA (≤6 $\log_{10}$ IU/mL, p=0.018) and early on-treatment viral kinetics (HCV RNA below the level of quantitation at week 1, p=0.003). **Conclusion:** Six weeks of sofosbuvir and ribavirin was safe and well tolerated, but efficacy was sub-optimal. Further research is needed to determine whether more potent interferon-free direct-acting antiviral regimens will allow treatment duration to be shortened in recent, predominantly asymptomatic, HCV infection.

The management of recent (acute or early chronic) hepatitis C virus (HCV) infection is not standardised, with uncertainty regarding the optimal regimen and treatment duration (1). As interferon (IFN)-free direct-acting antiviral (DAA) regimens are established as the standard-of-care for chronic HCV infection (2-4), their role and activity in recent HCV infection require evaluation. Most individuals with chronic HCV infection (>95%) will demonstrate a sustained virological response (SVR) following only eight to twelve weeks of DAA therapy (5-8). Shorter treatment durations should aim to optimise adherence and enhance cost-effectiveness. With IFN-based regimens, 12 weeks treatment is sufficient to cure greater than 90% of individuals with acute HCV infection (1), as compared with 24-48 weeks treatment for chronic HCV infection. The efficacy of ultra-short duration IFN-free DAA therapy in individuals with acute and recent HCV infection is not known.

As individuals with diagnosed recent HCV infection are keen to consider treatment (9), this initial assessment may represent an ideal opportunity for therapeutic intervention, offering both individual and population level benefits (10). Successful treatment of acute HCV infection in people who inject drugs (PWID) and men-who-have-sex-with-men (MSM), when combined with implementation of harm reduction strategies should prevent transmission (Treatment-as-Prevention) and contribute towards HCV elimination at a population level (10, 11).

Sofosbuvir and ribavirin for 12-24 weeks have been evaluated as treatment for chronic genotype 1-4 HCV infection, with variable efficacy (high in genotype 2 [SVR12 88-97%], moderate in genotypes 1 [SVR12 68-85%] and 3 [SVR12 56-89%]) (12-14). The aim of this

study was to assess the efficacy of sofosbuvir and ribavirin for six weeks in individuals with recent HCV infection (estimated duration of infection less than 12 months).

## Methods

*Study design*

DAA-based therapy for recent HCV II (DARE-C II) was a prospective, open-label multicenter pilot study in which adults with recent HCV infection received sofosbuvir 400mg daily and weight-based ribavirin (<75kg: 1000mg/day; ≥75kg: 1200mg/day) for six weeks. Recent HCV infection was defined as initial detection of serum anti-HCV antibody and/or HCV RNA within six months of enrolment and either (i) documented recent HCV seroconversion (anti-HCV antibody negative result in the 18 months prior to enrolment) or (ii) acute clinical hepatitis (jaundice or alanine aminotransferase [ALT] greater than 10 times the upper limit of normal [ULN]) within the previous 12 months with the exclusion of other causes of acute hepatitis (15), with calculated estimated duration of infection less than 12 months at screening.

The decision regarding HCV treatment suitability and timing of treatment initiation was made by the investigator on an individual basis at site level. Sites were instructed to observe participants for four to 12 weeks between screening and baseline (treatment day 1), providing an opportunity to assess for spontaneous clearance.

*Study participants and setting*

Participants were enrolled between October 2014 and May 2015 through a network of tertiary hospitals in Australia (n=4) and New Zealand (n=1).

Adults (age ≥18 years) with recent HCV infection and HCV RNA ≥10,000 IU/mL at screening were eligible for study inclusion. Individuals with HIV co-infection were eligible provided: 1.

HIV infection >6 months duration and 2. CD4 count >200 cells/mm$^3$ and HIV viral load <50 copies/ml on stable combination antiretroviral therapy (cART) or 3. CD4 count ≥500 cells/mm$^3$ and HIV viral load <100,000 copies/mL not on cART. The following antiretroviral agents were not permitted based on drug-drug interactions and potential adverse events: didanosine (16), zidovudine (17) and ritonavir-boosted tipranavir (18). Individuals with acute or chronic HBV co-infection were excluded. For the complete inclusion and exclusion criteria, see Supplementary Material.

*Study definitions*

HCV virological suppression was defined as HCV RNA below the lower limit of quantitation (LLoQ) (target not detected [TND] or target detected, not quantifiable [TDnq]). An end-of-treatment response (ETR) was defined as serum HCV RNA below the LLoQ at the end of treatment. HCV virologic failure was defined as non-response (failure of virological suppression on-treatment with quantifiable HCV RNA at all time points between baseline and end of treatment), breakthrough (an increase from non-quantifiable to quantifiable HCV RNA or to at least 1 $\log_{10}$ above nadir while on treatment) or post-treatment relapse (the presence of quantifiable HCV RNA after an ETR, confirmed as homologous virus on sequencing of Core-E2 and/or NS5B regions as described previously) (19, 20). Reinfection was defined by the presence of quantifiable HCV RNA after an ETR and detection of infection with an HCV strain that was distinct from the primary infecting strain (heterologous virus on sequencing of Core-E2 and/or NS5B regions). Loss of HIV virologic control was defined as a confirmed HIV RNA of at least 400 copies/mL in individuals on cART. For further study definitions, see Supplementary Material.

*Study assessments*

Study visits were undertaken at baseline (treatment day 1), treatment day 2 and treatment weeks 1, 2, 3, 4 and 6 and post-treatment weeks 4, 12 and 24. The presence of HCV RNA was assessed at all scheduled study visits using COBAS Taqman HCV RNA assay, version 2.0 (LLoQ, 25 IU/mL; lower limit of detection [LLoD] 15 IU/mL; Roche Diagnostics, Branchburg, NJ, USA), with centralised testing performed at SydPath (390 Victoria St, Darlinghurst, NSW, Australia). HCV genotype was assessed at screening and in the setting of HCV RNA recurrence. Participants with HCV RNA recurrence had HCV RNA sequencing of CoreE2 and/or NS5B regions performed on the first available detectable HCV RNA sample and the first available detectable HCV RNA sample indicating HCV RNA recurrence. All participants were genotyped for IFN-λ 3/4 (IFNL3/4) *rs8099917* and *rs12979860* single nucleotide polymorphisms (SNPs), as described previously (21, 22). Adverse events were recorded from screening until post-treatment week 12.

Behavioural questionnaires were administered at screening, baseline, end-of-treatment, post-treatment week 12 and post-treatment week 24. The behavioural questionnaire included sections on demographics (age, sex, ethnicity, education, main source of income and accommodation), opioid substitution treatment (including methadone and buprenorphine), and injecting drug use. At screening, injecting drug use history was collected for lifetime (ever), previous six months (current) and the previous month (recent). Recent (previous month) associated risk behaviours including use of a new sterile needle/syringe for all injections, needle/syringe borrowing and lending, and ancillary injecting equipment sharing were also collected. Follow-up information on injecting drug use and associated risk behaviours in the previous month were used for subsequent

longitudinal analyses. Social functioning was assessed using the Opiate Treatment Index Social Functioning Scale (23).

Study drug adherence was assessed by pill count and self-reported adherence questionnaires at treatment weeks 2, 4 and 6 (end-of-treatment). Plasma samples were taken and stored for therapeutic drug monitoring at baseline hour 4, treatment day 2 and treatment weeks 1, 2, 3, 4 and 6. Ribavirin plasma concentrations (mg/L) were quantified for all of these time points using a validated High-Performance Liquid Chromatography (HPLC) assay with UV detection (HPLC-UV; λ 235 nm) (24), with the following HPLC conditions: Atlantis C18 column (Waters, Milan, Italy), mobile phase 50 mM potassium phosphate buffer, pH 3.23 at 1 mL/min, isocratic mode, temperature 35ºC.

*Study Outcomes*

The primary efficacy endpoint was SVR12, defined as serum HCV RNA below the LLoQ (target not detected [TND] or target detected, not quantifiable [TDnq]) at post-treatment week 12. Secondary virological endpoints included ETR, SVR4 (defined as serum HCV RNA below the LLoQ at post-treatment week 4) and SVR24 (defined as serum HCV RNA below the LLoQ at post-treatment week 24).

*Study oversight*

All study participants provided written informed consent before study procedures. The study protocol was approved by St Vincent's Hospital, Sydney Human Research Ethics Committee (primary study committee), as well as local ethics committees at all study sites. The study was conducted according to the Declaration of Helsinki and International

Conference on Harmonization Good Clinical Practice (ICH/GCP) guidelines. The study was registered with clinicaltrials.gov registry (NCT02156570).



*Statistical analysis*

Primary efficacy and safety data were analysed based on intention-to-treat (ITT) population, including all participants who received at least one dose of therapy. Loss to follow-up was deemed treatment failure. Additional per-protocol efficacy analysis included all participants who completed the intended treatment course and who had follow-up virological data to post-treatment week 12.

Categorical parameters were summarised as number and proportion. Continuous variables were summarised by either mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate. For all efficacy endpoints, means and proportions with two-sided 95% confidence intervals (CI) were determined. Categorical data was analysed using Fisher's exact test. Continuous variables were analysed using the Mann-Whitney U test. The proportion of individuals engaging in IDU and associated risk behaviours during treatment and follow-up were assessed up until post-treatment week 12. Changes in injecting behaviour between screening, end of treatment and post-treatment week 12 were compared using the McNemar test (exact binomial probability). All statistical tests were two-sided with a significance level of 0.05. Analysis was performed using STATA (version 14.0; StataCorp, College Station, TX).

## RESULTS

### Participant disposition and overview of the study population

Between October 2014 and May 2015, 26 individuals were screened and 19 enrolled (**Figure 1**). Participants were predominantly male (89%), with genotype 1 (G1) (68%) and genotype (G3) (26%) infection (**Table 1**). HIV co-infection was documented in 74%. Sixty-eight percent (n=13) of participants had acute clinical hepatitis at the time of diagnosis and the remaining 32% (n=6) demonstrated asymptomatic anti-HCV antibody seroconversion. Twenty-one percent of those with acute clinical hepatitis had a symptomatic seroconversion illness (n=4, including 2 with jaundice) and 63% (n=12) had an ALT greater than 400 U/L. The predominant modes of acquisition were IDU (53%, n=10) and sexual exposure in men-who-have-sex-with-men (MSM) (47%, n=9). Median estimated duration of infection at screening and baseline were 32 (range 9–51) and 37 (range 12-55) weeks, respectively.

Eighty-four percent (n=16) of participants had ever injected drugs with 58% (n=11) reporting IDU within the last 6 months (**Table 2**). Among participants who reported IDU ever, median age at first injecting was 28 years (IQR 19-42), with older age at first injecting in those with HIV co-infection (median age 30 [IQR 27-42] vs 19 [IQR 18-19]; p=0.049). Among those reporting recent IDU at screening (n=6), the drugs most often injected were methamphetamine (83%) and heroin (17%).

### Efficacy

By ITT analysis, SVR12 was achieved in 32% (6/19; 95% CI 13%, 57%) (**Figure 2**). By genotype, SVR4 and SVR12 were 31% (4/13) and 23% (3/13), respectively, in G1, 100% (1/1) and 100% (1/1), respectively, in G2 and 60% (3/5) and 60% (3/5), respectively, in G3

(Supplementary Table 1). In those with HIV co-infection, SVR4 and SVR12 were 36% (5/14) and 21% (3/14), respectively, as compared with 60% (3/5) and 60% (3/5), respectively, in those with HCV mono-infection (SVR4, p=0.603; SVR12, p=0.262) (Supplementary Table 2). By per-protocol analysis, SVR12 was 33% (6/18; 95% CI 13%, 59%).

Virological suppression at end of treatment was documented in 89% (17/19) with similar results by HIV status (HCV mono-infection 100%, 5/5; HIV/HCV co-infection 86%, 12/14; p=1.000) (**Figure 2**). HCV RNA was below the LLoQ in 47%, 74% and 79% at weeks 1, 2 and 4 of treatment, respectively (**Figure 3** and Supplementary Figure 1). A rapid biochemical response on treatment was observed in the majority (Supplementary Figure 2); median ALT at baseline and end of treatment were 133 U/L (IQR 54-271) and 22 U/L (IQR 18-25), respectively (p<0.001).

SVR24 was 37% (7/19; 95% CI 16%, 62%) (**Figure 2**). SVR24 was confirmed in one HIV-positive G3 male participant who had detectable HCV RNA at end-of-treatment (HCV RNA 31 IU/mL). He developed a clinical acute hepatitis at post-treatment week 4 (ALT 780 U/L; HCV RNA 136400 IU/mL), which resolved by post-treatment week 12 (ALT 25 U/L; HCV RNA 50 IU/mL). HCV RNA was not detected (TND) at post-treatment week 24 and confirmed at post-treatment weeks 28 and 48.

Treatment efficacy was related to baseline HCV RNA (**Figure 4**) and early on-treatment viral kinetics (**Figure 3**). Median $\log_{10}$ HCV RNA at baseline and treatment day 2 were significantly lower in those achieving SVR12 (baseline: 4.47 IU/mL [IQR 3.48-4.87] vs 6.26 IU/mL [IQR 5.36-6.82], p=0.014; day 2: 3.08 IU/mL [IQR 2.06, 3.27] vs 4.85 IU/mL [IQR 4.07-

5.39]; p=0.008). Fifty-five percent (6/11) of participants with baseline HCV RNA ≤6 $\log_{10}$ IU/mL achieved SVR12 compared to 0% (0/8) of those with baseline HCV RNA >6 $\log_{10}$ IU/mL (p=0.018). Sixty-seven percent (6/9) of participants with HCV RNA below the LLoQ (TND or TDnq [<15 IU/mL]) at week 1 achieved SVR12 as opposed to 0% (0/10) with quantifiable HCV RNA at week 1 (p=0.006) (**Figure 3**). There was no association between SVR12 and HCV RNA below the LLoQ at week 2 (p=0.114) or week 4 (p=0.515). Only two of the study participants were female, both of whom achieved SVR12 (p=0.088). Similarly, both participants with jaundice at diagnosis of acute HCV infection achieved SVR12 (p=0.088). SVR12 was not associated with any other baseline or on-treatment factors, including duration of infection, HIV infection, or rs8099917/rs12979860 SNP.

**Virological Failure, Relapse and Reinfection**

At post-treatment week 12, 13 (68%) of 19 had treatment failure. One participant discontinued after only two weeks treatment and was lost to follow up (LTFU, HCV RNA TND at end of treatment). The remaining 12 participants demonstrated virological failure: two non-response, nine post-treatment relapse and one reinfection. Both participants with virological non-response had high HCV RNA at baseline (>6.8 $\log_{10}$ IU/mL). Virological failure (excluding the two participants with reinfection and LTFU) was associated with baseline HCV RNA >6 $\log_{10}$ IU/mL (100% [8/8] vs 33% [3/9], p=0.009).

Reinfection was documented in an HIV-positive male participant with recurrence of HCV viraemia between post-treatment weeks four and 12, with a switch from G3a at screening to G1a at post-treatment week 12, confirmed on sequencing of CoreE2. Ongoing IDU and high-risk sexual behaviour were reported.

**Treatment adherence**

Eighteen (95%) participants completed the scheduled six week treatment course. Adherence to therapy was high, by pill count and self-reported questionnaire. Sofosbuvir 90/90 and 100/100 adherence were 89% and 79%, respectively, with mean on-treatment sofosbuvir adherence 96.24% (SD 13.62%). Ribavirin 90/90 and 100/100 adherence were 89% and 84%, respectively, with mean on-treatment ribavirin adherence 98.18% (SD 4.73%) (Supplementary Figure 3).

Consistent with self-reported adherence, when sampled, all participants had detectable ribavirin plasma concentrations on treatment. Median plasma ribavirin concentration at week 4 was 2.1 mg/L (range 1.00, 5.68) (Supplementary Figure 4). Although median ribavirin plasma concentration was numerically lower in those who did not achieve SVR12, there was no significant association between SVR12 and plasma ribavirin concentration at week 4 ($p=0.745$) or week 6 ($p=0.688$), with a median week 4 RBV plasma concentration of 2.39 mg/L (IQR 1.00, 4.38) in participants achieving SVR12 as compared with 1.87 mg/L (IQR 1.35, 5.68) in participants with treatment failure. Both participants with virological non-response reported 100/100 adherence to sofosbuvir and ribavirin. At end of treatment, ribavirin plasma concentrations were 1.78 mg/L and 3.24 mg/L, respectively, with corresponding declines in haemoglobin of 22 and 24 g/L.

**Safety**

The safety profile was consistent with the reported side effects of sofosbuvir and ribavirin to date with no decompensated liver disease, death or treatment discontinuation due to

adverse events (**Table 3**). One or more clinical adverse events were reported by 14 participants (74%), with most adverse events of mild (68%) or moderate (29%) severity. No ribavirin dose modification was required. In those with HIV infection, median change in CD4 count at end of treatment was $57x10^6$/L (IQR -33–108) with no loss of HIV virological control. Two serious adverse events (SAE) were reported: acute kidney injury requiring hospitalisation; and *Escherichia coli* bloodstream infection (urinary source) requiring hospitalisation. Both SAEs resolved without sequelae and were deemed by the investigator to be unrelated to study drug administration.

**Longitudinal behavioural assessment**

Injecting drug use risk behaviour (in the past 30 days) was assessed during study follow up. The proportion reporting recent IDU was 32% (n=6) prior to enrolment, 42% (n=8) prior to baseline, 32% (n=6) prior to end of treatment, and 32% (n=6) prior to post-treatment week 12. The proportion reporting needle and syringe borrowing was 33% (n=2) prior to enrolment, 29% (n=2) prior to baseline and 20% (n=1) prior to end of treatment. No-one reported needle and syringe borrowing between end of treatment and post-treatment week 12. There was no significant change in injecting drug use (p=0.317) or needle and syringe borrowing (p=0.157) between enrolment and post-treatment week 12.

**Conclusions**

While six weeks of sofosbuvir and ribavirin was safe and well tolerated, efficacy was poor with SVR12 only 32%. This study conclusively demonstrates that while on-treatment virological suppression was achieved in the majority, a high proportion of participants demonstrated post-treatment relapse. Baseline HCV RNA ≤6 $\log_{10}$ IU/mL and subsequent rapid viral suppression (HCV RNA below the LLoQ [TND or TDnq] at week 1 and HCV RNA TND at week 2) were associated with SVR12, which supports further research with more potent DAA regimens in recent HCV infection. In this cohort, while those participants achieving SVR12 did have significantly lower HCV RNA at baseline, spontaneous clearance was unlikely, given the median duration of HCV infection of 37 weeks.

Very limited evidence exists for the use of DAA therapy in the setting of acute HCV infection with no published IFN-free DAA trials to date. Fierer et al performed a pilot study in HIV-positive MSM with recent HCV G1 infection and administered PEG-IFN, ribavirin and telaprevir (25). Risk factors for acquisition were not reported. By ITT, SVR12 was 84% (16/19). Treatment duration was response-guided; of those who achieved SVR12, most (13/16; 81%) received 12 weeks of therapy. In the DARE-C I open-label pilot study, response-guided therapy (8-24 weeks) with PEG-IFN, ribavirin and telaprevir for recent HCV infection (duration of infection 6-18 months) was effective in the majority (SVR12 71%; 10/14), regardless of HIV co-infection, although similar to that observed with PEG-IFN and ribavirin alone in this population (26).

IFN-free DAA therapy offers significant promise for individuals with recent HCV infection; a summary of registered IFN-free DAA clinical trials in recent HCV infection is presented in

Supplementary Table 5. DARE-C II is one of the first studies of IFN-free DAA therapy in this population although the regimen used in this study (a single DAA combined with ribavirin) is now regarded as suboptimal in patients with chronic HCV G1 and G3 infection (27). In contrast, combinations of 2 or more different classes of DAAs have achieved SVR in greater than 95% of individuals with chronic HCV infection (5, 7, 8). Preliminary results from recent pilot studies have reported encouraging results with short duration DAA therapy in acute HCV infection. In symptomatic acute HCV G1 mono-infection, very high SVR was demonstrated with 6 weeks of sofosbuvir/ledipasvir (SVR12 ITT 100%, 20/20); PWID were excluded (28). Notably, baseline HCV RNA was low in the majority (≤6 $\log_{10}$ IU/mL, 90%; ≤3 $\log_{10}$ IU/mL, 45%) and the proportion who may have demonstrated spontaneous clearance is unclear. Again, in acute HCV G1 mono-infection (PWID included), high SVR was demonstrated with 4 weeks of sofosbuvir/ledipasvir (SVR12 ITT 100%, 14/14) and 8 weeks of sofosbuvir plus simeprevir (SVR12 ITT 87%, 13/15) (29). In keeping with our findings, viral suppression was rapid in with HCV RNA below the limit of detection in 93% at week 1 in both arms. Similarly, in acute HCV G1 and G4 with HIV co-infection, SVR12 ITT was 77% (20/26; relapse, n=3; reinfection, n=1; LTFU, n=2) following 6 weeks of sofosbuvir/ledipasvir (SVR12 per-protocol analysis: 83% [20/24]) (30). Consistent with our results, the three participants with relapse had high baseline HCV RNA (>6.9 $\log_{10}$ IU/mL), suggesting that even with potent DAA regimens, viral suppression may be protracted in those with a high HCV burden. Again, these data support further research with contemporary DAA regimens in acute, particularly asymptomatic, HCV infection.

Internationally, increasing HCV incidence has been observed in HIV-positive MSM, largely associated with sexual and non-IDU behaviour (31, 32). High reinfection incidence following

SVR has been observed in some cohorts (33, 34). Prior to post-treatment week 12, one case of reinfection was demonstrated in an HIV-positive male, who reported concomitant IDU (with sharing of needles, syringes and other paraphernalia) and high-risk sexual behaviours (including multiple casual male partners, group sex and unprotected anal intercourse). The risk for reinfection following treatment for recent HCV infection in those individuals with ongoing high risk behaviour emphasises the need for post-treatment surveillance, harm reduction strategies and education (33, 35). Of note, no change in IDU behaviour was observed in participants longitudinally in this study; follow up is ongoing. Despite concerns regarding non-adherence and reinfection following treatment in PWID, the available evidence would indicate that active drug use does not significantly compromise the long term benefit of treating recent HCV infection with either IFN-containing or IFN-free regimens (36-38). Individuals with recent HCV infection should be offered antiviral therapy together with education and harm minimisation, in order to reduce high-risk behaviours (39) and the subsequent risk of reinfection.

The potential for broad access to highly effective, well tolerated IFN-free DAA regimens has stimulated discussion around HCV treatment-as-prevention and subsequent HCV elimination. Modelling suggests that substantial reductions in incidence and chronic HCV prevalence could be achieved by targeted DAA treatment scale-up amongst PWID and HIV-positive MSM at high risk of ongoing transmission (10, 40, 41). Importantly (and in opposition to many country and region specific DAA restrictions), a treatment scale-up model in HIV-positive MSM predicted that the greatest impact on HCV incidence and prevalence would be achieved if treatment was prioritised to those with recently diagnosed (<1 year) HCV infection, regardless of disease stage, and occurred when combined with

behavioural interventions (10). Further, modelling has demonstrated the cost-effectiveness of DAA treatment scale-up among active PWID (41). International guidelines recommend that "treatment should be prioritized regardless of the fibrosis stage for individuals at risk of transmitting HCV, including active injection drug users" (2). However, due to high drug pricing, access to IFN-free DAA therapy is restricted, even in high-income settings: by and within countries, by fibrosis stage and by former or current substance misuse (42). If HCV elimination is to be achieved, expedient, widespread access to DAA therapy, without liver disease stage or drug use restriction, will be required.

Sample size and the applicability of the DAA regimen used may be considered study limitations. Given the pace of change in HCV therapeutics, the DAA regimen used, sofosbuvir plus ribavirin, is suboptimal in chronic HCV G1 and G3 infection (27). However, DARE-C II was designed as a proof-of-concept study of IFN-free DAA therapy in individuals with recent HCV infection. It confirmed the feasibility of ultrashort IFN-free DAA therapy in those with recent HCV infection, regardless of treatment regimen, with excellent adherence in this historically difficult-to-treat population. DARE-C II has informed future trial design in this population, exemplified by an upcoming international randomised control trial of ultrashort versus standard duration sofosbuvir/velpatasvir for recent HCV infection (NCT02625909). Despite the small sample size, the study also conclusively demonstrated the suboptimal efficacy of sofosbuvir and ribavirin for only six weeks.

The treatment paradigm for individuals with HCV infection is evolving rapidly. Combining two or more potent DAAs from different classes has increased SVR (>95%) and shortened treatment duration to only 8 weeks in most populations with chronic HCV infection (5, 7, 8).

While these excellent results in chronic HCV infection have cast some doubt on any "efficacy advantage" of early treatment in acute HCV infection (4), the potential reduction in transmission amongst PWID and HIV-positive MSM will be beneficial (10, 11). HCV treatment-as-prevention strategies will be enhanced by early diagnosis and increased treatment uptake in recent HCV infection.

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**Figure Legends**

**Figure 1. Patient disposition.**

**Figure 2. Primary and secondary efficacy endpoints (intention-to-treat population).**

Total study population, n=19; HCV mono-infection, n=5; HIV/HCV co-infection, n=14.

Abbreviations: End of treatment response (ETR); sustained virological response (SVR)

**Figure 3. Proportion of participants with HCV RNA below the LLoQ (TND and TDnq, <15 IU/mL) on and post-treatment.**

** At week 1, 47% (n=9) of participants had HCV RNA TND or TDnq (<15 IU/mL) and 26% (n=5) had HCV RNA TND; 66% (n=6) and 80% (n=4), respectively, achieved SVR12 (HCV RNA TND or TDnq week 1, p=0.006; HCV RNA TND week 1, p=0.017).

* At week 2, 47% (n=9) of participants had HCV RNA TND; of these, 56% (n=5) achieved SVR12 (p=0.057). Excluding those participants with loss to follow up (n=1) and reinfection (n=1), SVR12 was associated with HCV RNA TND at week 2 (p=0.035), but not HCV RNA TND or TDnq (p=0.102).

Abbreviations: Baseline (BL); day 2 (D2); end of treatment (EOT); lower limit of detection (LLoD); lower limit of quantitation (LLoQ); PT (post treatment); screening (SCR); sustained virological response (SVR); week 1 (W1); week 2 (W2); week 4 (W4)

**Figure 4. Treatment outcome by baseline HCV RNA ($log_{10}$ IU/mL).**

Note, 1. ETR documented in participant prior to LTFU and 2. SVR4 documented in participant prior to reinfection at post-treatment week 12.

Abbreviations: Sustained virological response (SVR), lost to follow up (LTFU); end-of-treatment response (ETR)

**Table 1. Participant demographic, clinical and viral enrolment characteristics**

| Enrolment characteristics | Total study population (n=19) |
|---|---|
| Age (years), median (IQR) | 41 (31-50) |
| Male, n (%) | 17 (89) |
| Weight (kg), median (IQR) | 76.0 (64.0-89.7) |
| BMI (kg/m$^2$), median (IQR) | 22.6 (21.4-26.2) |
| Caucasian ethnicity, n (%) | 14 (74) |
| Higher education or qualification [a], n (%) | 15 (79) |
| Full or part time employment, n (%) | 14 (74) |
| Social functioning score, median (IQR) | 14 (8-16) |
| HIV infection, n (%) | 14 (74) |
| CD4 count ($10^6$/L), median (IQR) | 598 (486-752) |
| HIV VL ≤50 at screening, n (%) | 11 (79) [b] |
| On cART, n (%) | 12 (86) [c] |
| Mode of HCV acquisition, n (%) | |
| Injecting drug use | 10 (53) |
| Sexual exposure – MSM | 9 (47) |
| Estimated duration of infection (weeks) | |
| At screening, median (IQR) | 32 (23-35) |
| At baseline, median (IQR) | 37 (27-41) |
| Acute HCV [c], n (%) | 6 (32) |
| Presentation of recent HCV, n (%) | |

| | |
|---|---|
| Acute clinical illness – symptomatic | 4 (21) |
| Jaundice | 2 (11) |
| Nausea/vomiting | 3 (16) |
| Abdominal pain | 1 (5) |
| Fever | 1 (5) |
| Acute clinical illness - ALT >10x ULN | 12 (63) |
| Asymptomatic seroconversion | 6 (32) |
| ALT (U/L), median (IQR) | |
| Peak ALT prior to enrolment | 507 (228-959) |
| ALT at screening | 139 (78-323) |
| $Log_{10}$ HCV RNA at baseline, median (IQR) | 5.4 (4.4-6.8) |
| HCV genotype (and subtype), n (%) | |
| 1a | 13 (68) |
| 2b | 1 (5) |
| 3a | 4 (21) |
| 3, unable to subtype | 1 (5) |
| IFNL3/4 (formerly IL28B) SNPs, n (%) | |
| rs12979860 CC | 7 (37) |
| rs8099917 TT | 14 (74) |
| Median liver stiffness measurement (Fibroscan®), kPa (IQR) | 6.5 (5.3-7.4) |

[a] Completed higher technical qualification/TAFE/College/university degree

[b] HIV RNA >50 copies/mL in 1 HIV-positive participant on cART at screening (HIV RNA 149 copies/mL)

[c] Acute HCV infection (duration of infection <24 weeks) at screening

Abbreviations: Combination antiretroviral therapy (cART), men-who-have-sex-with-men (MSM), SNP (single nucleotide polymorphism)

**Table 2. History of substance use at enrolment**

| Substance use characteristics | Total study population (n=19) |
|---|---|
| Injecting drug use, n (%) | |
| Ever | 16 (84) |
| Current [a] | 11 (58) |
| In those reporting injecting drug use: | |
| Age at first injecting, median (IQR) | 28 (19-42) |
| Last injected within last month, n (%) | 6 (38) |
| Last injected between 1-6 months ago, n (%) | 5 (31) |
| Last injected >6 months ago, n (%) | 5 (31) |
| If injected in the previous 1 month, frequency (n, %): | |
| Daily | 1 (17) |
| More than weekly, not daily | 1 (17) |
| Less than weekly | 4 (67) |
| Drug injected most in last month, n (%) | |
| Amphetamines | 5 (83) |
| Heroin | 1 (17) |
| Opioid substitution therapy, n (%) | |
| Ever | 1 (5) |
| Current | 1 (5) |
| Alcohol use, n (%) | |
| None | 3 (16) |

| | |
|---|---|
| 1-2 standard drinks per week [b] | 7 (37) |
| >2 standard drinks per week [b] | 9 (47) |

[a] Current injecting drug use refers to use within 6 months of screening

[b] One standard drink equates to 10 grams of alcohol

**Table 3. Safety parameters**

| **Safety parameters** | **Total treated population (n=19)** |
|---|---|
| *Clinical parameters* | |
| Total number of adverse events | 41 |
| Grade 3 or 4, n (%) | 1 (2) |
| Serious adverse events, n (%) | 2 (11) |
| Discontinuation due to adverse event, n (%) | 0 |
| Clinical adverse events | |
| *Common (>10% of study population), n (%)* | |
| Headache | 4 (21) |
| Fatigue | 3 (16) |
| Insomnia | 3 (16) |
| Nausea | 3 (16) |
| Sleep disorder | 3 (16) |
| Diarrhoea | 2 (11) |
| Musculoskeletal chest pain | 2 (11) |
| Pruritus | 2 (11) |
| Rash | 2 (11) |
| *Laboratory parameters* | |
| Median reduction in Hb at end of treatment, g/L (IQR) | 14 (2-23) |
| Decrease in Hb >30g/L, n (%)* | 2 (11) |
| Decrease in Hb <100g/L, n (%) | 2 (11) |

*Decease in Hb >30g/L between baseline and end of treatment

Abbreviations: Absolute neutrophil count (ANC); haemoglobin (Hb); platelet (plt)

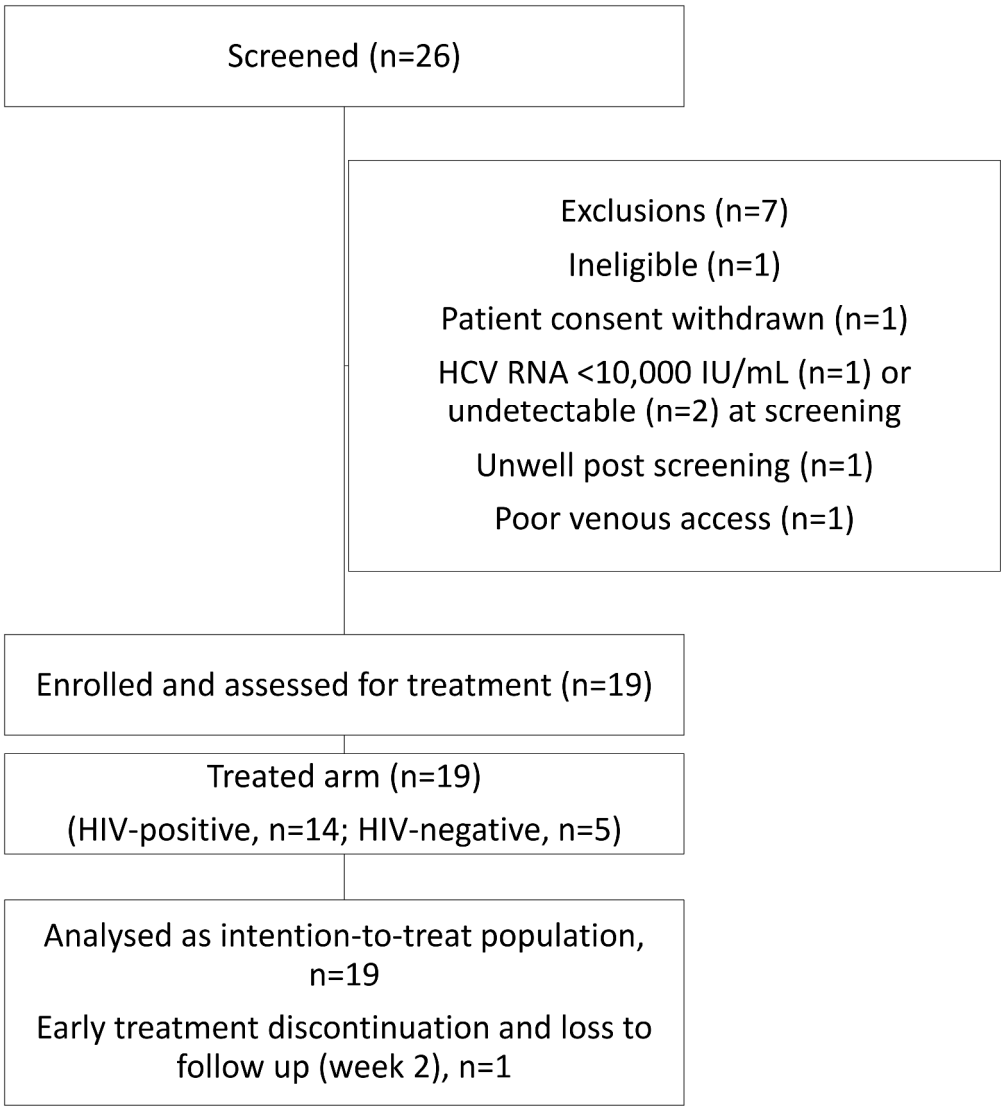


Figure 1. Patient disposition.

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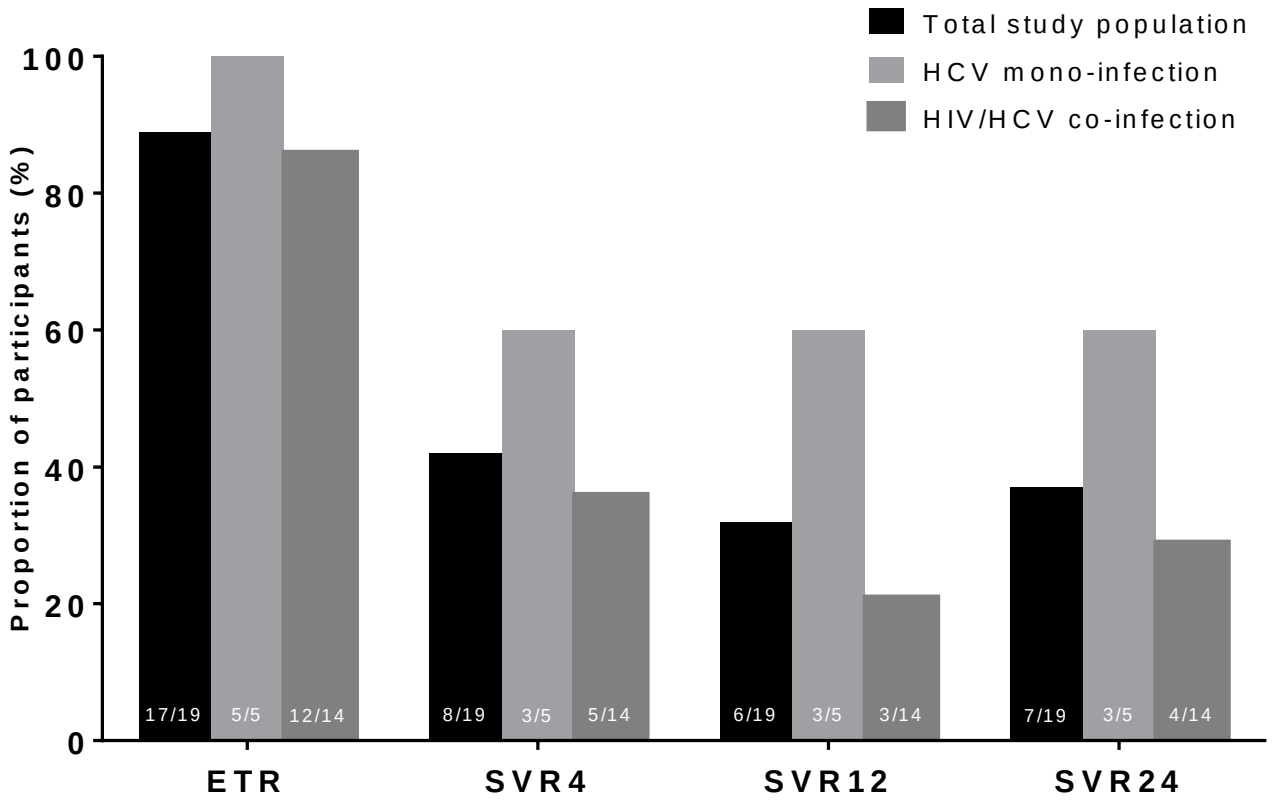

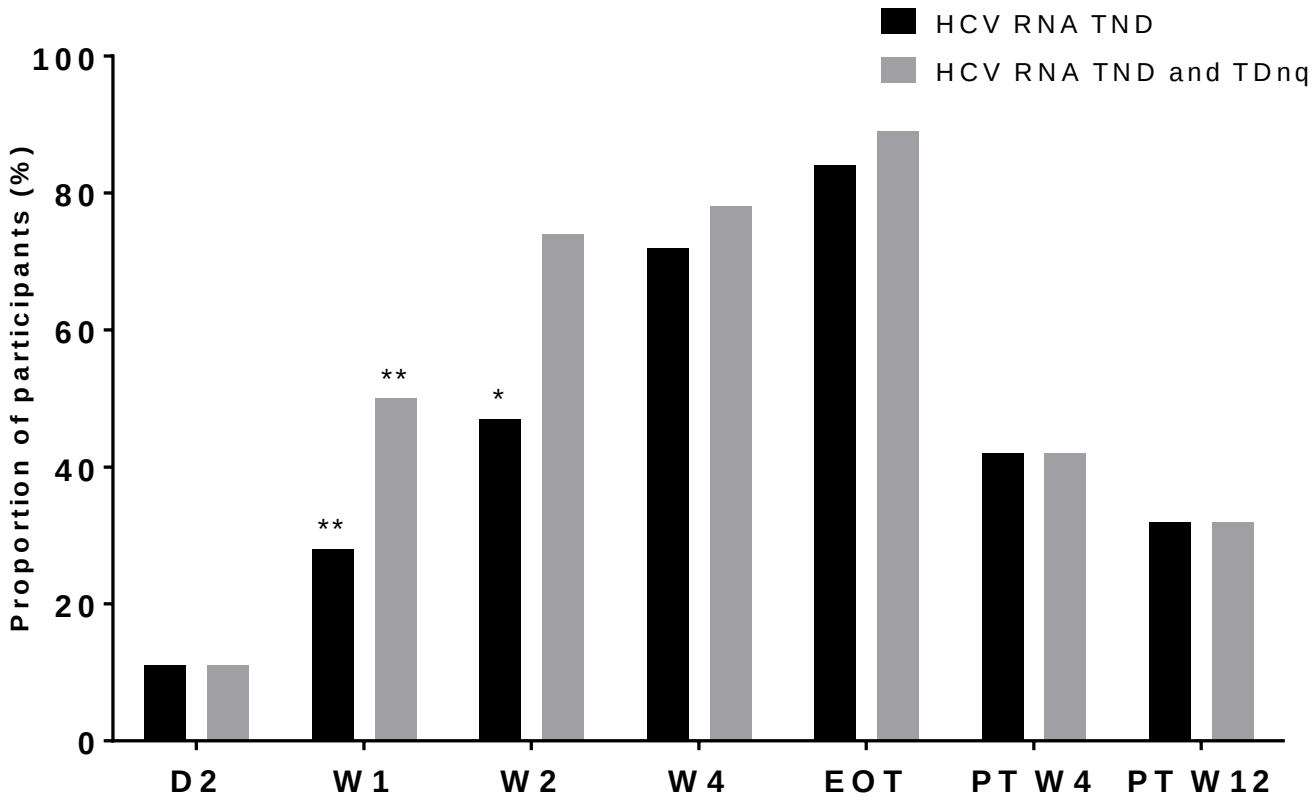

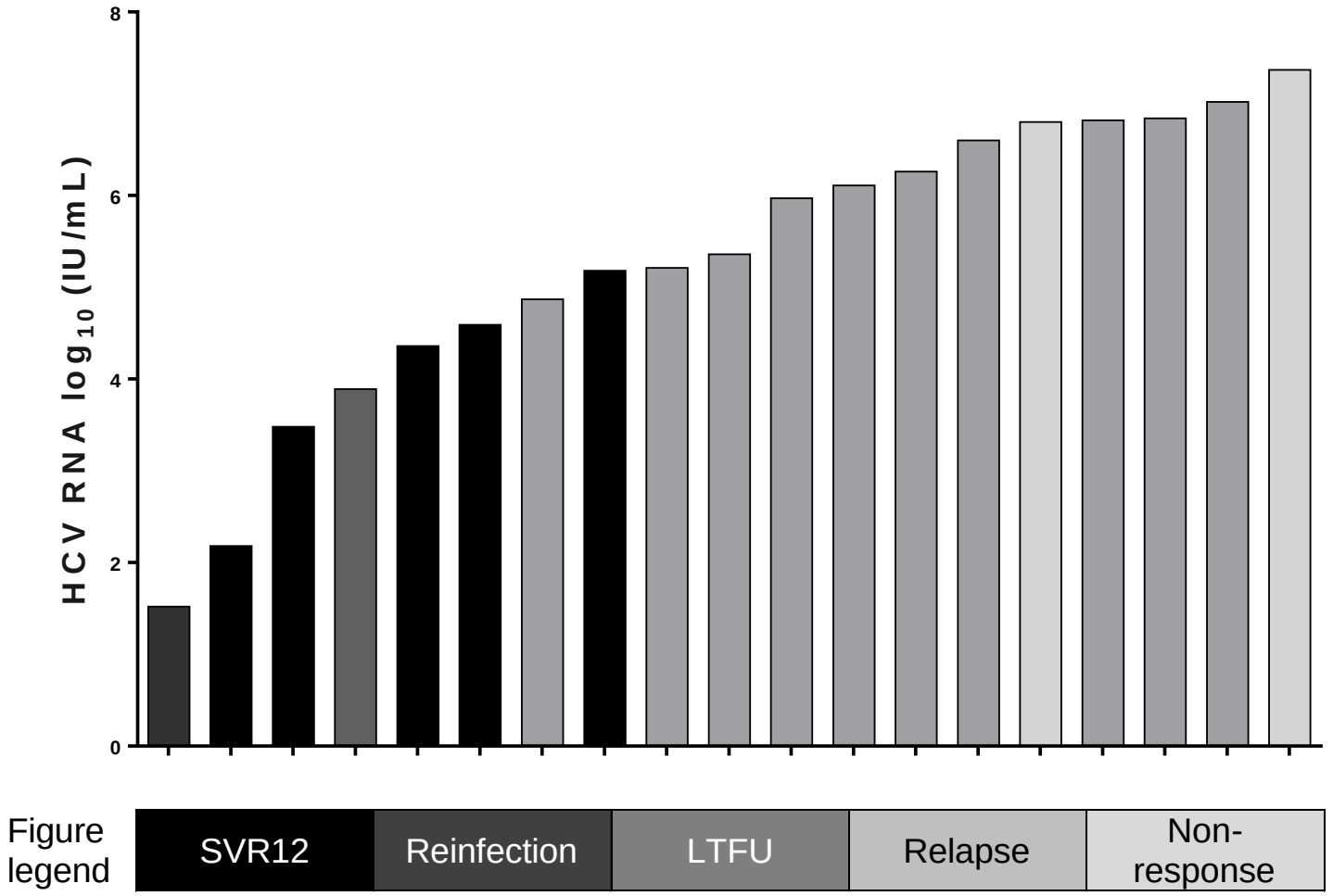

**Supplementary Material**

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# Sofosbuvir and ribavirin for six weeks is not effective among people with recent HCV infection: The DARE-C II study

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## Methods

*Inclusion and exclusion criteria*

Adults (age ≥18 years) with recent HCV infection, HCV RNA ≥10,000 IU/mL at screening and hepatitis B surface antigen negative were eligible for study inclusion. The following additional inclusion criteria were required for HIV-positive individuals: 1. HIV infection >6 months duration and 2. CD4 count >200 cells/mm$^3$ and HIV viral load <50 copies/ml on stable combination antiretroviral therapy (cART) or 3. CD4 count ≥500 cells/mm$^3$ and HIV viral load <100,000 copies/mL not on cART. The following antiretroviral agents were not permitted based on drug-drug interactions and potential adverse events: didanosine (1), zidovudine (2) and ritonavir-boosted tipranavir (3).

Exclusion criteria included: pregnant women or male partners of pregnant women; breast feeding; systemic anti-neoplastic or immunomodulatory therapy ≤6 months prior to first dose of study drug; any investigational drug ≤6 weeks prior to first dose of study drug; positive anti-HAV IgM Ab or anti-HBc IgM antibody at screening; alternative aetiology of chronic liver disease; decompensated liver disease; hepatocellular carcinoma (HCC); prior treatment with HCV protease or polymerase inhibitors; severe retinopathy; severe seizure disorder; immunologically mediated disease, chronic pulmonary disease with functional limitation, severe cardiac disease, organ transplantation (apart from corneal, skin or hair graft), malignancy, or other severe illness (including psychiatric) which in the opinion of the investigator would compromise the participants safety or ability to comply with the protocol; and the following lab values at screening: neutrophil count <1500 cells/mm3, platelet count <90,000 cells/mm3, creatinine >1.5 times the upper limit of normal (ULN), haemoglobin <12 g/dL in women or <13 g/dL in men, haemoglobin A1c ≥8.5%, International

Normalised Ratio (INR) ≥1.5, serum albumin <33 g/L, serum total bilirubin >1.8 times the ULN (unless isolated in subjects with Gilbert's syndrome).

*Study definitions*

The presentation of recent HCV infection at the time of diagnosis was classified as either acute clinical or asymptomatic infection. Acute clinical infection included participants with a documented clinical history of symptomatic seroconversion illness (including, but not limited to, the presence of jaundice, nausea/vomiting, abdominal pain, fever and hepatomegaly) and those without clinical symptoms but with a documented peak ALT >400 U/L within the 12 months prior to diagnosis. Asymptomatic infection included participants with anti-HCV antibody seroconversion but no acute clinical symptoms or documented peak ALT >400 U/L.

The duration of HCV infection at screening and baseline was calculated from the estimated date of infection. The estimated date of clinical infection was calculated as six weeks before onset of seroconversion illness or six weeks before the first ALT >400 U/L. The estimated date of asymptomatic infection was calculated as the midpoint between the last negative anti-HCV antibody and the first positive anti-HCV antibody. For participants who were anti-HCV antibody negative and HCV-RNA positive at screening, the estimated date of infection was six weeks before enrolment, regardless of symptom status.

On-treatment adherence was calculated for each medication individually (sofosbuvir and ribavirin) by subtracting the number of missed doses from the total number of doses prescribed for therapy duration and dividing by the total number of doses prescribed for

therapy duration. By pill count and self-reported questionnaire, compliance with sofosbuvir and ribavirin were individually calculated at the 90/90 and 100/100 adherence levels, defined as receipt of ≥90 or 100% of scheduled sofosbuvir or ribavirin doses for ≥90 or 100% of the scheduled treatment period, respectively.

HCV virological suppression was defined as HCV RNA below the lower limit of quantitation (LLoQ) (target not detected [TND] or target detected, not quantifiable [TDnq]). An end-of-treatment response (ETR) was defined as serum HCV RNA below the LLoQ at the end of treatment. HCV virologic failure was defined as non-response (failure of virological suppression on-treatment with quantifiable HCV RNA at all time points between baseline and end of treatment), breakthrough (an increase from non-quantifiable to quantifiable HCV RNA or to at least 1 $\log_{10}$ above nadir while on treatment) or post-treatment relapse (the presence of quantifiable HCV RNA after an ETR, confirmed as homologous virus on sequencing of Core-E2 and/or NS5B regions as described previously) (4, 5). Reinfection was defined by the presence of quantifiable HCV RNA after an ETR and detection of infection with an HCV strain that was distinct from the primary infecting strain (heterologous virus on sequencing of Core-E2 and/or NS5B regions). Loss of HIV virologic control was defined as a confirmed HIV RNA of at least 400 copies/mL in individuals on cART.

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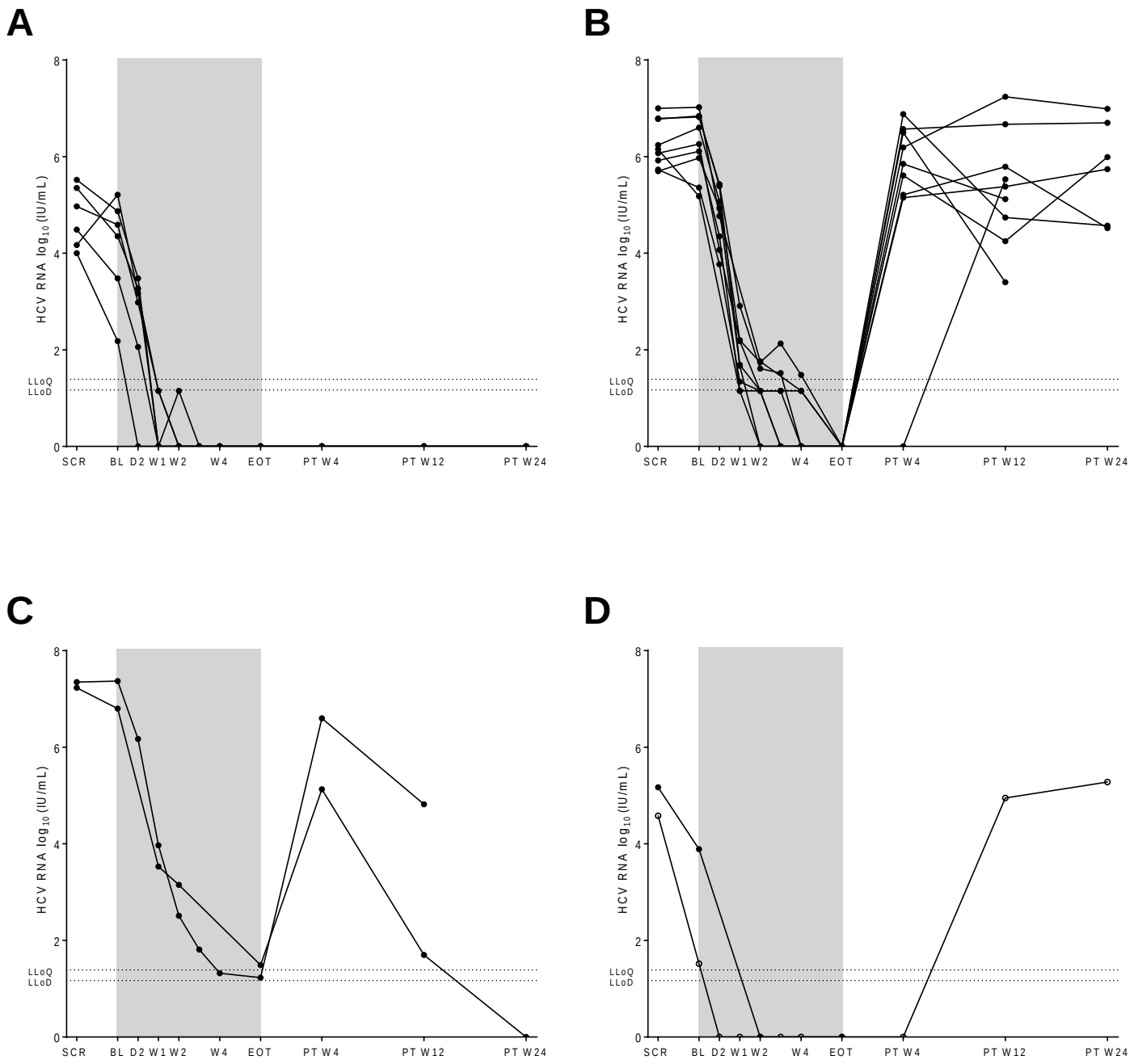
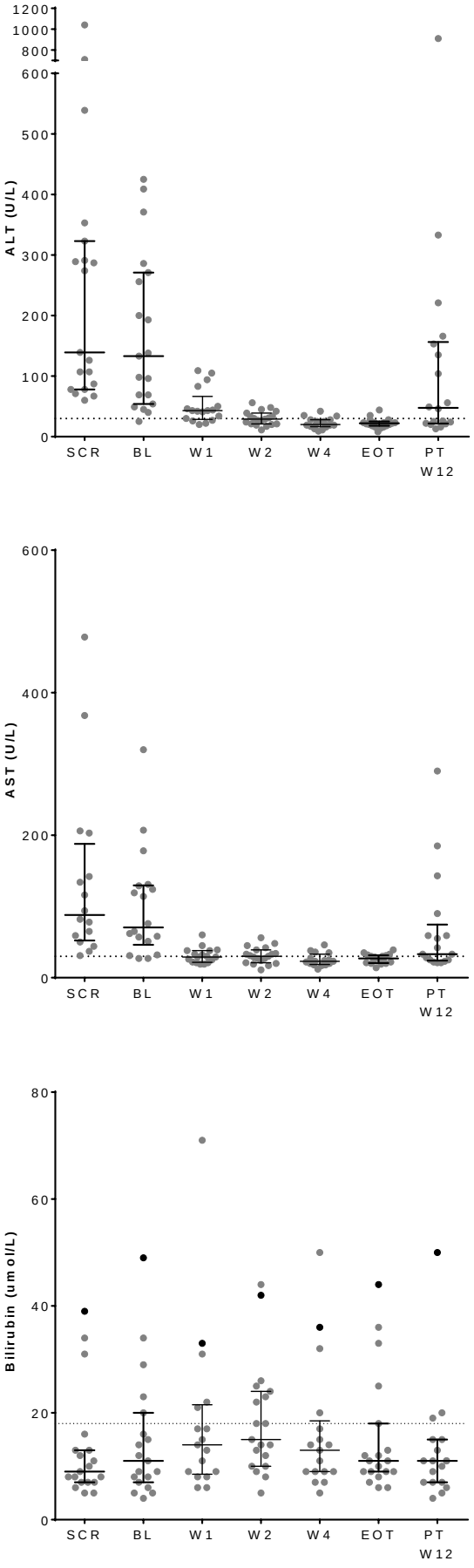
## Figures



**Supplementary Figure 1**. **On-treatment viral kinetics.**

Panel A: Sustained virological response (n=6). Panel B: Relapse (n=9). Panel C: Non-response (n=2). Panel D: Reinfection (n=1; depicted by open circles) and LTFU (n=1). Grey shaded area indicates period on treatment.

Abbreviations: Baseline (BL); day 2 (D2); end of treatment (EOT); lost to follow up (LTFU); lower limit of detection (LLoD); lower limit of quantitation (LLoQ); PT (post treatment); screening (SCR); sustained virological response (SVR); week 1 (W1); week 2 (W2); week 4 (W4)

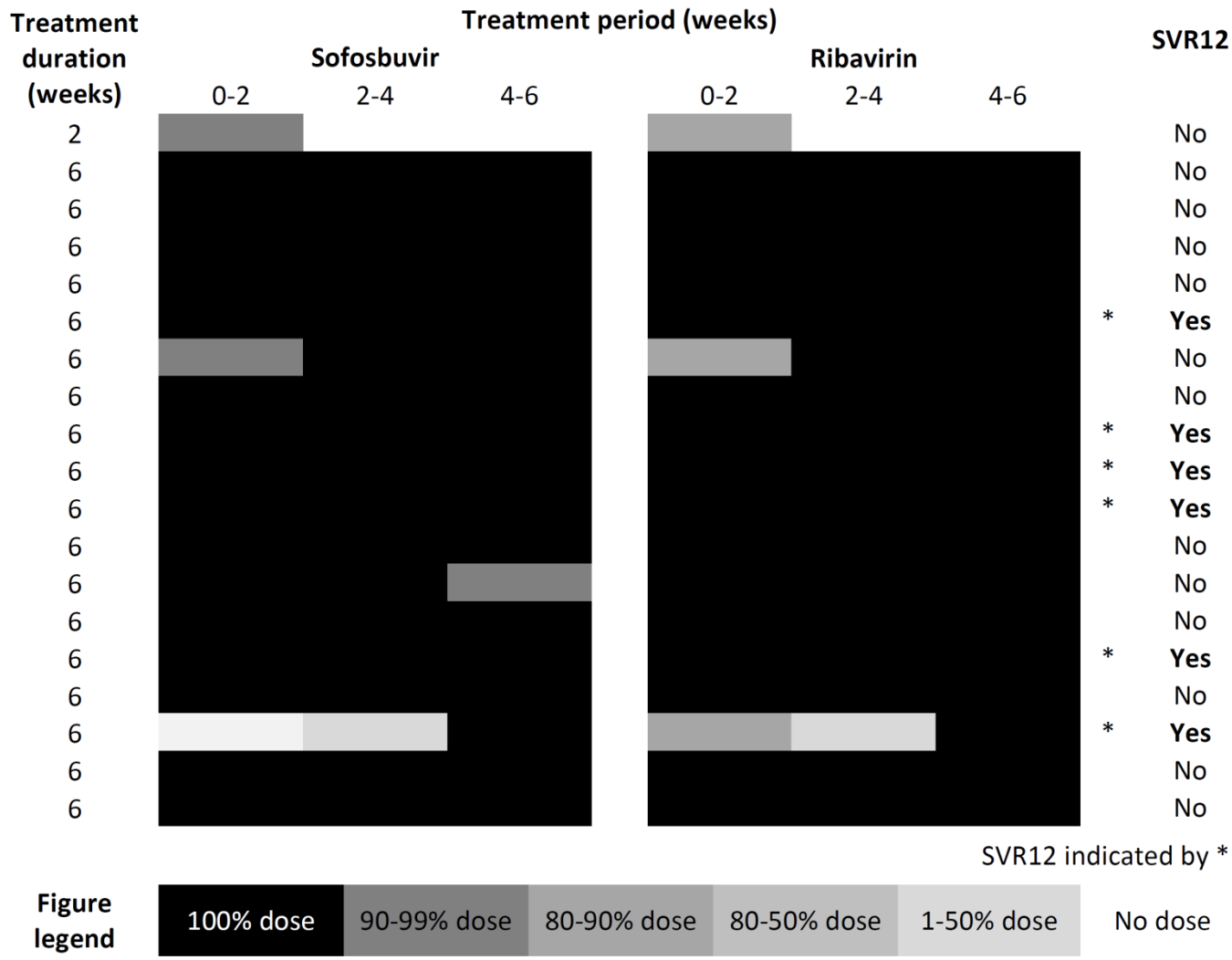


**Supplementary Figure 2. Change in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin prior to, on and post-treatment.**

Panel A: ALT. Panel B: AST. Panel C: Total bilirubin. Bars depict median with interquartile range. Dotted line at upper limit of normal (ULN) for each parameter - ALT and AST, ULN 30 U/L; total bilirubin ULN 18 umol/L. In Panel C, black circle indicates the single HIV-positive participant receiving ritonavir-boosted atazanavir.

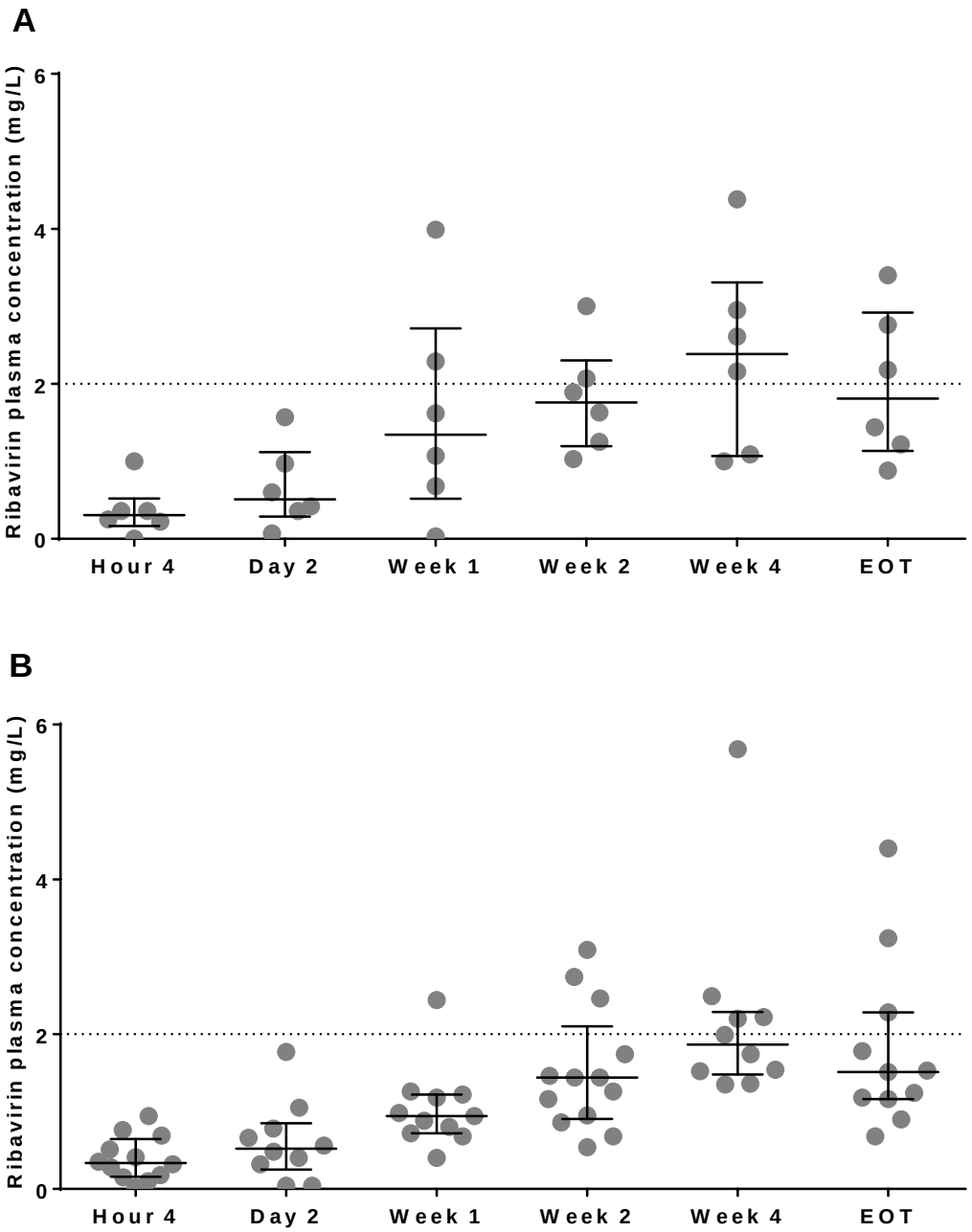
At end of treatment, two participants had ALT above the ULN. The first participant (ALT 35 U/L) achieved SVR12 with ALT within normal limits at post treatment week 12 (26 U/L). The second participant (ALT 44 U/L) demonstrated post treatment relapse with ALT rising to 153 U/L at post treatment week 12.

Abbreviations: Alanine aminotransferase (ALT); aspartate aminotransferase (AST); baseline (BL); end of treatment (EOT); post treatment week 12 (PT W12); screening (SCR); upper limit of normal (ULN)




**Supplementary Material Figure 3. On-treatment adherence**

Abbreviations: Sustained virological response (SVR)




**Supplementary Material Figure 4. Ribavirin plasma concentration (mg/L) by treatment time-point and virological outcome.**

Panel A: Participants achieving SVR12 (n=6). Panel B: Participants with virological failure (n=13). Bars depict median with interquartile range. Dotted line at ribavirin plasma concentration 2mg/L.

## Tables

**Supplementary Table 1. Clinical and virological characteristics of excluded participants**

| Site | Screen date | Gender | Age | HIV | HCV GT | Screening HCV RNA (IU/ml) | Reason for exclusion |
|---|---|---|---|---|---|---|---|
| Auckland, NZ | 23/10/2014 | Male | - | No | 3a | 64 919 | Ineligible (estimated duration of infection >12 months) |
| SVH, NSW | 25/11/2014 | Male | 48 | Yes | 2a | No sample taken | Poor venous access |
| SVH, NSW | 18/12/2014 | Male | 43 | Yes | 1a | 4600* | HCV RNA <10,000 |
| SVH, NSW | 19/12/2014 | Male | 36 | Yes | 1a | TND | HCV RNA TND |
| RAH, SA | 12/02/2015 | Female | 25 | No | 3a | TND | HCV RNA TND |
| RAH, SA | 26/02/2015 | Female | 37 | No | 1a | 3 240 000 | Psychiatric admission after screening |
| RAH, SA | 7/05/2015 | Female | 18 | No | 3a | 177 000 | Withdrawal of consent |

*HCV RNA <15 IU/mL (target detected, not quantifiable) on re-screening 4 weeks later

Abbreviations: Genotype (GT); target not detected (TND).

**Supplementary Table 2. Primary and secondary efficacy endpoints – by HCV genotype (ITT)**

| Efficacy | Genotype 1 (n=13) | Genotype 2 (n=1) | Genotype 3 (n=5) |
|---|---|---|---|
| ETR, n (%) | 12 (92) | 1 (100) | 4 (80) |
| SVR 4, n (%) | 4 (31) | 1 (100) | 3 (60) |
| SVR 12, n (%) | 3 (23) | 1 (100) | 2 (40) |
| Non-response | 1 (8) | 0 | 1 (20) |
| Relapse | 8 (62) | 0 | 1 (2) |
| Reinfection | 0 | 0 | 1 (20) |
| Lost to follow up | 1 (8) | 0 | 0 |
| SVR 24, n (%) | 3 (23) | 1 (100) | 3 (60)* |
| Viral kinetics on treatment, n (%) | | | |
| HCV RNA <LLoQ week 1 | 5 (38) | 1 (100) | 3 (60) |
| HCV RNA <LLoQ week 2 | 10 (77) | 1 (100) | 3 (6) |
| HCV RNA <LLoQ week 4 | 10 (77) | 1 (100) | 4 (80) |
| HCV RNA <LLoQ end of treatment | 12 (92) | 1 (100) | 4 (80) |

*Note, confirmed spontaneous clearance of HCV viraemia between post-treatment week 12 and 24 in one HIV-positive participant

**Supplementary Table 3. Primary and secondary efficacy endpoints – by HIV co-infection (ITT)**

| Efficacy | Total treated population (n=19) | HCV mono-infection (n=5) | HIV/HCV co-infection (n=14) |
|---|---|---|---|
| ETR, n (%) | 17 (89) | 5 (100) | 12 (86) |
| SVR 4, n (%) | 8 (42) | 3 (60) | 5 (36) |
| SVR 12, n (%) | 6 (32) | 3 (60) | 3 (21) |
| Non-response | 2 (11) | 0 | 2 (14) |
| Relapse | 9 (47) | 2 (40) | 7 (50) |
| Reinfection | 1 (5) | 0 | 1 (7) |
| Lost to follow up | 1 (5) | 0 | 1 (7) |
| SVR 24, n (%) | 7 (37)* | 3 (60) | 4 (29)* |
| Viral kinetics on treatment, n (%) | | | |
| HCV RNA <LLoQ week 1 | 9 (47) | 3 (60) | 6 (43) |
| HCV RNA <LLoQ week 2 | 14 (74) | 4 (80) | 10 (71) |
| HCV RNA <LLoQ week 4 | 15 (79) | 5 (100) | 10 (71) |
| HCV RNA <LLoQ end of treatment | 17 (89) | 5 (100) | 12 (86) |

*Note, confirmed spontaneous clearance of HCV viraemia between post-treatment week 12 and 24 in one HIV-positive participant

**Supplementary Table 4. Changes in key laboratory parameters between screening and post treatment week 12**

| Laboratory parameter, median (range) | Screening | Baseline | End of treatment | Post treatment week 12 |
|---|---|---|---|---|
| ALT, U/L | 139 (60 – 1040) | 133 (25 – 425) | 22 (8 – 44) | 48 (13 – 910) |
| AST, U/L | 88 (31-478) | 71 (27-320) | 27 (14-39) | 33 (21-290) |
| Bilirubin (total), umol/L | 9 (5-39) | 11 (4-49) | 11 (6-44) | 11 (4-50) |
| Haemoglobin, g/L | 153 (131-170) | 146 (126-171) | 134 (90-160) | 149 (133-173) |
| Absolute neutrophil count, $x10^9$/L | 3.4 (2.0-7.5) | 2.9 (1.7-7.4) | 3.4 (1.8-8.0) | 3.0 (1.7-9.4) |
| Platelet count, $x10^9$/L | 226 (149-423) | 231 (145-349) | 261 (158-404) | 230 (137-333) |
| CD4 count*, $x10^6$/L | 598 (370-986) | - | 614 (283-838) | 728 (415-1340) |

*HIV-positive participants only

Normal range for laboratory values: ALT, 0-30 U/L; AST, 0-30 U/L; absolute neutrophil count, 2.0 - 7.5 $x10^9$/L; Bilirubin, 0-18 umol/L; CD4 count, 500 - 1650 $x10^6$/L; Haemoglobin, female: 115 - 165 g/L, male: 130 - 180 g/L; Platelet count, 150-400 $x10^9$/L.

Abbreviations: Alanine aminotransferase (ALT), aspartate aminotransferase (AST)

**Supplementary Material Table 5. Registered clinical trials for interferon-free direct-acting antiviral therapy in recent HCV infection**

| PI +/- study group | Country, year commenced | Short title | Duration of infection (months) | Study population | N | Regimen | Treatment duration (weeks) | Status (SVR12*) |
|---|---|---|---|---|---|---|---|---|
| Matthews G; Kirby Institute | Australia, New Zealand 2014 | DARE-C II NCT02156570 | ≤12 | GT 1-6; HIV-positive and PWID eligible | 19 | SOF+RBV | 6 | Closed to recruitment (6/19; 32%) |
| Chung RT, Naggie S; ACTG | US 2014 | SWIFT-C NCT02128217 | ≤6 | HCV GT 1-6 and HIV-positive; PWID eligible | 17<br>27 | SOF+RBV<br>LDV/SOF | Arm A: 12<br>Arm B: 8 | Recruiting (Arm B) (Arm A: 10/17; 59%) |
| Wedemeyer H; HepNet | Germany 2014 | HepNet Acute HCV IV NCT02309918 | ≤4 | HCV GT 1; HIV-positive and PWID ineligible | 20 | LDV/SOF | 6 | Closed to recruitment (20/20; 100%) |
| Rockstroh J | Germany, UK 2015 | NCT02457611 | ≤6 | GT 1 or 4 and HIV-positive; PWID eligible | 26 | LDV/SOF | 6 | Closed to recruitment (20/26; 77%) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Matthews G; Kirby Institute | Australia, New Zealand, UK 2016 | TARGET3D NCT02634008 | ≤12 | GT 1 or 4; HIV-positive and PWID eligible | 60 | PrO + DSV +/- RBV | Arm A: 8 Arm B: 6 | Recruiting |
| Rijnders B | Netherlands, Belgium 2016 | DAHHS-2 NCT02600325 | ≤6 | GT 1 or 4 and HIV-positive; PWID eligible | 80 | GZR/EBR | 8 | Recruiting |
| Matthews G; Kirby Institute | International 2016 | REACT NCT02625909 | ≤12 | GT 1-6; HIV-positive and PWID eligible | 250 | SOF/VEL | 6 or 12 (RCT 1:1) | Not yet recruiting |

*SVR12 if applicable

Abbreviations: Dasabuvir (DSV), grazoprevir (GBR), elbasvir (EBR), ledipasvir (LDV), paritaprevir/ritonavir/ombitasvir (PrO), participants enrolled or to be enrolled (N), Principal Investigator (PI), randomised control trial (RCT), ribavirin (RBV), sofosbuvir (SOF)