

RESEARCH ARTICLE

Short-duration treatment with the novel non-nucleoside inhibitor CDI-31244 plus sofosbuvir/velpatasvir for chronic hepatitis C: An open-label study

Joel V. Chua¹  | Afua Ntem-Mensah¹ | Ameer Abutaleb² | Jennifer Husson¹ | Lydiah Mutumbi¹ | Ka Wing Lam¹ | Alip Ghosh¹ | Sara Romani¹ | Bhawna Poonia¹ | Sam Lee³ | M. Luz Pascual³ | Lyn R. Frumkin⁴ | Shyamsundaran Kottlil¹

¹Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA

²Division of Gastroenterology and Hepatology, University of Maryland School of Medicine, Baltimore, Maryland, USA

³Cocrystal Pharma, Inc., Bothell, Washington, USA

⁴Seattle, Washington, USA

Correspondence

Joel V. Chua, Institute of Human Virology, University of Maryland School of Medicine, 725 West Lombard St, Baltimore, MD 21201, USA.

Email: jchua@ihv.umaryland.edu

Funding information

Cocrystal Pharma, Inc.

Abstract

Combination regimens of direct-acting antiviral agents (DAAs) for chronic genotype 1 hepatitis C virus (HCV) infection given for 8 or 12 weeks have high cure rates. Shortened treatment durations that maintain high cure rates may lessen treatment barriers related to affordability and drug adherence. We enrolled 12 treatment-naïve adults with chronic genotype 1 HCV infection without cirrhosis in a single-center, open-label trial to receive 2 weeks of the highly potent and selective non-nucleoside inhibitor (NNI) CDI-31244 concurrent with 6 weeks of sofosbuvir/velpatasvir. The main efficacy endpoints were sustained virologic response at 12 (SVR12) and 24 (SVR24) weeks after treatment completion. In all patients, plasma HCV RNA levels rapidly decreased during the first 2 days of treatment and were below the lower limit of quantification by the end of the 6-week treatment period. Eight of 12 (67%) patients achieved both SVR12 and SVR24. Four patients had virological relapse at Week 10, 4 weeks after end of treatment. The most common adverse event was headache, occurring in five (42%) patients. Pharmacokinetic analysis showed no relevant drug interactions between CDI-31244, sofosbuvir, and velpatasvir. In this pilot study of short-duration combination therapy involving a novel NNI with a fixed-combination DAA, 8 of 12 treatment-naïve patients with chronic genotype 1 HCV infection without cirrhosis achieved virologic cure. Future trials might evaluate whether extending the NNI duration beyond 2 weeks with combination DAAs results in higher cure rates comparable with currently approved longer duration therapy.

KEYWORDS

chronic hepatitis C, clinical trial, direct-acting antiviral, therapeutics

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Journal of Medical Virology* published by Wiley Periodicals LLC

1 | INTRODUCTION

The global hepatitis report by the World Health Organization estimated that about 71 million people, or 1% of the world's population, were living with hepatitis C virus (HCV) infection.¹ Only 1.7 million individuals, or less than 3% of the estimated 72 million people chronically infected with HCV, were believed to have received treatment during 2015.^{1,2} The Lancet Gastroenterology & Hepatology Commissioners noted that in 2017 more people were infected with HCV than cured.³ While barriers to treatment of HCV infection are multifactorial, affordability and medication adherence are significant impediments to treatment in both low-income and high-income populations.¹⁻⁴

Because of cost and demand, insurance providers often use a risk stratification model to prioritize who should receive treatment, generally favoring those with cirrhosis over those without.⁵ This strategy excludes treatment for younger, less advanced HCV-infected people who comprise the bulk of the untreated HCV epidemic.⁶

Current approved therapies for chronic genotype 1 HCV infection using combination direct-acting antivirals (DAAs) given for 8 or 12 weeks achieve high rates (>95%) of virologic cure, defined as sustained virologic response at 12 weeks after completion of treatment (SVR12), across various viral and host characteristics.⁷⁻¹⁰ Development of a safe and highly effective regimen that shortens the current duration of HCV infection treatment by combining highly potent DAAs with different mechanisms of action could enhance both treatment adherence and affordability, the latter a significant contributor to restricted access.^{1,4}

CDI-31244 is an investigational agent that is a novel, highly potent, and selective non-nucleoside inhibitor (NNI) of the HCV RNA-dependent RNA polymerase with excellent antiviral activity across all genotypes. CDI-31244 is designed to deliver high drug concentrations to the liver with no relevant *in vitro* effects against human DNA and RNA polymerases (Cocrystal Pharma, Inc., data on file). In a phase 1b study that evaluated patients with HCV genotype 1, CDI-31244 administered at a daily dose of 400 mg (either 200 mg twice daily or 400 mg once daily) or 600 mg for 7 days resulted in mean HCV RNA viral load reductions of 3.0 log₁₀ IU/ml by 96 h.¹¹ This reduction is greater than with dasabuvir (0.95 log₁₀), the only NNI that is currently approved for use as part of an HCV combination medication regimen (ombitasvir/paritaprevir/ritonavir).¹² As such, CDI-31244 may be a strong candidate to combine with DAAs to evaluate its potential as a highly effective and safe short-duration regimen for the treatment of chronic HCV infection. We conducted an open-label pilot study in treatment-naïve patients with chronic genotype 1 HCV infection without advanced fibrosis or cirrhosis to evaluate the effects of treatment with 2 weeks of CDI-31244 concurrent with 6 weeks of sofosbuvir/velpatasvir (Epclusa; Gilead Sciences, Inc.), a fixed-dose combination HCV nucleotide analog NS5B polymerase inhibitor and HCV NS5A inhibitor approved for the 12-week treatment of all HCV genotypes in adults without cirrhosis or with compensated cirrhosis.¹³

2 | METHODS

2.1 | Study design and selection of patients

Twelve patients were enrolled in this single-center, open-label study at the Institute for Human Virology, University of Maryland, Baltimore, MD between June 26 and September 4, 2018 (ClinicalTrials.gov: NCT03501550). Treatment was given for 6 weeks followed by a 24-week nontreatment period. Eligible patients were at least 18 years of age with chronic HCV genotype 1a or 1b infection for at least 6 months, serum HCV RNA greater than 1000 IU/ml at screening, and absence of advanced fibrosis or cirrhosis by liver biopsy, FibroScan less than 8 kPa, or FibroTest/FibroSure (F2 or lower) within 1 year of screening. Key exclusion criteria included active hepatitis B infection, HIV infection, coinfection with a genotype other than 1, chronic liver disease of a non-HCV etiology, nursing or pregnancy, drug or alcohol abuse, predefined laboratory abnormalities, and history of use of any HCV DAA therapy.

Written informed consent was obtained from all patients. The study was approved by the University of Maryland Institutional Review Board and conducted in compliance with good clinical practice guidelines, the Declaration of Helsinki, and regulatory requirements. An independent safety monitoring committee comprised of five members reviewed the progress of the study.

2.2 | Procedures

Patients received sofosbuvir 400 mg/velpatasvir 100 mg (Epclusa) as a combination tablet and CDI-31244 400 mg as capsules (50 mg x 8) once daily for 2 weeks. Sofosbuvir 400 mg/velpatasvir 100 mg alone was then given once daily for an additional 4 weeks for a total of 6 weeks of treatment. During the 6-week treatment period, the criterion for stopping study drug was virologic failure (breakthrough), defined as either HCV RNA greater than the lower limit of quantification (LLOQ) after two consecutive HCV RNA values below the LLOQ or greater than 1 log₁₀ increase in HCV RNA from nadir. Patients who completed the 6-week treatment were considered virologic failures (relapse) if HCV RNA decreased and remained undetectable during treatment but became detectable after cessation of treatment at any time during the 24-week posttreatment period. Patients with virologic failure, whether during the 6-week study drug treatment or the 24-week nontreatment follow-up period, were to receive sofosbuvir/velpatasvir/voxilaprevir (Vosevi, Gilead Sciences, Inc.) 400 mg/100 mg/100 mg once daily for 12 weeks with additional follow-up through SVR12.

Plasma HCV RNA concentrations to evaluate SVR were measured using COBAS AmpliPrep/COBAS TaqMan HCV Test v 2.0 (Roche Diagnostics) with LLOQ of 15 IU/ml. HCV RNA concentrations were measured at screening, baseline (0 h), during treatment at Days 1, 2, 3, 7, 10, 14, 21, and 42 (end of 6-week treatment), and posttreatment at Weeks 8, 10, 14, 18 (SVR12), and 30 (SVR24). HCV genotype and subtype were determined at screening using the

Versant HCV Genotype INNOLiPA 2.0 assay (Siemens Healthcare Diagnostics).

IL28B genotyping was done at baseline by polymerase chain reaction amplification and sequencing of the rs12979860 single-nucleotide polymorphism (Applied Biosystems). Deep sequencing of the HCV NS5A and NS5B regions (Monogram Biosciences) to identify resistant-associated variants (RAVs) was performed on blood samples collected at baseline for all patients, and again at the time of virologic failure if HCV RNA concentration was greater than 500 IU/ml.

Blood for pharmacokinetic analysis of plasma CDI-31244, sofosbuvir, GS-331007 (main metabolite of sofosbuvir), and velpatasvir levels was obtained before the first dose (Day 1) and at 0 (pre-dose), 1, 2, 4, 6, 8, 12, and 24 h after Day 14 dosing. Plasma drug concentrations were determined using validated liquid chromatography-tandem mass spectrometry (PRA, Raleigh). The LLOQ was 0.08 ng/ml for CDI-31244, 0.07 ng/ml for sofosbuvir, 3.5 ng/ml for GS-331007, and 0.59 ng/ml for velpatasvir. Blood for plasma HCV levels to assess viral kinetics was obtained at 2, 4, and 6 h after Day 1 dosing.

Blood was stored at baseline and end-of-treatment (Week 6) to conduct exploratory analyses of the association of specific immune biomarkers with SVR or relapse. Immunotyping with antibody staining and flow cytometry as well as degranulation assays to investigate T and natural killer (NK) cell subsets are described elsewhere.¹⁴

Clinical laboratory, physical examination (including vital signs), and electrocardiogram data were obtained at screening, baseline, and throughout the 30-week study. Treatment-emergent adverse events (TEAEs) were recorded through 4 weeks after study drug completion. Study drug compliance was assessed by pill counts, examination of patient diaries at each study visit, and plasma CDI-31244, sofosbuvir, GS-331007, and velpatasvir levels.

2.3 | Outcomes

The primary efficacy endpoint was the proportion of patients who achieved SVR₁₂ as defined by HCV RNA below the LLOQ (15 IU/ml). Secondary efficacy endpoints were proportion of patients who achieved SVR₂₄, time to achieve HCV RNA levels below the LLOQ, proportion of patients with HCV RNA below the LLOQ after Day 7 and 14 of study drug treatment, and proportion of patients with HCV RNA below the LLOQ after Day 7 and 14 of study drug treatment by genotype 1a or 1b. The main safety assessments were frequency and severity of adverse events. Exploratory endpoints included the relationship between terminally differentiated effector memory CD8⁺ T cells, naïve CD8⁺ T cells, and NK cell cytotoxic phenotypes between patients achieving SVR versus those who relapsed.

2.4 | Statistical analyses

No formal sample size calculation was performed for this exploratory study. All analyses were done on an intent-to-treat basis using all

patients who received study drug. All TEAEs were classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities, v.22.1 and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.1 (March 2017).

Plasma concentration-time profiles of CDI-31244, sofosbuvir, GS-331007, and velpatasvir were determined over a 24-h period on Day 14 at the following time intervals: 0 (pre-dose), 1, 2, 4, 6, 8, 12, and 24 h after dosing. A sample was also collected before the first dose on Day 1. The following pharmacokinetic parameters were computed for each patient using non-compartmental analysis: maximum plasma concentration (C_{max}), minimum plasma concentration (C_{min}), time to maximum concentration (t_{max}), and area under the plasma concentration-time curve from 0 to 24 h (AUC_{0-24}). Individual patient plasma concentrations and pharmacokinetic parameters were listed and summarized by descriptive statistics. Individual patient and mean plasma concentration-time data were to be presented graphically. Relationships between the change from baseline in \log_{10} RNA at Day 14 and C_{min} and AUC_{0-24} were examined graphically. If suggested by the graphs, appropriate pharmacokinetic or pharmacodynamic models were fit to the data.

HCV viral kinetics modeling was applied retrospectively using virologic data collected prospectively. The mathematical model used to predict treatment duration required to achieve cure has been previously described.¹⁵ This model assumed that HCV viral kinetics under therapy followed the standard biphasic model. The effect of overall treatment on HCV clearance was estimated by considering the effectiveness of the regimen in inhibiting HCV RNA synthesis ($\epsilon\alpha$) and virion secretion or export ($\epsilon\delta$), with $\epsilon = 1$ being 100% effective and enhancing intracellular HCV RNA decay (by a factor κ).

3 | RESULTS

Of 16 patients screened, 12 (75%) met the eligibility criteria and were enrolled in the study. All 12 enrolled patients completed the 6-week study drug treatment period and the planned study visits.

3.1 | Baseline characteristics

Demographic and baseline characteristics of the 12 enrolled patients are presented in Tables 1 and 2. The median age of the patients was 45.5 years (range, 28–62) and most had genotype 1a infection (83%). Patients were predominantly African-American or of African descent (58%) and 50% were male. The mean (SD) baseline plasma HCV level for all patients was 6.59 (0.62) \log_{10} IU/ml with six (50%) patients with levels greater than 6×10^6 IU/ml. Nine (75%) patients had no fibrosis (F0) and three (25%) had fibrosis scores greater than F0. Resistance-associated variants were detected at baseline in seven patients (58%); six with NS5A and one with NS5B. Overall, IL28B genotype was CT in five (42%) patients, CC in four patients (33%), and TT in three patients (25%).

TABLE 1 Subject demographics

| Subject ID | Age | Gender | Race | Ethnicity |
|------------|-----|--------|-------------------------------------|--------------|
| S03-01 | 62 | Male | African American or African descent | Not Hispanic |
| S01-02 | 43 | Male | Caucasian (White) | Not Hispanic |
| S04-03 | 29 | Female | Caucasian (White) | Not Hispanic |
| S08-04 | 56 | Female | African American or African descent | Not Hispanic |
| S07-05 | 51 | Male | Others | Hispanic |
| S05-06 | 40 | Female | African American or African descent | Not Hispanic |
| S09-07 | 54 | Female | African American or African descent | Not Hispanic |
| S10-08 | 34 | Male | African American or African descent | Not Hispanic |
| S11-09 | 48 | Female | Caucasian (White) | Not Hispanic |
| S13-10 | 35 | Male | African American or African descent | Hispanic |
| S16-11 | 49 | Female | Asian | Not Hispanic |
| S14-12 | 28 | Male | African American or African descent | Hispanic |

3.2 | Virologic response

Eight of 12 (67%) patients achieved sustained virologic response 12 weeks after completing study treatment (SVR12), the primary endpoint of the study. The same eight patients had sustained virologic response 24 weeks after completing treatment (SVR24). Table 3 presents a summary of efficacy endpoints; a listing of efficacy outcome by subject is shown in Table 2.

3.3 | Viral kinetics

Rapid reduction in HCV RNA levels was observed in all patients over the first two days of treatment as shown in Table 4. By Day 14, the end of the CDI-31244 treatment period, 6 of 12 patients (50%) had HCV RNA levels that were below the LLOQ. By the end of the 6-week study treatment period, all patients had HCV RNA levels below the LLOQ. In 4 of 12 patients (33%), virologic relapse occurred at study week 10. In addition, there was no significant relationship between SVR12 and age, gender, race, or ethnicity. Baseline disease characteristics (HCV genotype, IL28B polymorphism, RAVs, and baseline HCV RNA level) were also not significantly related to SVR12 in this small study population.

3.4 | Virologic failure

Four of 12 patients who had virologic (treatment) failure received additional treatment ("retreatment") with a standard of care regimen

using sofosbuvir/velpatasvir/voxilaprevir 400 mg/100 mg/100 mg once daily; three patients completed the 12-week retreatment regimen. Table 5 presents a summary of HCV characteristics in these patients. None had postrelapse changes in HCV genotype, including RAVs. Three of four patients (S07-05, S05-06, and S13-10) had undetectable HCV RNA at Week 4 of retreatment. In contrast, subject S10-08 only took sofosbuvir/velpatasvir/voxilaprevir for 3 days and had an HCV RNA level of 3,900,000 IU/ml at Week 4. The subject was removed from retreatment due to noncompliance. The three patients who completed retreatment achieved SVR4; two (S07-05, S13-10) achieved SVR12 and one (S05-06) was lost to follow-up after the SVR4 assessment visit.

3.5 | Pharmacokinetics

The pharmacokinetics of CDI-31244, sofosbuvir, GS-331007 (sofosbuvir metabolite), and velpatasvir evaluated by plasma concentrations were available for 11 patients. The geometric mean plasma CDI-31244 concentrations after 14 days of oral administration at a dose of 400 mg once daily was 712 ng/ml, occurring at a median T_{max} of 2.10 h (range, 1.03–4.20), consistent with findings from a phase 1 study evaluating the effects of a single dose of CDI-31244 400 mg (Cocrystal Pharma, data on file). The geometric mean trough value was 7.92 ng/ml, and AUC over the 24-h dosing interval averaged 2136 h*ng/ml. Examination of predose concentrations on Days 14 and 15 and at Weeks 3 and 4 indicated that steady state had been reached for all 4 moieties by Day 14. The exposures to sofosbuvir, GS-331007, and velpatasvir were consistent with reported values and suggest that coadministration of CDI-31244 did not affect their pharmacokinetics. There was also no meaningful relationship between the decreases in \log_{10} HCV RNA and CDI-31244 exposure on Day 14 as measured by AUC_{0-24} .

3.6 | Safety

Ten of 12 patients (83%) treated with CDI-31244 in combination with sofosbuvir and velpatasvir experienced at least one TEAE. There were 38 TEAEs reported, with 13 (34%) judged by the investigator to be related to treatment with study drug (CDI-31244, sofosbuvir/velpatasvir, or both). All but one of the TEAEs were Grade 1 (mild) in severity, and none led to discontinuation of study drug. Headache was the most commonly reported TEAE, occurring in 5 of 12 patients (42%), followed by nausea in 3 of 12 patients (25%), and back pain, diarrhea, and nasopharyngitis in 2 of 12 patients (17%) each. An overview of TEAEs is presented in Table S1, with a listing of the most commonly reported TEAEs in Table S2. There were no clinically significant observations or trends noted in laboratory assessments, vital signs, physical exam findings, or electrocardiography.

One patient, a 40-year old woman with a history of hypertension and fetal loss, experienced a treatment-emergent serious adverse event of spontaneous abortion that was judged to be unrelated to

TABLE 2 Baseline disease characteristics and efficacy outcome by subject

| Subject ID | HCV genotype ^a | IL28B polymorphism | NS5A RAV ^b | NS5B RAV ^b | Fibrosis | HCV Load (IU/ml) ^b | Treatment outcome |
|------------|---------------------------|--------------------|-----------------------|-----------------------|----------|-------------------------------|------------------------------|
| S03-01 | 1a | CT | None | G307G/R, S556S/G | F0-F1 | 457,000 | Achieved SVR12 and SVR24 |
| S01-02 | 1a | CC | Y93Y/N | none | F0 | 6,670,000 | Achieved SVR12 and SVR24 |
| S04-03 | 1a | TT | H58P | none | F0 | 2,340,000 | Achieved SVR12 and SVR24 |
| S08-04 | 1a | CT | none | none | F0 | 6,500,000 | Achieved SVR12 and SVR24 |
| S07-05 | 1a | CT | M28V | none | F0-F1 | 173,000 | Virologic failure at week 10 |
| S05-06 | 1a | CT | none | none | F0 | 558,000 | Virologic failure at week 10 |
| S09-07 | 1a | TT | none | none | F0 | 444,000 | Achieved SVR12 and SVR24 |
| S10-08 | 1a | CC | M28V, Q30H | none | F0 | 8,120,000 | Virologic failure at week 10 |
| S11-09 | 1a | CC | K24K/R | none | F1-F2 | 6,710,000 | Achieved SVR12 and SVR24 |
| S13-10 | 1a | CT | none | none | F0 | 7,450,000 | Virologic failure at week 10 |
| S16-11 | 1b ^c | CC | NR ^c | NR ^c | F0 | 13,200,000 | Achieved SVR12 and SVR24 |
| S14-12 | 1b | TT | P58T | none | F0 | 716,000 | Achieved SVR12 and SVR24 |

Abbreviations: HCV, hepatitis C virus; NR, not reportable; RAV, resistance-associated variants; SVR12, SVR at posttreatment Week 12; SVR24, SVR at posttreatment Week 24.

^aAt screening.

^bAt baseline (Day 1).

^cScreening HCV genotype detected as 1b (using 5'UTR and core region amplification). HCV NS5A and NS5B drug resistance assay (using full-length gene amplification and sequencing) done on Day 1. Visit did not fit any of 6 major genotypes but was distantly related to HCV genotype 1 and 6 sequencing and may represent a novel HCV strain. Categorized as phenotype 1b throughout the analyses.

TABLE 3 Summary of efficacy endpoints

| | n/N | % | 90% CI | 95% CI |
|---------------------------------------|-------|--------|-------------|-------------|
| Primary efficacy endpoint | | | | |
| SVR12 | 8/12 | 67 | (39%, 88%) | (35%, 90%) |
| Secondary efficacy endpoints | | | | |
| SVR24 | 8/12 | 67 | (39%, 88%) | (35%, 90%) |
| HCV RNA < LLOQ on: | | | | |
| Day 7 | 0/12 | 0 | (0%, 22%) | (0%, 23%) |
| Day 14 | 6/12 | 50 | (25%, 76%) | (21%, 79%) |
| Genotype 1a HCV RNA < LLOQ on: | | | | |
| Day 7 | 0/10 | 0 | (0%, 22%) | (0%, 23%) |
| Day 14 | 6/10 | 60 | (25%, 76%) | (21%, 79%) |
| Genotype 1b HCV RNA < LLOQ on: | | | | |
| Day 7 | 0/2 | 0 | (0%, 22%) | (0%, 23%) |
| Day 14 | 0/2 | 0 | (0%, 22%) | (0%, 23%) |
| | Mean | Median | Range | |
| Time to achieve HCV RNA < LLOQ (days) | 23 | 12 | 9–42 | |
| Additional efficacy endpoints | | | | |
| HCV RNA < LLOQ on: | | | | |
| Day 10 | 3/12 | 25 | (7%, 53%) | (6%, 57%) |
| Week 3 | 8/12 | 67 | (39%, 88%) | (35%, 90%) |
| Week 6 (end of treatment) | 12/12 | 100 | (78%, 100%) | (74%, 100%) |

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; LLOQ, lower limit of quantification (15 IU/ml for HCV RNA assay used in this study); SVR, sustained virologic response; SVR12, SVR at posttreatment week 12; SVR24, SVR at posttreatment week 24.

study drug. The patient initially responded well to study treatment with an HCV RNA concentration that was below the LLOQ by Day 10 and undetectable by Week 3. Despite reporting that she used contraception as required by the protocol, she had a positive serum pregnancy test at Week 6 (negative at Week 3). She experienced a

virologic relapse at Week 10 and had a spontaneous abortion during study Week 11. Estimated date of conception based on ultrasound was August 18, 2018, or approximately 4 weeks after study drug initiation.

TABLE 4 Treatment outcome by HCV RNA threshold over time

| | All N | SVR12 N (%) | Virologic failure N (%) |
|-------------------------|----------|----------------|-------------------------------|
| All patients | 12 | 8 (67) | 4 (33) |
| HCV RNA < 500 IU/ml at | | | |
| Day 3 | 6 | 4 (67) | 2 (33) |
| Day 7 | 9 | 6 (67) | 3 (33) |
| Day 14 | 12 | 8 (67) | 4 (33) |
| HCV RNA < 15 IU/ml at | | | |
| Day 7 | 0 | - | - |
| Day 14 | 6 | 4 (67) | 2 (33) |
| Week 3 | 7 | 6 (86) | 1 (14) |
| End of treatment | 12 | 8 (67) | 4 (33) |
| HCV RNA not detected at | | | |
| Day 14 | 1 | 1 (100) | 0 (0) |
| Week 3 | 4 | 3 (75) | 1 (25) |
| End of treatment | 10 | 7 (70) | 3 (30) |

Abbreviation: HCV, hepatitis C virus.

3.7 | Immunology

We compared immune cell phenotypes at baseline and end of treatment (Week 6) between patients who achieved SVR (i.e., those who achieved both SVR12 and SVR24) with those patients with virologic failure, including exploratory analyses of T cell exhaustion and activation, T cell function, and NK cell function. There were no differences in frequencies of naïve, central memory, or effector memory subsets of CD8+ or CD4+ T lymphocytes between the groups at either time. The frequencies of terminally differentiated CD8+ T lymphocytes, however, were significantly higher in the SVR group at both times ($p < .01$, baseline; $p < .05$, Week 6).

Chronic HCV infection is associated with high levels of expression of T cell exhaustion markers, including PD-1 (programmed cell death protein 1) and 2B4 (CD244) on global and virus-specific T cells. Expression of PD-1 on virus-specific cells is associated with a decline in the antiviral potential of CD8+ T cells and is associated with poor virus control. We determined PD-1 and 2B4 expression on global CD8 T cells. No difference in expression of either marker or coexpression of both markers was

TABLE 5 Hepatitis C profiles at baseline and relapse in the retreatment population

| | Subject ID | | | |
|---------------------|------------|---------|------------|-----------|
| | S07-05 | S05-06 | S10-08 | S13-10 |
| IL-28B polymorphism | CT | CT | CC | CT |
| Fibrosis | F0-F1 | F0 | F0 | F0 |
| HCV RNA (IU/ml) | | | | |
| At baseline | 173,000 | 558,000 | 8,120,000 | 7,450,000 |
| Before retreatment | 1,060,000 | 314,000 | 9,060,000 | 3,200,000 |
| Genotype | | | | |
| At baseline | 1a | 1a | 1a | 1a |
| Before retreatment | 1a | 1a | 1a | 1a |
| NS5A RAV | | | | |
| At baseline | M28V | NJone | M28V, Q30H | None |
| Before retreatment | M28V | None | M28V, Q30H | None |
| NS5B RAV | | | | |
| At baseline | None | None | None | None |
| Before retreatment | None | None | None | None |

Abbreviations: HCV, hepatitis C virus; IL, interleukin; RAV, resistant-associated variant.

observed on CD8+ T cells at baseline or end-of-treatment (Week 6) in the SVR or relapse groups.

The functional response of HCV-specific CD8+ T cells was determined by measuring cytokine production in response to HCV peptide stimulation of peripheral blood mononuclear cells. The group achieving SVR had significantly higher levels of tumor necrosis factor-alpha single cytokine and interferon gamma-plus tumor necrosis factor-alpha double cytokine-producing CD8+ T cells at baseline compared with the relapse (failure) group ($p < .05$). The frequency of interferon gamma-plus single positive CD8+ T cells was higher in the SVR group at baseline but was not statistically significant. This result correlates well with higher frequencies of terminally differentiated CD8+ T cells present in the SVR group (Figure 1). NK cell function determined by degranulation assay measuring CD107a expression was not significantly different between SVR and relapse groups, suggesting that this immune cell function is not a correlate of HCV clearance after antiviral treatment.

4 | DISCUSSION

In this proof-of-concept phase 2a study, treatment-naïve patients with HCV genotype 1 without cirrhosis received 2 weeks of a highly potent and selective NNI (CDI-31244) concurrent with 6 weeks of a fixed-dose combination of sofosbuvir, an HCV nucleotide analog NS5B polymerase inhibitor, and velpatasvir, an HCV NS5A inhibitor. Treatment was well tolerated; however, only 67% (8/12) of patients achieved SVR12. This SVR12 rate is lower than currently approved HCV therapies requiring longer treatment durations (8 or 12 weeks), and unapproved oral 3-DAA regimens of 6 weeks duration for treatment-naïve genotype 1 infection without cirrhosis (86%–100%).^{16–21}

In the four patients with virologic failure, relapse first occurred 4 weeks after completion of treatment and could not be explained by

study drug compliance, viral resistance, or reinfection. In the absence of historical control data evaluating 6 weeks of sofosbuvir/velpatasvir treatment, the influence of CDI-31244 on cure rates (SVR12 and SVR24) in the present cohort also cannot be determined. Rapid reduction in HCV RNA levels was observed during the first 2 days of treatment in all patients. In addition, serum HCV RNA was either undetectable or below the LLOQ in all patients at the end of the 6-week treatment period, suggesting that a longer course of CDI-31244 beyond 2 weeks might result in a higher proportion of patients with SVR12. The lack of treatment-emergent resistance in the NS5A and NS5B regions of patients who relapsed suggests that the failure of persistent viral suppression rather than viral resistance or reinfection was likely to be the most important contributor to outcome in these patients.

We did not find a relationship between baseline factors or time to not detected target and treatment outcomes, which may have been limited by our small sample size. Pharmacokinetic analyses showed predictable drug levels consistent with no relevant drug interactions to explain differences in efficacy. Our homogenous population also did not enroll patients with differing baseline covariates (genotypes, altered hepatic metabolism due to significant liver disease, prior HCV treatment) that can affect outcome. In addition, study drug compliance as assessed by serum drug levels, pill counts, and patient diaries was also excellent for all patients. It is not apparent what host or viral factors, if any, contributed to those achieving SVR12 compared with those who did not. In exploratory analyses, CD8+ effector T cell phenotypes were associated with a successful response to therapy. In humans, terminally differentiated memory T cells are capable of immediate inflammatory cytokine production and cytotoxicity. It is possible that at treatment initiation and viral load decline these cells control the residual virus.

In contrast, circulating frequencies of T cell and NK subsets did not predict treatment response, as occurred in a prior study

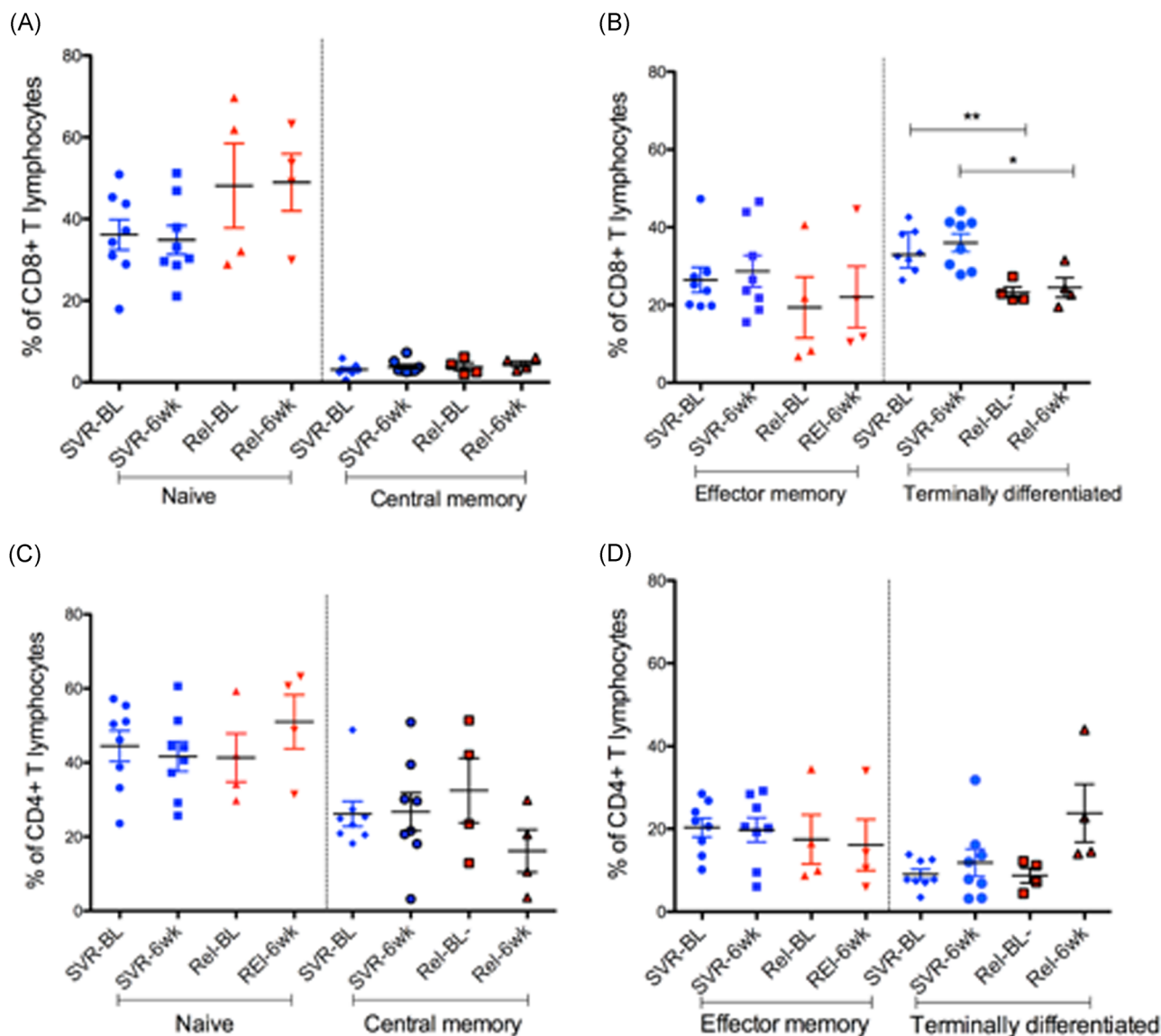


FIGURE 1 Immune cell phenotypes comparison between subjects who achieved sustained virologic response versus subjects with virologic failure. Description: peripheral blood frequencies of CD8+ and CD4+ T cell subsets in SVR or relapsed patient groups. Frequencies of (A and C) naïve (CCR7+CD45RO-) and central memory (CCR7+CD45RO+) CD8+, and (B and D) effector memory (CCR7-CD45RO+) and terminally differentiated effector (CCR7-CD45RO-) CD8+ T lymphocytes in patients that achieved SVR (blue) or those who relapsed (red). BL, baseline; 6wk, end of therapy time point; Rel, relapse; SVR, sustained virologic response at Week 24. Statistical significance determined by Mann-Whitney *U* Test. * denotes statistically significant at $p < .05$. ** denotes statistically significant at $p < .01$

of patients with HCV infection who had previously failed a course of pegylated interferon or ribavirin and were retreated with asunaprevir/daclatasvir for 24 weeks.²²

In summary, the 3-DAA regimen of CDI-31244 for 2 weeks coadministered with sofosbuvir/velpatasvir for 6 weeks resulted in virologic cure in 8 of 12 (67%) treatment-naïve HCV genotype-1 patients without cirrhosis or advanced fibrosis. This regimen also had a favorable safety profile with predictable pharmacokinetics. This pilot study is limited by the small sample size and an inability to distinguish the effects of the 3-DAA regimen from sofosbuvir/velpatasvir alone. The evaluation of safety and efficacy of this combination regimen with a longer treatment

duration of CDI-31244 (e.g., 4 or 6 weeks) may still allow the development of a highly effective and safe short-duration pan-genotypic HCV therapy.

ACKNOWLEDGMENTS

The authors had complete access to all data and total control over content and submission of the manuscript. The authors thank Dr. William Kramer for his analysis and interpretation of pharmacokinetic data, Dr. Harel Dahari for modeling of HCV kinetics, Dr. Curtis Scribner for regulatory support, Pam Walentynowitz for study monitoring, and Dr. Søren Bentzen for statistical support.

CONFLICT OF INTERESTS

Sam Lee and M. Luz Pascual are employees of and own stocks in Cocrystal Pharma, Inc. SL is also the coinventor on the patent for CDI-31244. Lyn R. Frumkin receives consulting fees from Cocrystal Pharma, Inc. The other authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Joel V. Chua and Lyn R. Frumkin drafted the manuscript. Joel V. Chua is the principal investigator that conducted the study. Joel V. Chua, Afua Ntem-Mensah, Ameer Abutaleb, Jennifer Husson, Lydiah Mutumbi, Ka Wing Lam, and Shyamsundaran Kottlil cared for the patients and collected the data. Alip Ghosh, Sara Romani, and Bhawna Poonia conducted the immunology work. Joel V. Chua, Lyn R. Frumkin, Sam Lee, M. Luz Pascual, and Shyamsundaran Kottlil designed the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Joel V. Chua  <https://orcid.org/0000-0002-7579-4672>

REFERENCES

- WHO. Hepatitis C. 9 July 2019. Geneva, Switzerland: World Health Organization. <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c>. Accessed March 20, 2020.
- WHO. Global hepatitis report, 2017. Geneva, Switzerland: World Health Organization.
- Cooke GS, Andrieux-Meyer I, Applegate TL, et al. Lancet Gastroenterology & Hepatology Commissioners. Accelerating the elimination of viral hepatitis: a lancet gastroenterology & hepatology commissioners. *Lancet Gastroenterol Hepatol*. 2019;4:135-184.
- Emmanuel B, Wilson EM, O'Brien TR, Kottlil S, Lau G. Shortening the duration of therapy for chronic hepatitis C infection. *Lancet Gastroenterol Hepatol*. 2017;2:832-836.
- Chua JV, Kottlil S. Are we nearing the end in the fight against hepatitis C? *Expert Rev Gastroenterol Hepatol*. 2017;11:499-500.
- Gowda C, Lott S, Grigorian M, et al. Absolute insurer denial of direct-acting antiviral therapy for hepatitis C: a national specialty pharmacy cohort study. *Open Forum Infect Dis*. 2018;5:5.
- Feld JJ, Jacobson IM, Hézode C, et al. Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infection. *N Engl J Med*. 2015;373:2599-2607.
- Puoti M, Foster GR, Wang S, et al. High SVR12 with 8-week and 12-week glecaprevir/pibrentasvir therapy: an integrated analysis of HCV genotype 1-6 patients without cirrhosis. *J Hepatol*. 2018;69:293-300.
- Kowdley KV, Gordon SC, Reddy KR, et al. ION-3 Investigators. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. *N Engl J Med*. 2014;370:1879-1888.
- AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. *Hepatology*. 2019;62:932-954.
- Cocrystal Pharma. Inc. *Cocrystal announces positive data from the successful completion of phase 1a/1b trial of the non-nucleoside polymerase inhibitor CC-31244 for the treatment of chronic hepatitis C infection [press release]*. Atlanta, GA and Bothell, WA. Cocrystal Pharma; 2017. <https://www.cocrystalpharma.com/news/press-releases/detail/38/cocrystal-announces-positive-data-from-the-successful>. Accessed March 20, 2020.
- Pockros PJ. Non-nucleoside analogue polymerase inhibitors in development. *Clin Liver Dis*. 2013;17:123-128.
- Eplusa [U.S. package insert]. Gilead sciences: Foster City, CA; 2019.
- Shrivastava S, Bhatta M, Ward H, et al. Multitarget direct-acting antiviral therapy is associated with superior immunologic recovery in patients coinfecting with human immunodeficiency virus and hepatitis C virus. *Hepatol Commun*. 2018;2:1451-1466.
- Goyal A, Lurie Y, Meissner EG, et al. Modeling HCV cure after an ultra-short duration of therapy with direct acting agents. *Antiviral Res*. 2017;144:281-285.
- Gane EJ, Schwabe C, Hyland RH, et al. Efficacy of the combination of sofosbuvir, velpatasvir, and the NS3/4A protease inhibitor GS-9857 in treatment-naïve or previously treated patients with hepatitis C virus genotype 1 or 3 infections. *Gastroenterology*. 2016;151:448-456.
- Lawitz E, Poordad F, Gutierrez JA, et al. Short-duration treatment with elbasvir/grazoprevir and sofosbuvir for hepatitis C: a randomized trial. *Hepatology*. 2017;65:439-450.
- Gane EJ, Stedman CA, Schwabe C, et al. Short-duration AL-335, odalasvir, with or without simeprevir, in patients with HCV GT1 or 3 infection without cirrhosis. *Hepatology*. 2018;68:2145-2157.
- Zeuzem S, Bourgeois S, Greenbloom S, et al. JNJ-4178 (AL-335, odalasvir, and simeprevir) for 6 or 8 weeks in hepatitis C virus-infected patients without cirrhosis: OMEGA-1. *Hepatology*. 2019;29:2349-2363.
- Kohli A, Osinusi A, Sims Z, et al. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet*. 2015;385:1107-1113.
- Sulkowski MS, Feld JJ, Lawitz E, et al. Efficacy and safety of 6 or 8 weeks of simeprevir, daclatasvir, sofosbuvir for HCV genotype 1 infection. *J Viral Hepat*. 2018;25:631-639.
- Alao H, Cam M, Keembiyehetty C, et al. Baseline Intrahepatic and peripheral innate immunity are associated with hepatitis C virus clearance during direct-acting antiviral therapy. *Hepatology*. 2018;68:2078-2088.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Chua JV, Ntem-Mensah A, Abutaleb A, et al. Short-duration treatment with the novel non-nucleoside inhibitor CDI-31244 plus sofosbuvir/velpatasvir for chronic hepatitis C: An open-label study. *J Med Virol*. 2021;93:3752-3760. <https://doi.org/10.1002/jmv.26652>