IFN-free therapy is associated with restoration of type I IFN response in HIV-1 patients with acute HCV infection who achieve SVR

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Summary

Interferon (IFN)-free direct-acting antiviral agents (DAAs) have revolutionized chronic hepatitis C virus (HCV) treatment; early studies suggest excellent efficacy in acute HCV. However, changes in innate immune responses during DAA therapy for acute HCV are unknown. We studied interferon-stimulated gene (ISG) expression and related cytokines/chemokines in HIV-infected patients with acute HCV receiving sofosbuvir plus ribavirin (SOF+RBV) as part of the A5327 clinical trial. ISG expression was determined from PBMCs, and circulating cytokines/chemokines were quantified from serum from study participants. The overall sustained virologic response (SVR) was 57%; all treatment failures were due to virologic relapse. Apart from *NOS2a*, baseline ISG/chemokine/cytokine levels were similar irrespective of treatment outcome. Downregulation of ISGs was observed at treatment week four and end of treatment (EOT), implicating HCV in establishing elevated ISGs early during HCV infection. Levels of many of these ISGs increased at post-treatment week 12 (PTW12) in relapsers only, coinciding with recurrent HCV RNA. Eleven ISGs were differentially expressed in responders vs relapsers. On-treatment viral suppression was also associated with a reduction in IP-10, CXCL11 and MIP-1β levels. In contrast, circulating IFN-α levels were significantly higher at EOT and PTW12 in responders vs relapsers. Upregulation of peripheral ISG expression is established early in the course of HCV infection during acute HCV infection, but did not predict subsequent treatment outcome with SOF+RBV. ISGs were downregulated during therapy and increased post-therapy in relapsers. IFN-α levels

were higher in responders at EOT/PTW12, suggesting that impaired type I IFN production/ secretion may contribute to relapse.

Keywords

acute hepatitis C; cytokines/chemokines; direct-acting antiviral (DAA) therapy; HCV/HIV coinfection; IFN-free; interferon-stimulated gene (ISG) expression; treatment response

1 | INTRODUCTION

Hepatitis C virus (HCV) infects over 71 million people worldwide and is a significant etiological agent in the development of chronic liver disease.[1] HCV establishes chronic infection in the majority of patients, while only a minority of patients (15%−25%) with acute HCV spontaneously clear the virus. Long-term chronic HCV infection results in persistent liver injury that can progress to cirrhosis after 15–20 years and risks of complications of end-stage liver disease such as decompensated liver failure, hepatocellular carcinoma and death.[2] Human immunodeficiency virus (HIV) coinfection with HCV occurs in approximately 30% of patients owing to similar transmission routes and has been associated with accelerated liver fibrosis progression and more frequent liver-related complications, such as decompensation.[3] In the postantiretroviral era, liver disease is now the second leading cause of non-AIDS-related mortality in HIV patients and therefore represents a significant health issue in these individuals.[3]

The development of direct-acting antiviral agents (DAAs) that target specific steps in the HCV replication cycle have revolutionized antiviral therapy for chronic HCV, allowing combination interferon (IFN)-free DAA regimens that are not only highly efficacious (>95% sustained virologic response [SVR] rate) but also have a significantly improved adverse event profile. However, studies of their efficacy in acute HCV are limited. In addition, there is little known about the host-virus interactions in the setting of DAAs and how the immune system will respond to the inhibition of replication without concomitant exogenous interferon administration. It is well established that chronic HCV is associated with changes in innate immune responses characterized by the upregulation of interferon-stimulated genes (ISGs) [4–6] and elevations in several relevant IFN-sensitive chemokines and cytokines.[7–10] The degree of host immune dysregulation has been associated with treatment outcomes with IFN-based therapy, and HCV eradication with IFN-based regimens has been shown to restore ISG and type I IFN responses.[4–6] There are extremely limited data regarding reversal of impaired immune responses in the context of DAA therapy. Several small studies have demonstrated improvement in liver ISG expression, restoration of type I IFN signalling, and natural killer and T cell function following IFN-free DAA therapy in the setting of chronic infection.[11–14]

The aims of this study were to characterize the effect of viral antigen removal on peripheral ISG and interferon-effector gene (IEG) expression and circulating chemokines/cytokines in participants enrolled in the A5327 study who were receiving sofosbuvir (SOF) and ribavirin (RBV) for the treatment of acute HCV infection. Furthermore, we planned to assess levels of ISGs/IEGs or chemokines/cytokines for any association with viral eradication and treatment

response. In the parent study, only 58% of individuals achieved an SVR, with the remaining patients relapsing following the completion of IFN-free therapy, representing a unique and well-characterized cohort to study differences in immune profiles between patients who ultimately clear virus and those who relapse following DAA therapy in the context of acute HCV infection.

2 | MATERIALS AND METHODS

2.1 | Patients and study design

A5327 was an open-label multicenter clinical trial to determine the safety and efficacy of IFN-free DAA therapy in HIV-1 subjects with confirmed acute HCV infection (NCT02128217). This study focuses on cohort one of this trial, where patients were treated with sofosbuvir plus weight-based ribavirin for 12 weeks. Key inclusion/exclusion criteria for entry into this study are summarized in Table S1 (see NCT02128217 for additional detail). All patients were HIV-1 positive on antiretroviral therapy (ART) and demonstrated HIV viral suppression throughout the study.

All subjects consented to collection of serum and peripheral blood mononuclear cells (PBMCs) at baseline, on-treatment week 4 (TW4), end of treatment (EOT) and at post-treatment week 12 (PTW12). Immune profiling was completed at each time point. Per the parent protocol, SVR was defined as HCV RNA less than the lower limit of quantification (<LLoQ) target not detected (TND) at PTW12, and relapse was defined as HCV RNA undetectable (<LLoQ and TND) at EOT but quantifiable HCV RNA (LLoQ) at any time during post-treatment follow-up. Participants achieving SVR will be referred to as responders in this study. The study was approved by each local institutional review board, and all participants provided written consent. The study was conducted according to the Declaration of Helsinki.

2.2 | *IFNL3* rs12979860 genotyping

Genomic DNA was extracted from PBMCs using the QuickExtract DNA Extraction Protocol (EPICENTRE® Biotechnologies, Madison, WI, USA), as per the manufacturer's instructions. rs12979860 genotyping was performed by real-time PCR and direct PCR Sanger sequencing (primers: sense $5'$-AGAAGCAGAGATGCGGC $-3'$ and antisense $5'$-TCTGGGATTCCTGGACG $-3'$). Sequence alignment was performed using CodonCode v5.1.5 (CodonCode Corporation, Centerville, MA, USA).

2.3 | HCV RNA quantification and HIV RNA quantification

HCV RNA was measured using the AmpliPrep/COBAS® assay (Roche, California, USA), where the lower limit of detection (LLoD) is 15 IU/mL. HIV RNA was measured using the LCx HIV RNA quantitative assay (Abbott Molecular, Rungis, France), with a dynamic range of 50–1 000 000 copies/mL.

2.4 | RNA extraction and gene expression profiling

RNA was extracted from rapidly thawed cryopreserved PBMCs using the RNeasy Mini Kit (Qiagen, Hilden, Germany), as per the manufacturer's instructions. Using 300 ng of total

RNA, mRNA expression of a custom nCounter® codeset of 47 ISGs and nine IEGs was measured (NanoString® Technologies, Seattle, WA, USA). These ISGs/IEGs were chosen based on previously published literature indicating their relevance in the context of chronic HCV infection.[4–6,15] Data were normalized to four housekeeping genes using the nSolver software v2.6 (NanoString® Technologies). Baseline $\log_2$ normalized mRNA expression and differential gene expression at each treatment time point were compared to baseline levels and according to subsequent treatment response. Gene expression profiles were also compared at each time point to baseline, both as normalized gene expression levels and expressed as fold change from baseline and time point of interest.

2.5 | Cytokine/chemokine quantification

Plasma collected at each study time point and immediately frozen at −80°C was used to quantify a panel of chemokines and relevant cytokines. Levels and fold change in levels at each treatment time point were compared to baseline levels and also according to subsequent treatment response. Interleukin (IL)-6, IL-8, IL-10, IL-12p70, CXCL11, macrophage inflammatory protein (MIP)-1α, IFN-γ and TNF-α were measured using the human high-sensitivity T cell panel kit (Millipore, Billerica, MA, USA). Interferon-gamma-induced protein 10 (IP-10), IFN-α, monocyte chemoattractant protein-1 (MCP-1) and MIP-1β were measured using the human cytokine/chemokine magnetic bead kit (Millipore). ENA78 and IL-18 were measured using the human ProcartaPlex custom panel (Thermo Fisher Scientific, Waltham, MA), and chemokine (C-C motif) ligand five (RANTES) was measured using the human RANTES ELISA kit (eBioscience, San Diego, CA, USA).

2.6 | Statistical analyses

Change in immune profiles from baseline to each study time point was calculated. Categorical data are presented as frequencies, and continuous data are presented as medians with interquartile ranges. The chi-squared and Fisher's exact t tests were used to compare categorical data, and continuous data were compared with the MannWhitney U test and the Wilcoxon signed-rank test for unpaired and paired samples, respectively. GraphPad Prism v6 (GraphPad Software, Inc., La Jolla, CA) and STATA v11 were used to compare baseline demographics and levels of chemokines/cytokines. Peripheral gene expression data were generated using the nSolver software (NanoString Technologies), and R Studio v0.99.902 (RStudio, Inc., Boston, MA) and STATA v11 (StataCorp LP, College Station, TX) were used to determine differentially expressed genes in responders vs relapse. A *P*-value of <.05 was considered significant, and a *P*-value with Bonferroni correction was set at .001.

3 | RESULTS

3.1 | Patients

Fourteen of the 17 patients enrolled in the original parent study were included in this substudy. Three patients were excluded for the following reasons: two patients were found to have detectable HCV RNA at screening but at baseline the HCV RNA was subsequently found to be undetectable or extremely low at baseline (<100 IU/mL) and therefore these patients were excluded as they were felt likely to have different immune profiles to the

remaining patients with high HCV RNA levels; the third patient did not have stored paired PBMC and plasma samples available for all study time points.

The baseline demographics and patient characteristics are presented in Table 1. In brief, all patients were male, with a median age of 46 years, and predominantly infected with HCV genotype one (93%). The majority of patients had the non-C/C *IFNL3* genotype (8% C/C, 71% C/T and 21% T/T). Median baseline HCV RNA level was 6.39 $\log_{10}$ IU/mL. All patients were receiving ART with suppression of HIV RNA <50 copies/mL, and the median CD4 count was 452 cells/mm^3. There were no significant differences in baseline demographics and patient characteristics between responders and relapsers (Table 1).

In the parent study, the overall SVR rate for cohort one was only 58% (10 of 17), and all virologic failures were due to virologic relapse.[16] In this substudy, two of the 14 patients had high HCV RNA levels at baseline, defined as HCV RNA levels >6 000 000 IU/mL. At TW4, 86% had HCV RNA levels below the LLoD, and all patients had undetectable HCV RNA levels at EOT (Figure 1). The SVR rate was similar to the overall study (57%, eight of 14) (Figure 1). As observed in the parent study, all treatment failures were virologic relapse and no patient experienced viral breakthrough or viral nonresponse.

3.2 | Gene expression

We determined the gene expression profiles of a panel of biologically relevant ISGs (n = 45) and lEGs (n = 9) previously reported to be important in HCV[4–6,17,18] in PBMCs at baseline, TW4, EOT and PTW12. Of the 14 patients from this substudy, 13 were included in this gene expression analysis; one patient was excluded as their baseline RNA sample failed stringent NanoString quality control parameters.

3.2.1 | Baseline ISG/IEG expression profiles—Figure 2 displays the heat map depicting the normalized $\log_2$-transformed peripheral ISG and IEG expression levels for all 13 patients at baseline. Of the 45 ISGs and nine lEGs examined, only *NOS2a* gene expression was differentially expressed >1.5-fold in responders compared to relapsers (P = .016); however, this failed to meet statistical significance when correcting for multiple testing. There were no other significant differences at baseline according to subsequent treatment response. Baseline ISG/IEG expression analysis according to *IFNL3* genotype was not able to be performed as only one patient carried the C/C *IFNL3* genotype.

3.2.2 | On-treatment and post-treatment ISG/IEG expression profiles

Treatment week 4 (TW4): At TW4, the majority of ISGs were downregulated (37 of 47) in the setting of on-treatment viral clearance in all patients, whereas only three of the nine IEGs were downregulated at TW4 (4 upregulated, two unchanged). However, only five genes (all ISGs) demonstrated >1.5-fold differential expression compared to baseline levels with a *P*-value <.05, including *CXCL10, CXCL11, IFI44L, IFIT1* and *IFIT3* (Figure 3, Table S2). In addition, 10 genes were upregulated at TW4, but none were differentially expressed >1.5-fold above baseline levels (Figure 3, Table S2). When stratified according to treatment outcome, only two genes were found to be differentially expressed >1.5-fold

among responders and relapsers at TW4, including *NOS2a* (downregulated in relapsers) and GUCY1B3 (upregulated in relapsers) (all *P*-values <.05) (Table S3).

End of Treatment (EOT): At EOT, the majority of ISGs were downregulated (40 of 47), 6 of which were >1.5-fold differentially expressed with a *P*-value <.05 (Figure 3, Table S2). Only four of the nine IEGs were downregulated, but none were differentially expressed >1.5-fold above baseline. Of the remaining IEGs, two IEGs were unchanged and three were upregulated but were <1.5-fold above baseline.

In addition, the gene expression levels of the ISGs *DDIT4, IFI27, IFI44, IFIT2* and *RSAD2*, as well as the lEGs *GCKR* and *GUCY1B3*, were differentially expressed >1.5-fold between relapsers compared to responders (*P*< .05) (Table S3).

Post-Treatment Week 12 (PTW12): In the context of viral relapse, gene expression profiles differed in responders compared to relapsers, with upregulation of 32 of the 47 ISGs in relapsers (Figure 3, Table S2), 11 of which were differentially expressed more than 1.5-fold with a *P*-value <.05. No lEGs were found to have differential expression according to treatment outcome at PTW12 (Figure 3, Table S3).

3.3 | Changes in plasma levels of chemokines/cytokines with DAA treatment

3.3.1 | Baseline—We determined the levels of a panel of circulating chemokines and cytokines from plasma samples at each time point reported to be associated with treatment response in chronic HCV infection. Figure 4 shows the median levels for six of the 14 quantified chemokines/cytokines according to treatment outcome (SVR vs relapsers) that demonstrated significant differences between responders and relapsers or significant changes during therapy. There was no difference in the median baseline level of any of the 14 chemokines or cytokines in responders compared to relapsers, including in IP-10 levels which have been associated with subsequent treatment outcome with IFN-based therapy in chronic HCV (Figure 4).

3.3.2 | On-treatment and post-treatment

IFNα levels: Interestingly, although levels of IFNα did not significantly change during treatment in responders, there was a trend towards increased IFNα at TW4 in responders, and levels were significantly higher in responders at EOT and PTW12 compared to relapsers (Figure 4), suggesting impaired IFNα production or secretion in patients that subsequently relapse with SOF+RBV.

IP-10, CXCL11, MIP-1β, IL-18 and IL-8 levels: IP-10 levels were significantly lower at TW4 and EOT compared to baseline in both responders and relapsers (Figure 4). IP-10 levels remained low at PTW12 in responders, but returned to pretreatment levels in the context of relapse (Figure 4). Similarly, CXCL11 levels were significantly lower at TW4 and EOT compared to baseline in responders and nonresponders, and significantly lower at PTW12 than at baseline in responders (Figure 4). Compared to baseline, circulating levels of MIP1β were significantly lower at EOT and PTW12 in responders and at EOT only in relapsers (Figure 4). There was trend towards lower IL-18 levels at TW4 and EOT, with

significantly lower levels at PTW12 compared to baseline in responders (Figure 4). In addition, levels were also significantly lower in responders compared to relapsers at PTW12. At TW4, levels of IL-8 were significantly lower in responders compared to baseline (Figure 4). There were no other differences in IL-8 at other time points.

4 | DISCUSSION

There are limited data regarding peripheral ISG/IEG expression and circulating cytokine/chemokine profiling in the context of acute HCV infection in patients coinfected with HIV, especially in the context of the newer DAA-based therapies. It has been well established that intrahepatic ISG expression is associated with subsequent treatment outcome during IFN-based therapy for chronic HCV.[4,6] In contrast, baseline peripheral ISG/IEG expression is not always associated with subsequent treatment outcome; however, changes in ISG expression in PBMCs during IFN-based therapy of chronic HCV mirror on-treatment changes in the intrahepatic compartment.[6] In addition, levels of several circulating chemokines/cytokines, in particular IP-10, have been associated with subsequent treatment response to IFN-based therapy.[7–9,19] In the present study, 14 HIV-1-positive ART-treated patients with confirmed acute HCV deemed unlikely to spontaneously clear (>12 weeks acute HCV infection) received SOF+RBV IFN-free DAA therapy for 12 weeks. All patients were male and demonstrated adequate HIV RNA suppression with ART. The SVR rate with this IFN-free DAA regimen was unexpectedly low at only 57% (8/14), due to a high relapse rate, representing a unique cohort to interrogate the effect of DAA-induced viral suppression and re-appearance on innate immune responses in HIV-1 patients with acute HCV.

Contrary to the available data in chronic HCV patients, baseline IP-10 levels and levels of other circulating chemokines and cytokines did not differ significantly according to subsequent treatment outcome in this study. Peripheral *NOS2a* expression, an ISG previously shown to be a restriction factor for HCV replication,[20] was the only ISG/IEG that was differentially expressed in patients who subsequently relapsed compared to responders. These data suggest that the equilibrium patterns of differential cytokine/chemokine induction and ISG expression demonstrated in CHC may not yet be fully established in acute HCV. However, we did observe that the majority of ISGs did become down-regulated at TW4 and EOT compared to baseline in the setting of on-treatment viral suppression, suggesting that upregulation of ISGs that is observed in CHC may already be established very early on in the natural history of infection during acute HCV that is able to be modulated and reversed following successful viral eradication with IFN-free DAA therapy. This is further supported by an increase in ISGs at PTW12 in patients who experienced relapse, suggesting a virally mediated increase in ISG expression.

The timing of ISG downregulation that we observed during DAA therapy in this study was different to patterns reported with prior standard of care (IFN-based) therapy for CHC where upregulation of ISGs was observed at TW4 in individuals who subsequently achieved SVR. In contrast, we observed downregulation of the majority of ISGs at TW4 (37 of 47 ISGs) and EOT (40 of 47 ISGs). Although the DAA regimen of SOF+RBV resulted in an SVR rate of only 58%, this regimen was still more effective in rapidly suppressing HCV than IFN-based therapy. We observed that 86% of our acute HCV patients treated with SOF+RBV had

an HCV RNA level less than the LLoD compared to historical rates of only 20%−25% during IFN-based therapy. This increased early potency of therapy may therefore have altered changes in patterns of ISG expression with more rapid normalization of ISGs with more rapid viral suppression. Therefore, earlier time points during DAA therapy may be useful to follow changes in patterns of ISGs and determine if sets of ISGs may predict relapse with IFN-free DAA therapy. Interestingly, patterns of IEG expression were different to ISGs where there was less alteration in IEG expression during viral suppression, although it should be noted that only nine IEGs were examined.

As previously reported in chronic HCV patients, we also observed a reduction in levels of several circulating chemokines/cytokines in the context of on-treatment viral suppression, several of which rebounded to pretreatment levels in relapsers but remained low in responders, suggestive of a direct viral-mediated response. Circulating IFNα levels demonstrated a different pattern, where we observed that IFNα levels were significantly lower in relapsers at EOT and PTW12 compared to responders. The downregulation of ISGs at TW4 and their rebound at PTW12, accompanied by reduced IFNα levels, suggests that the ISG phenotype is only partially reversed, and that IFNα production or secretion may be impaired in relapsers, which may in turn impair type I IFN responses that are critical for optimal cell-mediated viral clearance and prevention of viral relapse. Conversely, the ISG downregulation of ISGs at TW4 that is sustained, together with greater circulating IFNα levels in responders, suggests that there is reversal of the ISG phenotype and that there is resetting of IFN-responsiveness that enables long-term viral eradication. These results are supported by prior findings in the intrahepatic compartment in chronic HCV patients treated with the same IFN-free regimen, where successful HCV clearance was associated with strong and early intrahepatic ISG induction, but increased type I IFN responses at EOT, suggesting HCV eradication and prevention of relapse were facilitated by restoring type I IFN responses in the liver. [11]

This study is limited by the small number of patients and serves primarily as proof-of-concept that reversal of the "exhausted" ISG phenotype does occur during IFN-free DAA therapy for acute HCV, and that improved IFNα secretion or production at EOT and PTW12 may be important to prevent relapse. In addition, patients in this study were all coinfected with HIV, and although all maintained controlled HIV RNA levels on ART, immune reconstitution is not universal in all compartments, and normal immune health may not be achieved with HIV suppression alone. This study requires validation in larger cohorts of acute HCV patients with HIV coinfection, and similar studies should be conducted in acute HCV patients without HIV infection to determine the contribution of HIV to these findings. It would also be important to compare peripheral and intrahepatic ISG/IEG expression profiles, although with the advent of noninvasive biomarkers of fibrosis and the morbidity/mortality associated with liver biopsy, it is becoming increasingly difficult to obtain liver tissue for intrahepatic studies, particularly in acute HCV where there is no clinical indication for liver biopsy. Owing to the small number of patients and the low frequency of the "good-responder" *IFNL3* genotype in this cohort, we were unable to perform a dedicated subanalysis according to *IFNL3* genotype, which is relevant given the historical association between *IFNL3* genotype, baseline circulation chemokine/cytokine levels and ISG expression. Given the exploratory nature of this study, and the fact that only 56 ISGs/IEGs

were tested, many of which are known to be antiviral within limited pathways, a more comprehensive panel of ISGs/IEGs should be examined in larger cohorts where more robust statistical analyses can be performed.

This study is the first to demonstrate that peripheral ISGs are already elevated in the context of acute HCV and can be reversed with DAAs. Taken together, our data suggest that IFN-free DAA therapy induces innate immune responses in the periphery of HIV-1 patients with acute HCV infection, and that ISGs and IEGs may play important and potentially differing roles in DAA-induced viral clearance, suggesting their antiviral biological functions may be distinctly different. We also demonstrate that despite the high relapse rate with this weak DAA combination, patterns of changes in ISG expression differ from those previously observed in chronic HCV patients treated with the suboptimal prior standard of care therapy (IFN + RBV), whereby there was downregulation of the vast majority of ISGs by TW4. Apart from *NOS2a*, we did not identify any differences in ISG/IEG expression or circulating cytokine/chemokine levels according to subsequent treatment response in our acute HCV cohort. The sustained prolonged downregulation of ISGs in responders suggests that there is reversal of the exhausted ISG phenotype and improved type I IFN secretion/production at EOT/PTW12 with DAAs even in the context of acute HCV infection. Similar studies in larger acute HCV cohorts and with more potent regimens should be performed to confirm these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

ALT	alanine aminotransferase
ART	antiretroviral therapy
AST	aspartate aminotransferase
DAAs	direct-acting antivirals
EOT	end of treatment
HCV	hepatitis C virus
HIV	human immunodeficiency virus

IEG	interferon-effector gene
IFN	interferon
IL	interleukin
IP-10	interferon-gamma-induced protein 10
ISG	interferon-stimulated gene
LLoD	lower limit of detection
LLoQ	lower limit of quantification
MCP-1	monocyte chemoattractant protein-1
MIP	macrophage inflammatory protein
PBMCs	peripheral blood mononuclear cells
PTW12	post-treatment week 12
RBV	ribavirin
SOF	Sofosbuvir
SVR	sustained virologic response
TND	target not detected
TW4	treatment week 4

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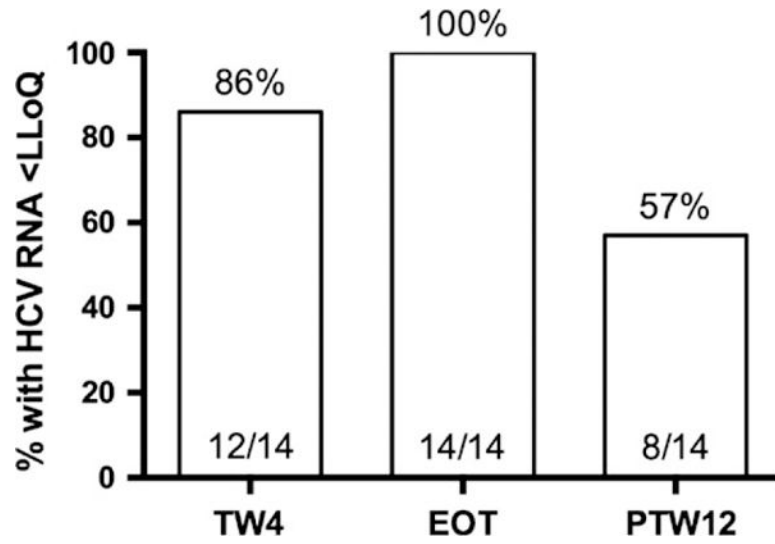
FIGURE 1.
On-treatment and post-treatment virologie response. All virologie failures were relapses.
HCV, hepatitis C virus; LLoQ, lower limit of quantification; TW4, treatment week 4; EOT,
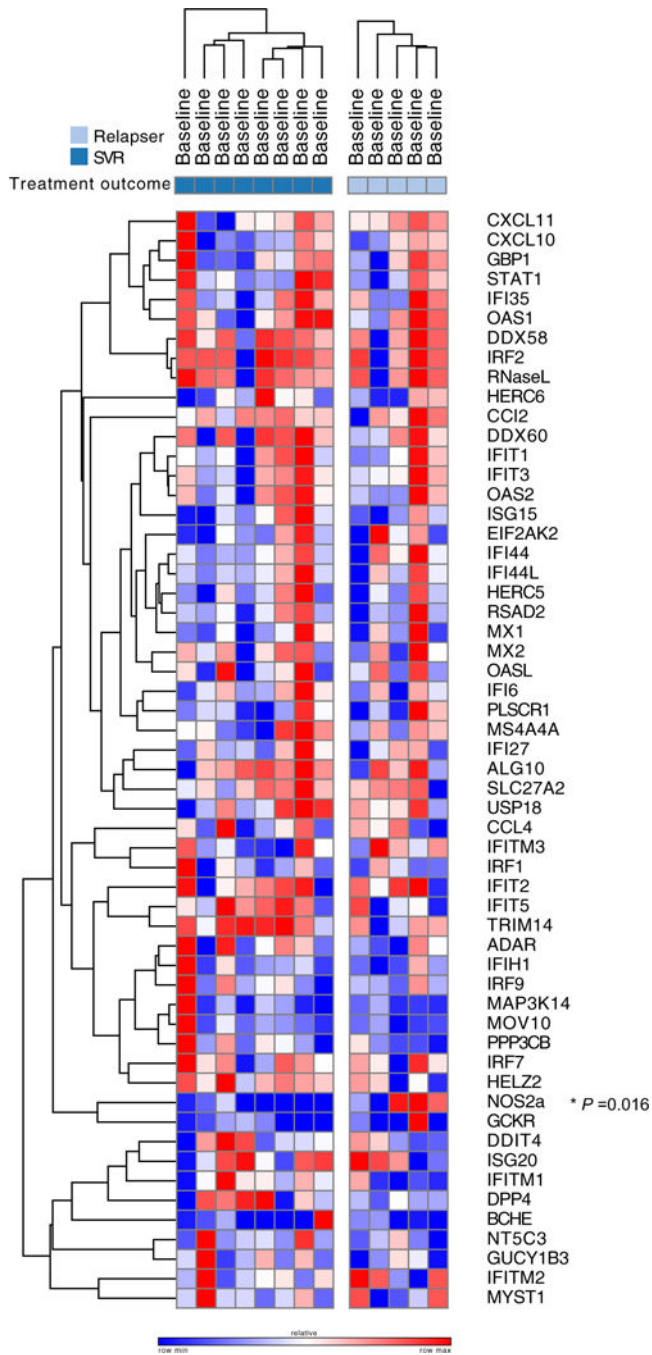end of therapy; PTW12, post-treatment week 12

FIGURE 2.
Heat map demonstrating baseline interferon-stimulated gene (ISG)/interferon-effector gene (IEG) log$_2$-normalized gene expression among responders (SVR, dark blue) and relapsers (light blue). Red on the heat map represents upregulation and blue represents downregulation
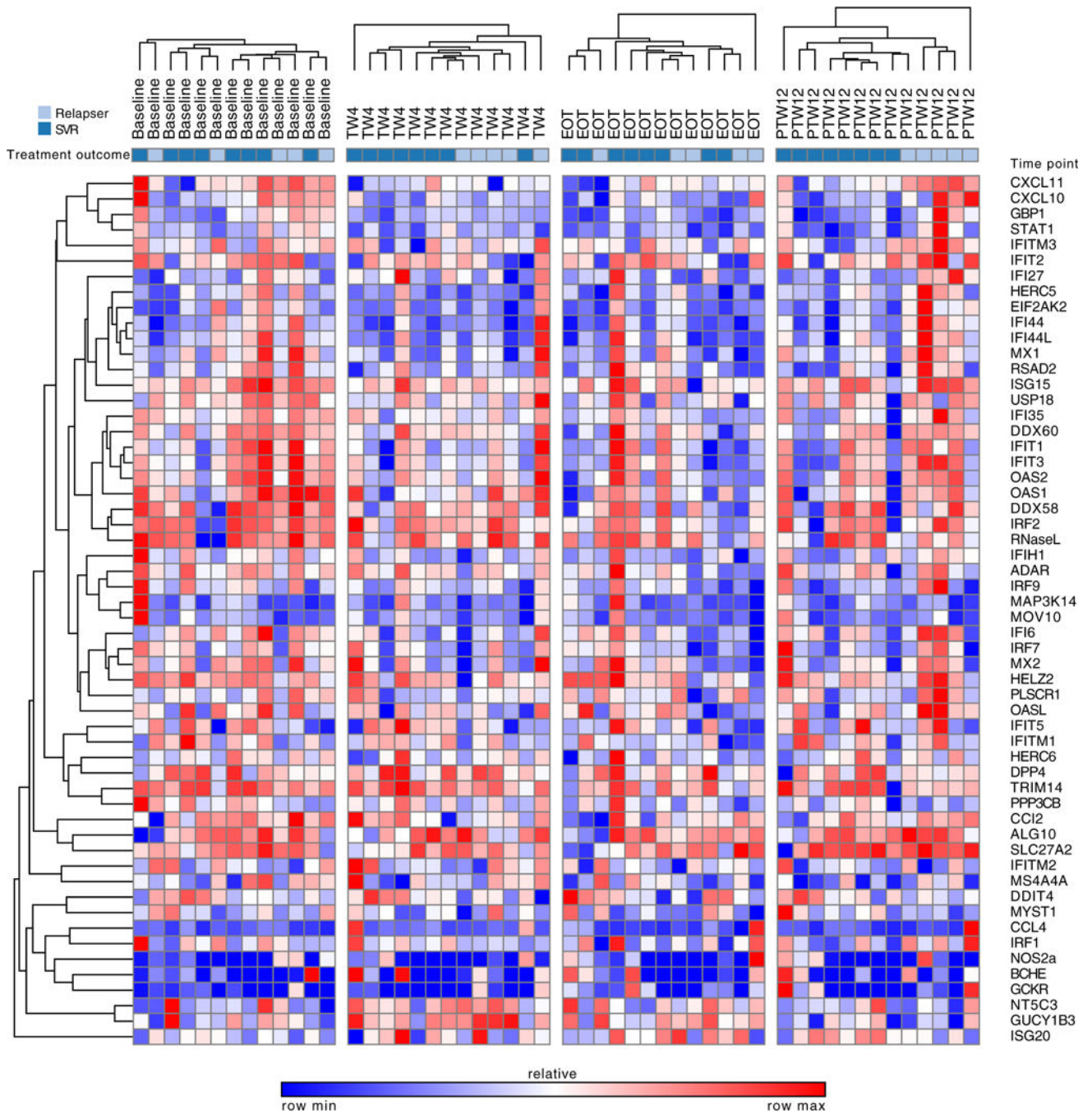
FIGURE 3.

Heat map demonstrating changes in interferon-stimulated gene (ISG)/interferon-effector gene (IEG) $\log_2$-normalized gene expression during sofosbuvir plus ribavirin therapy. Red on the heat map represents upregulation and blue represents downregulation

Note: SVR = sustained virologic response

TW4 = treatment week 4

EOT = end of treatment
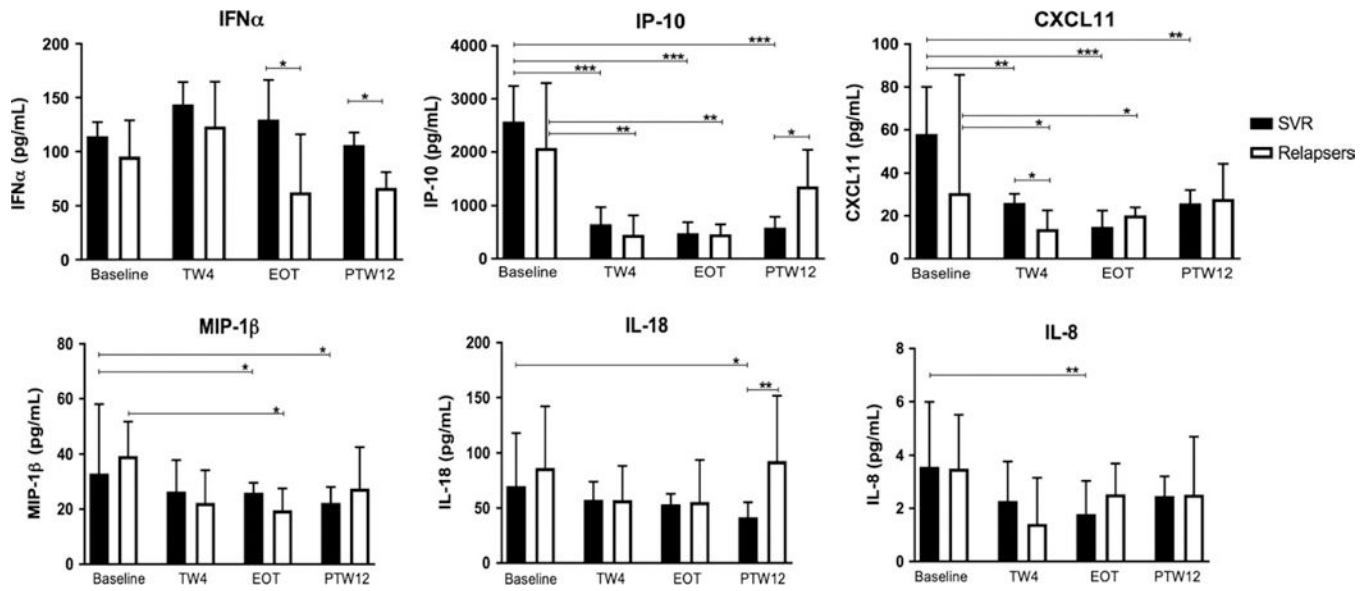
PTW12 = post-treatment week 12

FIGURE 4.

Levels of circulating cytokines and chemokines at baseline and during/after treatment according to treatment outcome. TW4, treatment week 4; EOT, end of treatment; PTW12, post-treatment week 12; *$P < .05$, **$P < .005$, ***$P < .0005$

TABLE 1

Baseline demographics and patient characteristics

	Responders (n = 8)	Relapsers (n = 6)	*P*-value
Age, years (median, range)	46.5 (21–65)	45.5 (29–48)	.6040
Male sex (n, %)	8 (100%)	6 (100%)	-
ALT, mg/dL (median, range)	206.5 (92–564)	183.0 (201–284)	.5181
AST, mg/dL (median, range)	134.0 (56–395)	103.5 (83–162)	.6982
Total bilirubin, mg/dL (median, range)	0.7 (0.4–2.1)	0.7 (0.6–1.1)	.6455
IFNL3 genotype (CC) (n, %)	1 (12.5%)	0 (0)	1.0000
IFNL3 genotype (CT/TT) (n, %)	7/1 (88%/12%)	6/0 (100%/0%)	.5590
HIV viral load, copies/mL (median, range)	<50 (<50–80)	<50 (<50–<50)	.3865
CD4 count, cells/mm^3 (median, range)	458.0 (331–652)	473.5 (213–618)	.5186
HCV genotype 1 (n, %)	7 (88%)	6 (100%)	1.0000
HCV viral load, $\log_{10}$ IU/mL (median, range)	6.22 (4.48–6.87)	6.50 (5.82–7.28)	.3662