# Multitarget Direct-Acting Antiviral Therapy Is Associated With Superior Immunologic Recovery in Patients Coinfected With Human Immunodeficiency Virus and Hepatitis C Virus

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**Patients coinfected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) have higher levels of immune activation, impaired antigen-specific responses, and accelerated fibrogenesis compared to patients monoinfected with HCV. Whether different direct-acting antiviral (DAA) combinations have differential effects on immunophenotypes and functions following successful HCV therapy remain unknown. Therefore, we aimed to assess the peripheral T-cell immunophenotypes and functions in patients coinfected with HIV/HCV who were successfully treated with combination DAA treatment regimens. We analyzed peripheral blood mononuclear cells (PBMCs) at baseline and at the time of sustained viral response (SVR) from subjects treated with three different combination DAA regimens: daclatasvir (DCV) and asunaprevir (ASV) for 24 weeks (CONQUER 2-DAA), DCV/ASV/beclabuvir (BCV) for 12 weeks (CONQUER 3-DAA), and sofosbuvir (SOF) and ledipasvir (LDV) for 12 weeks (ERADICATE study). We used flow cytometry to assess T-cell phenotypes (activation and exhaustion) and HCV-specific T-cell functions (cytokine secretion and cytotoxicity). Statistical analyses were conducted using the Wilcoxon matched-pairs signed-rank test with $P < 0.05$ considered significant. Overall, there was an improvement in T-cell exhaustion markers, a decrease in T-cell activation, an increase in the effector memory population, and improved T-cell function after achieving SVR, with the largest effects noted with CONQUER 3-DAA treatment. *Conclusion:* Treatment with DCV/ASV/BCV in patients coinfected with HIV/HCV resulted in greater restoration of the T-cell impairments and perturbations associated with HIV/HCV coinfection to an extent that was greater than that observed in either two-drug regimens. We showed that different DAA-based therapies have different immunologic outcomes after successful HCV treatment in patients coinfected with HIV/HCV. This information will be beneficial for providers when selecting the regimens for patients coinfected with HIV/HCV. (*Hepatology Communications* 2018;2:1451-1466).**

HIV/HCV coinfection is associated with accelerated hepatic fibrosis progression and higher rates of liver decompensation and death compared to HCV monoinfection.[1,2] The mechanisms associated with accelerated fibrosis progression rates among patients coinfected with HIV/HCV are not well understood, but multiple hypotheses have been proposed. These include viral mechanisms, such as a

*Abbreviations: 2B4, clusters of differentiation 244; APC, allophycocyanin; ASV, asunaprevir; BCV, beclabuvir; BLIMP-1, B lymphocyte-induced maturation protein 1; BV, Brilliant Violet; CCR7, chemokine (C-C motif) receptor 7; CD, clusters of differentiation; CONQUER 2-DAA, treatment with daclatasvir and asunaprevir; CONQUER 3-DAA, treatment with daclatasvir, asunaprevir, and beclabuvir; Cy5, cyanine-5; DAA, direct-acting antiviral; DCV, daclatasvir; Eomes, eomesodermine; ERADICATE study, treatment with sofosbuvir and ledipasvir; FBS, fetal calf serum; FITC, fluorescein isothiocyanate; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IL, interleukin; LDV, ledipasvir; NS, not significant; NS5/NS3, nonstructural protein 5/3; PBMC, peripheral blood mononuclear cell; PD1, programmed death 1; PE, phycoerythrin; PEG-IFN, peginterferon; PerCP, peridinin chlorophyll protein; SOF, sofosbuvir; SVR, sustained viral response; T-bet, T-cell-specific T-box transcription factor; TIGIT, tyrosine-based inhibition motif domain; TNF-α, tumor necrosis factor alpha.*

direct viral effect on hepatocytes and/or hepatic stellate cells that may lead to increased rates of HCV replication and increased hepatocyte apoptosis, and immunologic alterations, including diminished HCV-specific T-cell responses and increased T-cell exhaustion.[3]

Treatment of HIV with antiretroviral therapy and treatment of HCV have independently been shown to delay the progression of fibrosis and end-stage liver disease among coinfected patients.[4,5] However, the rates of SVR with immune-based methods based on peginterferon (PEG-IFN) and ribavirin therapy have been significantly inferior among coinfected patients compared to patients monoinfected with HCV, the reasons for which have not been fully elucidated.[6,7] Some groups have demonstrated a decline in the HCV-specific clusters of differentiation (CD)8 T-cell population, characterized by functional defects, reduced antiviral efficacy, and lack of full CD8+ T-cell maturation that cannot be restored by PEG-IFN therapy,[8-12] but the effects of viral eradication cannot be fully separated from the effects of IFN-based therapies which stimulates interferon-responsive genes and may contribute to inflammation independent of its antiviral effects. The advent of highly effective DAAs to treat HCV offers a unique opportunity to explore the impact of different DAA combinations on the restoration of HCV-specific T-cell immunity in the absence of IFN-based therapies in order to define the best treatment paradigm for coinfected patients. We have established that in patients monoinfected with HCV, DAA therapies are highly effective in inducing SVR, with a concomitant increase in HCV-specific immunity.[13] Studies also have demonstrated similar success rates in the treatment of patients monoinfected with HCV compared to patients coinfected with HIV/HCV.[14,15] We hypothesized that different combinations of DAA regimens (two- and three-drug combinations) may have differential effects on immune cell phenotypes (activation and exhaustion) and lymphocyte function in subjects coinfected with HIV/HCV who achieve SVR, defined as undetectable HCV RNA at least 12 weeks after completion of HCV therapy. We evaluated the effects of three different combination DAA therapies on peripheral T-cell immunophenotypes (including activation, exhaustion, and memory subsets) and immune function (including cytokine secretion, cytotoxic activity, and cytolytic functions) in subjects coinfected with HIV/HCV who were successfully treated for HCV.

# Patients and Methods

## PARTICIPANTS

Two prospective single-center phase II studies were conducted at the National Institutes of Health (NIH)

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Clinical Center, using three different DAA-only regimens in participants coinfected with HIV/HCV genotype 1: the CONQUER 2-DAA and 3-DAA arms (NCT02124044[15]) and the ERADICATE study (NCT01878799[13]). All patients provided written informed consent. In CONQUER 2-DAA, 10 patients coinfected with HIV/HCV genotype 1 were treated with the oral nonstructural protein (NS)5A inhibitor DCV and the NS3 inhibitor ASV for 24 weeks; in CONQUER 3-DAA, 20 patients coinfected with HIV/HCV genotype 1 were treated with DCV, ASV, and the investigational NS5B inhibitor BCV for 12 weeks. In ERADICATE, 50 patients coinfected with HIV/HCV were treated with the NS5A inhibitor LDV and the nucleotide NS5B inhibitor SOF. The SVR rates were 98% for the ERADICATE study,[14] 90% for the CONQUER 3D study, and 80% for the CONQUER 2D study.[16] A subset of successfully treated patients from each study (n = 6 from ERADICATE, n = 8 from the CONQUER 2-DAA arm, and n = 8 from the CONQUER 3-DAA arm) were selected, and stored samples, including PBMCs and plasma samples, from baseline and 12 weeks following end of therapy (SVR12) were used for immunologic assays. The National Institute of Allergy and Infectious Diseases (NIAID) Institutional Review Board approved the protocols for each study; these protocols are in accordance with the Declaration of Helsinki 1975.

## VIRAL LOAD MEASUREMENT

Plasma HCV RNA levels were measured in all patients at each visit, using the real-time HCV assay (Abbott Molecular) with a lower limit of quantification of 12 IU/mL and a lower limit of detection of 3 IU/mL.[14,16]

## PBMC ISOLATION

PBMCs from peripheral blood collected in heparinized tubes were separated using Ficoll-Paque (GE Health Care Life Sciences) density centrifugation. Cells were counted using trypan blue exclusion and were stored in liquid nitrogen until use.

## HCV PEPTIDE AND TETRAMERS

Genotype 1a or 1b HCV 15-mer to 18-mer peptides with 11 or 12 amino acid overlaps spanning the entire HCV polyprotein (peptide array, hepatitis C virus; BEI Resources, NIAID, NIH) were reconstituted in 5% sterile dimethyl sulfoxide and pooled consecutively into 21 groups. Peptides were aliquoted and stored at −80°C until use. Human leukocyte antigen (HLA)-A∗02 tetramers corresponding to HCV $NS3_{1073\text{-}1081}$ CINGVCWTV (MBL International Corporation, Woburn, MA) were used for analyzing the changes in HCV-specific CD8 T-cell responses.

## FLOW CYTOMETRY ANTIBODIES AND REAGENTS

For immunophenotyping analyses, flow cytometry was performed with anti-human surface and intracellular fluorochrome-conjugated antibodies. For surface and intracellular or intranuclear staining different flurochrome conjugated antibodies were used in 5 different panels (Supporting Tables S1 and S2). Multiparameter flow cytometry was performed and all samples were run using a BD FACS ARIA II instrument equipped with blue (488 nm), red (633 nm), and violet (405 nm) lasers (BD Biosciences). BD CompBeads (BD Biosciences) were used as individual compensation tubes for each fluorophore in the experiment, and data analysis was performed using FlowJo version 9.7.7 (TreeStar, Ashland, OR). To define positive and negative populations, we employed fluorescence minus one controls for each fluorophore used in this study.

## IMMUNOPHENOTYPING

PBMCs from day 0 and SVR12 time points were thawed for each patient, and immunophenotypes were assessed by multicolor flow cytometry analysis. The expressions of immune cell markers before and following treatment were assessed by staining cells with fluorescently labeled antibodies in two different 10-color panels. Two exhaustion panels assessed T-cell and specific exhaustion markers. The first included anti-CD3-Alexa Fluor 700, anti-CD4-PerCP-Cy5.5, anti-CD8-PE-Cy5, anti-CCR7-FITC, anti-CD45RO-APCef780, anti-PD1-PE-Cy7, anti-2B4-PerCP-Cy5.5, and intranuclear transcription factors anti-Eomes-PE, anti-T-bet-BV421, and anti-BLIMP-1-APC. A second panel used anti-CD3-Alexa Fluor 700, anti-CD4-BV605, anti-CD8-PE-Cy5, anti-CCR7-FITC, anti-CD45RO-APCef780, anti-TIGIT-APC, and anti-PD1-PE-Cy7 (Supporting Table S1). PBMCs were stained with the surface antibody-associated dyes, washed, and permeabilized. Intranuclear permeabilization was done with eBioscience Transcription Factor Fixation/Permeabilization concentrate and

diluent solutions (Cat. No. 00-5521) according to the manufacturer's instructions. Antibodies for intranuclear transcription factors anti-Eomes-PE, anti-T-bet-BV421, and anti-BLIMP-1-APC were then added to the cells, incubated for an additional 30 minutes on ice, washed, and fixed in 1% paraformaldehyde. Cells were then acquired in the BD FACS Aria, and data were analyzed using FlowJo version 9.7.7 (TreeStar, Inc.).

## TETRAMER STAINING FOR HCV-SPECIFIC CD8 T-CELL RESPONSE

A total of $1 \times 10^6$ cells per well on a 96-well plate were incubated with HLA-A∗02-matched tetramers. Cells were then incubated at 37°C and 5% $CO_2$ for 15 minutes. Cells were washed 3 times with phosphate-buffered saline containing 1% fetal bovine serum (FBS) and surface stained with anti-CD3 and anti-CD8 antibody for 15 minutes followed by 3 times washing. Cells were then acquired in the BD FACS Aria, and data were analyzed using FlowJo version 9.7.7 (TreeStar, Inc.).

## MULTIPARAMETER FLOW CYTOMETRY ASSAY TO ASSESS HCV PEPTIDE-SPECIFIC T-CELL FUNCTIONS

HCV-specific T-cell functions were assessed by measuring the frequency of degranulating and cytokine-secreting CD8 T cells by multiparameter intracellular cytokine staining. Frozen PBMCs from individuals at day 0 and SVR12 time points were thawed and counted using the trypan blue exclusion method. PBMCs were resuspended at $10^6$ cells/mL in Roswell Park Memorial Institute 1640 medium (Sigma) containing 10% FBS (Atlanta Biologicals), 2 mM l-glutamine (Cellgro), and 50 IU/mL penicillin (Cellgro). Cells were incubated with either genotype-specific overlapping HCV peptide pools (2 μg/mL/peptide), phorbol-12-myristate-13-acetate (2.5 μg/mL) and ionomycin (0.5 μg/mL) (Sigma) as a positive control or medium alone, which served as the negative control, for 5 days at 37°C. At day 4, cells were restimulated. To measure the T-cell degranulation or T-cell cytotoxic activity, anti-CD107A-BV650 antibody was added directly at 20 μL/mL during the restimulation. Cells were then incubated for 2 hours at 37°C in 5% $CO_2$; this was followed by the addition of brefeldin A (Sigma) at a final concentration of 1 μg/mL as well as 1 μL of monensin (Golgi-Stop; BD Biosciences) at a final concentration of 1 μg/mL and incubation for an additional 10 hours at 37°C in 5% $CO_2$. While brefeldin A prevents the exocytosis of cytokine-containing vesicles, allowing for the visualization of cytokine production following stimulation, monensin prevents the acidification of endocytic vesicles, avoiding the degradation of reinternalized CD107a proteins from the surface and allowing for the visualization of this marker following stimulation. Following incubation, PBMCs were harvested and stained with Live/Dead-Near Infrared (Invitrogen) for 30 minutes on ice and washed. Cells were then stained with surface and intracellular antibodies by using the procedure described above except that intracellular permeabilization was done with the eBioscience Intracellular Fixation & Permeabilization Buffer Set (catalog number 88-8824-00) according to the manufacturer's instructions. Cells were then acquired in the BD FACS Aria, and data were analyzed using FlowJo version 9.7.7 (TreeStar, Inc.). HCV peptide-specific T-cell functionality was assessed with three multiparameter flow panels. The first panel used surface anti-CD3-Alexa Fluor 700, anti-CD4-PerCP-Cy5.5, anti-CD8-PE-Cy5, anti-CD45RO-PE-Cy7, anti-CCR7-BV510, anti-CD38-PE-Texas Red, anti-PD1-APC, and intracellular antibodies anti-IFN-γ-BV421 and anti-TNF-α-PE. The second panel used surface antibodies of anti-CD3-Alexa Fluor 700, anti-CD4-PerCP-Cy5.5, anti-CD8-PE-Cy5, anti-CD45RO-PE-Cy7, anti-CCR7-BV510, anti-CD38-PE-Texas Red, and intracellular antibodies anti-perforin-BV421, anti-granzyme B-APC, anti-TGF-β-PE, and anti-IL-2-BV605. The third panel used surface anti-CD3-Alexa Fluor 700, anti-CD4-PerCP-Cy5.5, anti-CD8-PE-Cy5, and intracellular antibodies anti-IFN-γ-BV421, anti-TNF-α-PE, and anti-IL-2-BV510.

## STATISTICAL ANALYSIS

Paired Wilcoxon signed-rank tests were used to assess changes in the percentage of T-cell subsets expressing exhaustion markers before and after treatment. Changes in T-cell functions prior to treatment and at SVR 12 were also calculated using the Wilcoxon matched-pairs signed-rank test. All statistical analyses were conducted using GraphPad Prism version 6.0 with $P < 0.05$ considered significant.

# Results

## PATIENT CHARACTERISTICS

Baseline characteristics of the patients included from each study are shown in Table 1. Patient characteristics

were comparable among studies, with most patients being African-American, having a normal CD4 count, receiving highly active antiretroviral therapy, and having an HIV viral load below the limit of detection as measured by standard clinical assays (<40 for our laboratory). Patients in CONQUER 3-DAA had the highest baseline HCV RNA; 12.5% of patients in CONQUER 2-DAA, 12.5% in CONQUER 3-DAA, and none in ERADICATE had HCV viral loads >6,000,000 IU/mL. Rates of cirrhosis were also similar among groups, with 20%-25% of patients having a fibrosis score between 3 and 4 in all studies. All patients from whom samples were used for conducting these immunologic studies achieved SVR12.

## DECREASED CHRONIC IMMUNE ACTIVATION AND INCREASED EFFECTOR MEMORY PHENOTYPE ASSOCIATED WITH SVR

Chronic immune activation, a major hallmark of HIV monoinfection and HIV-HCV coinfection, is characterized by increased expression of activation markers on lymphocytes, such as CD4+ T cells and cytotoxic CD8+ T cells, associated with elevated levels of proinflammatory cytokines and chemokines, leading to accelerated disease progression. CD38 has been well established as one of the best biomarkers for T-cell immune activation.[17-19] To investigate the changes in T-cell immunophenotypes following successful combination DAA-based therapy for HCV in individuals coinfected with HIV/HCV and to determine whether treatment with different DAA therapy combinations was associated with different effects, we evaluated the changes in CD38 expression before and after treatment in the three different study groups. We observed a significant decline in CD38 expression on both CD4 and CD8 T cells in CONQUER 2-DAA (CD4 T cells, $P = 0.04$; CD8 T cells, $P = 0.04$) and CONQUER 3-DAA (CD4 T cells, $P = 0.04$; CD8 T cells, $P = 0.03$), while the CD38 expression in ERADICATE did not decline significantly (Fig. 1; Table 2).

T-cell differentiation usually follows a progression from naive to effector memory or central memory T cells, which play distinct roles in immunity against pathogenic agents. Perturbations in the homeostasis of different T-cell subsets during viral infections lead to

**TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SUBJECTS**

| | CONQUER 2 DAA | CONQUER 3 DAA | ERADICATE |
|---|---|---|---|
| Study characteristics | | | |
| n | 8 | 8 | 6 |
| Drugs | DCV/ASV | DCV/ASV/BCV | SOF/LDV |
| Drug class | NS5A/NS3 | NS5A/NS3/NS5B | NS5A/NS5B |
| Weeks of treatment | 24 | 12 | 12 |
| Patient characteristics | | | |
| Age, median (IQR) | 59 (56.5, 61) | 49 (48.5, 56.5) | 63.5 (60.75, 64.75) |
| Sex, % male | 37.5% | 50% | 83.3% |
| Race, % African-American | 87.5% | 50% | 66.6% |
| Race, % Caucasian Hispanic | 12.5% | 25% | 16.6% |
| Race, % Caucasian Non-Hispanic | 0% | 25% | 16.6% |
| HCV disease characteristics | | | |
| Log HCV RNA, median (IQR), IU/mL | 5.89 (5.73, 6.03) | 6.02 (5.78, 6.36) | 6.05 (5.6, 6.5) |
| Liver fibrosis/cirrhosis | | | |
| F0-F2, number (%) | 75% | 75% | 75% |
| F3-F4, number (%) | 25% | 25% | 25% |
| ALT, median (IQR), IU/mL | 35 (24, 43) | 39 (34, 61.5) | 57 (44.25, 70) |
| HIV disease characteristics | | | |
| Viral load, copies/mL (median) | <40 | <40 | <40 |
| CD4 count pretreatment, median (IQR), cells/mm$^3$ | 812 (664.75, 919.75) | 732 (509, 928.25) | 750 (714, 800) |
| On HAART | 100% | 100% | 100% |

Abbreviations: ALT, alanine transaminase; HAART, highly active antiretroviral treatment; IQR, interquartile range.

increased disease pathogenesis.[20,21] To investigate the effect of HCV treatment on T-cell differentiation, we analyzed the changes in different T-cell subset populations before and after therapy. Our results demonstrated a decrease in naive T cells (CD4 T cells, $P = 0.03$; CD8 T cells, $P = 0.04$) with CONQUER 3-DAA and an increase in effector memory cells with CONQUER 3-DAA (CD4 T cells, $P = 0.02$; CD8 T cells, $P = 0.03$) and ERADICATE (CD4 T cells, $P = 0.01$; CD8 T cells, $P = 0.004$) following treatment, while no changes were observed in central memory T-cell populations with any treatment arm (Fig. 1; Table 2).

## REVERSAL OF T-CELL EXHAUSTION FOLLOWING TREATMENT WITH 3-DAA THERAPY WITH DCV/ASV/BCV

In chronic infections, including HCV and HIV infection, persistent antigenic stimulation leads to immune exhaustion. This phenomenon is characterized by dysregulated expression of inhibitory receptors on antigen-specific effector T cells, resulting in a dysfunctional effector phenotype marked by deficits in their proliferative capacity, secretion of proinflammatory

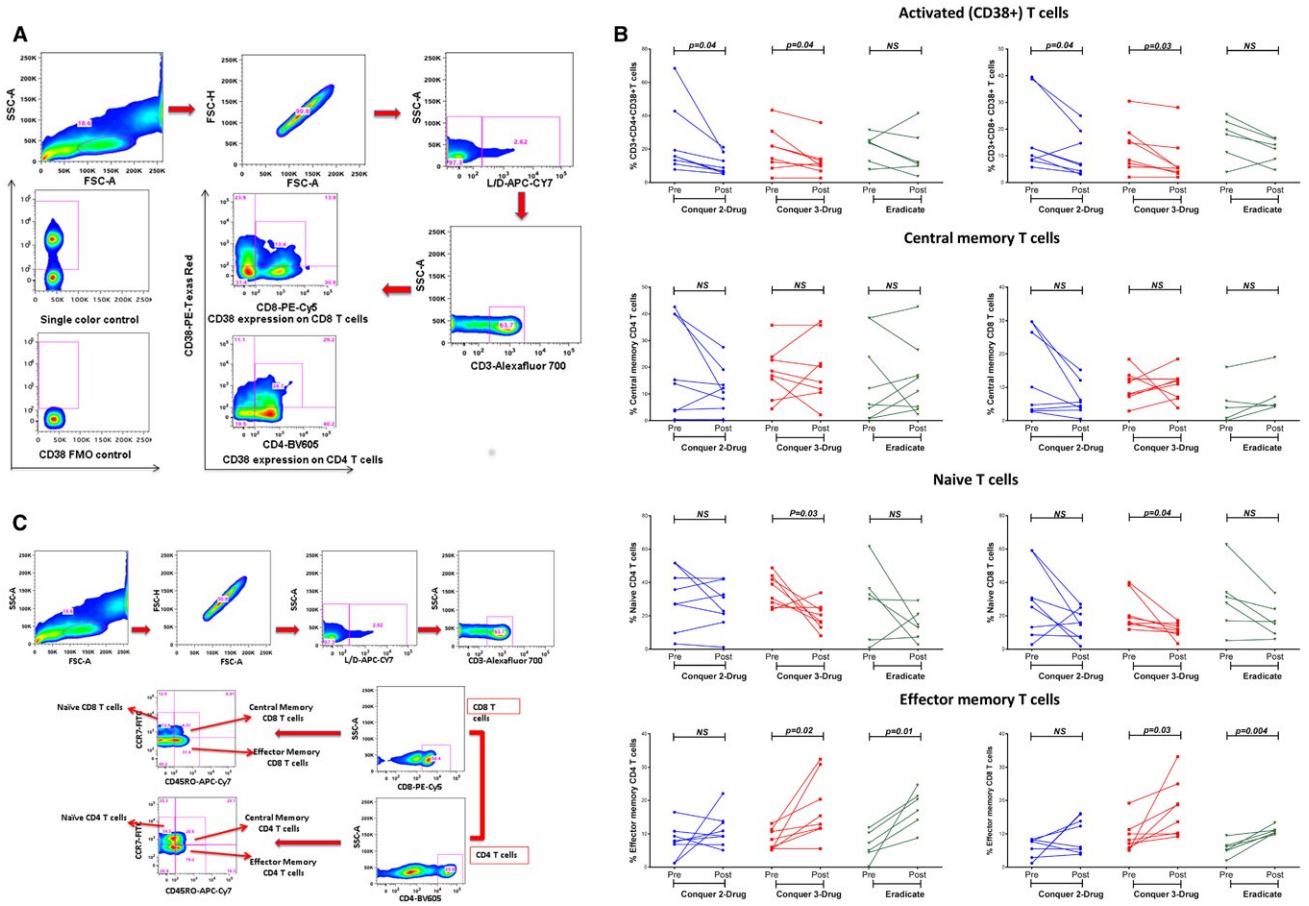


**FIG. 1.** Improved T-cell phenotypes marked by increased effector memory phenotype and decreased chronic immune activation. (A) Gating strategy for determining the expression of CD38 on CD4 and CD8 T cells in patients coinfected with HIV/HCV successfully treated with three different combination DAA regimens in CONQUER 2 DAA, CONQUER 3 DAA, and ERADICATE studies at baseline and SVR 12. (B) Line graphs showing comparative paired changes in the frequencies of activated (CD38+) (upper panel), central, naive, and effector memory (lower panels) CD4 and CD8 T cells before and after therapy. Changes in T-cell phenotypes at baseline and SVR 12 were analyzed using the paired Wilcoxon signed-rank test in GraphPad Prism version 6. $P < 0.05$ was considered significant. NS represents non significant ($P > 0.05$). Each pair represents one sample. (C) Gating strategy for determining the frequencies of central, naive, and effector memory CD4 and CD8 T cells.

**TABLE 2. CHANGES IN THE FREQUENCIES OF PHENOTYPIC AND FUNCTIONAL T CELL MARKERS IN HIV/HCV COINFECTED PATIENTS TREATED WITH DIFFERENT DAA COMBINATIONS**

| | | CONQUER 2 DAA | | | CONQUER 3 DAA | | | ERADICATE | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Methodology | | | | | | | | | | |
| Drugs | | DCV/ASV | | | DCV/ASV/BCV | | | SOF/LDV | | |
| Drug class | | NS5A/NS3 | | | NS5A/NS3/NS5B | | | NS5A/NS5B | | |
| Weeks of treatment | | 24 | | | 12 | | | 12 | | |
| Number of patients | | 8 | | | 8 | | | 6 | | |
| | | Pretherapy (% ± SEM) | Posttherapy SVR12 (% ± SEM) | *P* | Pretherapy (% ± SEM) | Posttherapy SVR12 (% ± SEM) | *P* | Pretherapy (% ± SEM) | Posttherapy SVR12 (% ± SEM) | *P* |
| T-cell phenotypes | | | | | | | | | | |
| T-cell activation CD38 | CD4 | 24.3 ± 7.3 | ↓10.6 ± 2.16* | 0.04 | 19.4 ± 4.6 | ↓13.03 ± 3.5* | 0.04 | 20.8.0 ± 3.1 | 17.5 ± 4.9 | NS |
| Naive T cells | CD8 | 17.1 ± 4.8 | ↓10.4 ± 2.9* | 0.04 | 12.8 ± 3.2 | ↓8.3 ± 3.04* | 0.03 | 16.8 ± 2.8 | 12.1 ± 1.6 | NS |
| | CD8 | 28.5 ± 7.5 | 14.9 ± 3.2 | NS | 21.4 ± 3.9 | ↓12.4 ± 1.78* | 0.04 | 29.6 ± 6.8 | 17.3 ± 3.5 | NS |
| Central memory | CD4 | 19.9 ± 6.3 | 11.9 ± 2.9 | NS | 18.2 ± 3.4 | 19.2 ± 4.3 | NS | 12.0 ± 5.4 | 10.8 ± 3.2 | NS |
| | CD8 | 13.7 ± 4.4 | 6.3 ± 1.7 | NS | 10.2 ± 1.6 | 10.4 ± 1.6 | NS | 9.8 ± 4.4 | 8.8 ± 2.1 | NS |
| Effector memory | CD4 | 7.6 ± 1.7 | 11.3 ± 1.8 | NS | 5.6 ± 1.4 | ↑16.5 ± 1.8* | 0.02 | 6.5 ± 1.4 | ↑17.6 ± 2.0* | 0.01 |
| | CD8 | 5.3 ± 1.1 | 9.7 ± 1.8 | NS | 9.02 ± 1.6 | ↑17.3 ± 2.9* | 0.03 | 5.6 ± 0.8 | ↑10.9 ± 0.4† | 0.004 |
| T-cell exhaustion | | | | | | | | | | |
| PD1 | CD4 | 27.9 ± 5.6 | 21.9 ± 4.5 | NS | 31.9 ± 4.2 | ↓22.5 ± 3.2* | 0.04 | 17.7 ± 4.0 | 14.8 ± 2.8 | NS |
| | CD8 | 57.5 ± 9.7 | 52.6 ± 10.4 | NS | 58.1 ± 9.5 | ↓50.6 ± 8.0* | 0.04 | 52.5 ± 8.2 | 49 ± 8.1 | NS |
| 2B4 | CD4 | 6.1 ± 1.8 | 5.7 ± 1.6 | NS | 11.6 ± 4.7 | 10.4 ± 4.2 | NS | 9.0 ± 2.5 | 6.1 ± 1.6 | NS |
| | CD8 | 55.1 ± 6.3 | 42.7 ± 4.5 | NS | 54.8 ± 4.1 | 47.8 ± 4.0 | NS | 55.4 ± 5.2 | 45.5 ± 4.4 | NS |
| TIGIT | CD4 | 15.0 ± 5.6 | 19.0 ± 7.3 | NS | 20.6 ± 6.7 | ↓8.9 ± 3.1* | 0.01 | 23.3 ± 5.6 | 23.4 ± 14.0 | NS |
| | CD8 | 59.0 ± 9.6 | 60-0 ± 8.2 | NS | 67.0 ± 9.0 | ↓56.0 ± 9.0* | 0.01 | 59.2 ± 8.0 | ↓48.0 ± 7.1* | 0.01 |
| Eomes hi T-bet lo | CD4 | 13.0 ± 3.7 | 10.6 ± 2.8 | NS | 14.9 ± 5.0 | ↓8.7 ± 3.0† | 0.002 | 13.5 ± 4.7 | 7.7 ± 2.7 | NS |
| | CD8 | 25.2 ± 4.2 | 26.2 ± 2.5 | NS | 23.3 ± 5.1 | ↓12.3 ± 1.7* | 0.04 | 17.0 ± 5.1 | 14.2 ± 4.5 | NS |
| T-bet hi Eomes lo | CD4 | 9.1 ± 3.9 | 9.4 ± 3.1 | NS | 9.0 ± 3.3 | ↑18.6 ± 3.7† | 0.005 | 7.3 ± 2.5 | 14.8 ± 5.3 | NS |
| | CD8 | 10.1 ± 3.0 | 11.2 ± 3.1 | NS | 9.9 ± 3.2 | ↑20.3 ± 3.7* | 0.02 | 18.1 ± 1.3 | 21.1 ± 9.2 | NS |
| BLIMP-1 | CD4 | 14.0 ± 5.0 | 7.0 ± 2.2 | NS | 11.1 ± 3.5 | ↓8.9 ± 3.1† | 0.005 | 10.0 ± 5.0 | 6.2 ± 2.0 | NS |
| | CD8 | 35.3 ± 9.7 | 29.7 ± 8.5 | NS | 34.4 ± 9.9 | 33.0 ± 9.7 | NS | 27.0 ± 7.5 | 28.0 ± 8.0 | NS |
| CD8 T-cell functionality | | | | | | | | | | |
| IL-2+ (CD3+CD8+) | | 3.8 ± 0.8 | 6.67 ± 1.8 | NS | 5.2 ± 0.6 | ↑8.4 ± 0.4† | 0.005 | 4.5 ± 1.1 | ↑7.6 ± 0.9† | 0.004 |
| IFN-γ+ (CD3+CD8+) | | 4.8 ± 1.2 | ↑8.3 ± 1.9* | 0.01 | 7.2 ± 1.6 | ↑17.8 ± 2.9‡ | 0.0006 | 6.7 ± 1.2 | 13.6 ± 2.5 | NS |
| TNF-α+ (CD3+CD8+) | | 5.5 ± 1.7 | 4.6 ± 0.6 | NS | 7.0 ± 1.8 | 10.8 ± 2.0 | NS | 6.7 ± 1.2 | 12.2 ± 1.7 | NS |
| IFN-γ+/TNF-α+ (CD3+CD8+) | | 1.5 ± 0.5 | ↑3.0 ± 0.8* | 0.02 | 2.5 ± 0.8 | ↑7.0 ± 1.9* | 0.03 | 2.7 ± 0.2 | 4.1 ± 0.9 | NS |
| CD107A+ (CD3+CD8+) | | 7.9 ± 2.7 | 14.2 ± 2.8 | NS | 11.3 ± 3.8 | ↑16.5 ± 3.6† | 0.006 | 13.9 ± 1.9 | 19.6 ± 2.8 | NS |
| Perforin+(CD3+CD8+) | | 6.7 ± 1.7 | 8.4 ± 2.3 | NS | 9.7 ± 2.7 | ↑16.6 ± 3.7 | 0.04 | 5.0 ± 1.0 | ↑15.0 ± 2.5* | 0.03 |
| Granzyme B+ (CD3+CD8+) | | 6.9 ± 1.8 | 9.6 ± 2.1 | NS | 12.8 ± 1.8 | ↑22.6 ± 2.8† | 0.001 | 11.1 ± 2.3 | ↑18.9 ± 2.8* | 0.03 |

Up arrow indicates increased expression; down arrow indicates decreased expression.
*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ and NS (non significant) indicates $P > 0.05$.

cytokines, and cytotoxic activity. Several markers of immune exhaustion are described, including PD1, cytotoxic T lymphocyte antigen 4, T-cell immunoglobulin and mucin-domain-containing 3, lymphocyte-activation gene 3, TIGIT, CD160, B- and T-lymphocyte attenuator, and 2B4.[22-30] TIGIT has also emerged as an important coinhibitory receptor that is preferentially up-regulated on T cells during chronic viral infections. TIGIT has a T-cell-intrinsic inhibitory function, with its ligation directly inhibiting T-cell proliferation and cytokine production.[31-33] We examined the frequency of various exhaustion markers on both CD4 and CD8 T cells before and after treatment, measured by changes in expression of PD1, 2B4, and TIGIT on treatment. Both the CD4 and CD8 T-cell compartments showed similar trends. The frequencies

of most of the exhaustion markers declined with CONQUER 3-DAA. The changes in the expression of PD1 (CD4 T cells, $P = 0.04$; CD8 T cells, $P = 0.04$) and TIGIT (CD4 T cells, $P = 0.01$; CD8 T cells, $P = 0.01$) decreased with CONQUER 3-DAA treatment. Detailed results are shown in Fig. 2 and Table 2.

To quantify the improvement in T-cell exhaustion following treatment, we analyzed the differences in the intracellular expression of the transcription factors T-bet, Eomes, and BLIMP-1 at pretreatment and SVR. We noted a significant decline in $Eomes^{hi}$ $T\text{-}bet^{lo}$ and an increase in $T\text{-}bet^{hi}$ $Eomes^{lo}$ populations in individuals treated with CONQUER 3-DAA ($Eomes^{hi}$ $T\text{-}bet^{lo}$ CD4 T cells, $P = 0.002$; CD8 T cells, $P = 0.04$; $T\text{-}bet^{hi}$ $Eomes^{lo}$ CD4 T cells, $P = 0.005$; CD8 T cells, $P = 0.02$), while no differences were observed with 2-DAA combination therapy in the CONQUER 2-DAA or ERADICATE trials. Studies in the past also showed that during chronic infection, CD4 T-cell populations expressed elevated levels of transcription factor BLIMP-1 concomitant with reduced functionality. BLIMP-1 is associated with T-cell exhaustion and deletion, resulting in reversal of dysfunction and improved pathogenic control.[34] BLIMP-1 expression was decreased on CD4 T cells ($P = 0.005$) following treatment with 3-DAA only (Fig. 3; Table 2). Thus, treatment with the CONQUER 3-DAA regimen is associated with decreased expression of transcription factors BLIMP-1 and Eomes and increased expression of T-bet.

## ENHANCED HCV-SPECIFIC CD8 T-CELL FUNCTIONS FOLLOWING TREATMENT WITH CONQUER 3-DAA

In order to investigate whether the improved T-cell phenotype was also associated with an enhanced T-cell functional profile, we evaluated the HCV-specific T-cell functions cytokine secretion, polyfunctionality (secretion of more than one cytokine), cytotoxic activity measurement, and cytolytic functions before and after therapy. Our results demonstrated enhancement of HCV-specific CD8 T-cell functions in CONQUER 3-DAA treatment, specifically increased cytokine production of IL-2 and IFN-γ (IL-2, $P = 0.005$; IFN-γ, $P = 0.0006$) (Fig. 4) and increased polyfunctional responses (co-expression of IFN-γ and TNF-α, $P = 0.03$) (Fig. 5); however, we did not see any significant differences in the triple cytokine-positive responses (Supporting Material). We also observed improvement in the cytotoxic activity or cytolytic functions of CD8 T cells, demonstrated by increases in CD107A ($P = 0.006$) production in CONQUER 3-DAA, an increase in perforin secretion ($P = 0.04$), and an increase in granzyme B secretion ($P = 0.001$) in the CONQUER 3-DAA study (Fig. 5). Detailed results are shown in Table 2.

## INCREASED HCV TETRAMER-SPECIFIC CD8 T-CELL RESPONSE FOLLOWING CONQUER 3-DAA THERAPY

To further investigate whether there were any improvements in virus-specific CD8 T-cell response, we also evaluated the differences in tetramer-positive HCV-specific CD8 T cells before and after therapy in patients with HLA-A*02 genotype from the CONQUER 2-DAA (n = 3), CONQUER 3-DAA (n = 3), and ERADICATE (n = 4) studies. We observed a greater increase in HCV-specific CD8 T cells, demonstrated by a greater increase in the frequency or percentage of tetramer-positive HCV-specific CD8 T cells with CONQUER 3-DAA therapy (CONQUER 2-DAA pretreatment, 0.29% ± 0.07% versus posttreatment, 0.64% ± 0.13%; $P = 0.25$; CONQUER 3-DAA pretreatment, 0.26% ± 0.08% versus posttreatment, 1.80% ± 0.59%; $P = 0.04$; and ERADICATE pretreatment 1.00% ± 0.50% versus posttreatment, 1.40% ± 0.50%; $P = 0.12$) (data represents mean ± SEM). In addition, fold changes evaluated by calculating the ratio of frequency of tetramer-positive HCV-specific CD8 T cells before and after therapy were higher in the CONQUER 3-DAA study (Fig. 6).

# Discussion

Various immunologic mechanisms have been proposed to account for accelerated liver disease in patients coinfected with HIV/HCV, including diminished HCV-specific T-cell responses associated with loss of effector and proliferative functions as well as chronic immune activation and immune exhaustion during HIV/HCV coinfection. For reasons that are not fully understood, past immune-based HCV treatment regimens that incorporated IFN resulted in significantly lower rates of SVR in coinfected patients compared to patients monoinfected with HCV. The advent of highly effective DAAs to treat HCV offers a unique opportunity to explore the immunologic impact of IFN-free, nonimmune-based, HCV treatment combinations

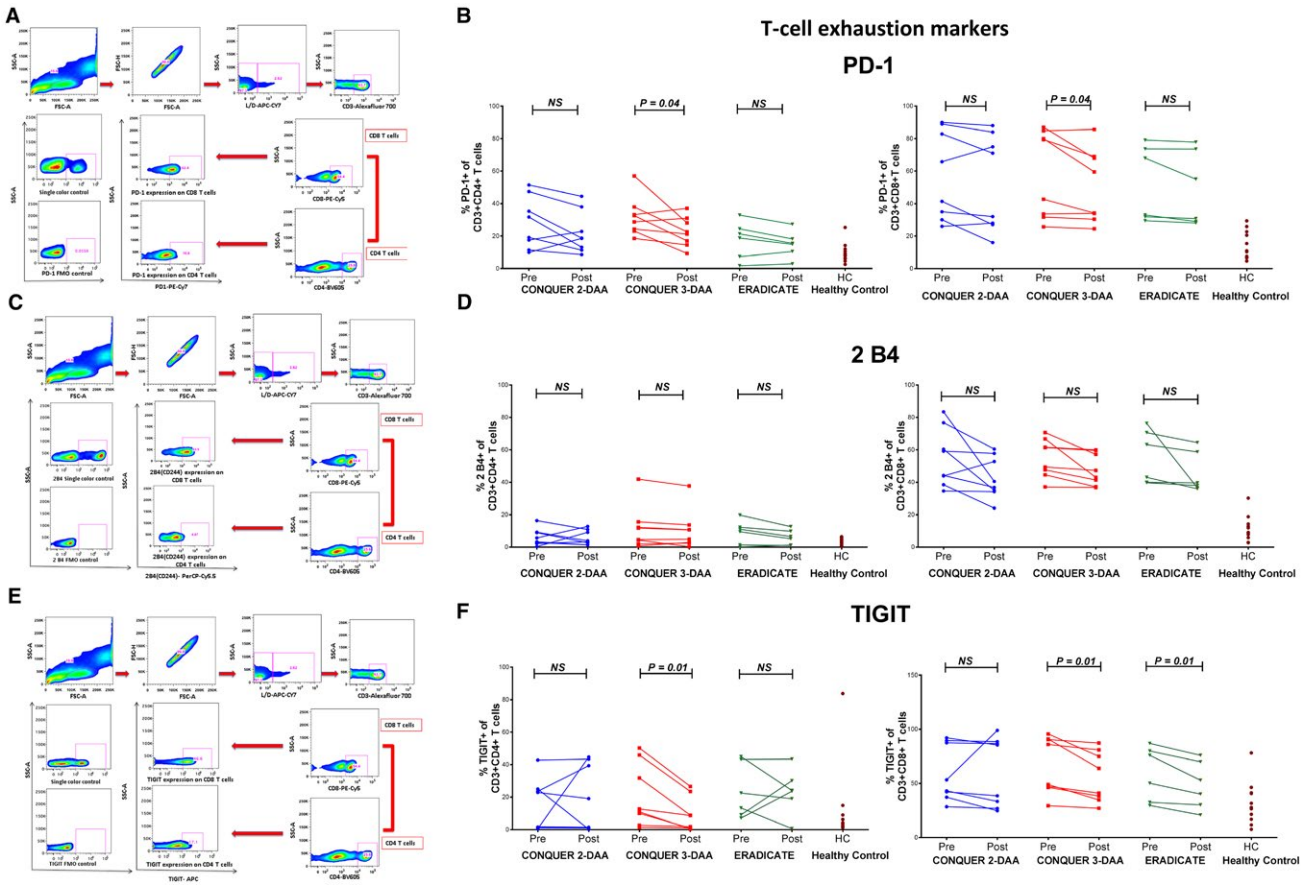


**FIG. 2.** Reversal of T-cell exhaustion following treatment with CONQUER 3 DAA (DCV/ASV/BCV). (A-F) T-cell immunophenotyping was performed to assess the changes in expression of exhaustion markers PD1, 2B4, and TIGIT on CD4+ and CD8+ T cells in patients coinfected with HIV/HCV successfully treated with three different combination DAA regimens in CONQUER 2 DAA, CONQUER 3 DAA, and ERADICATE studies at baseline and SVR 12. (A,C,E) Gating strategy for determining the expression of (A) PD1, (C) 2B4, and (E) TIGIT on CD4 and CD8 T cells. (B,D,F) Line graphs showing the comparative paired changes in the frequencies of CD4 and CD8 T cells expressing different exhaustion markers before and after therapy: (B) PD1, (D) 2B4, and (F) TIGIT. Changes in percentage of T-cell subsets expressing different markers at baseline and SVR 12 were analyzed using the paired Wilcoxon signed-rank test in GraphPad Prism version 6. $P < 0.05$ was considered significant. NS represents non significant ($P > 0.05$). Each pair represents one sample.

and to describe the effects of eradication of a chronic viral infection on the immune systems of patients with HIV/HCV coinfection.

In this study, we explored the impact of combination DAA-based therapy on immune phenotypes and functions in coinfected patients. By comparing coinfected patients successfully treated with different combination DAA-based regimens, we hope to discern whether treatment combinations with different viral targets have differential effects on T-cell immunophenotypes and the restoration of HCV-specific T-cell immunity that has been observed with successful HCV clearance. Our data suggest that DAA combination therapy targeting three steps in the HCV replication cycle is more effective in improving T-cell immunophenotypes than that targeting only two viral targets, as shown by the more significant declines in chronic immune activation, T-cell exhaustion, and improvements in T-cell subsets with increased effector memory phenotypes.

Our results showed improvement in T-cell activation phenotypes as evidenced by the subsequent decrease in CD38 expression and resolution of T-cell activation following treatment with CONQUER 2-DAA and 3-DAA therapies. We further investigated the changes in T-cell differentiation phenotypes because perturbations in the homeostasis of memory T cells may play an important role in disease pathogenesis; we were able to demonstrate a decrease in the naive T-cell subset with a corresponding increase in the effector memory T-cell subset following treatment with the CONQUER

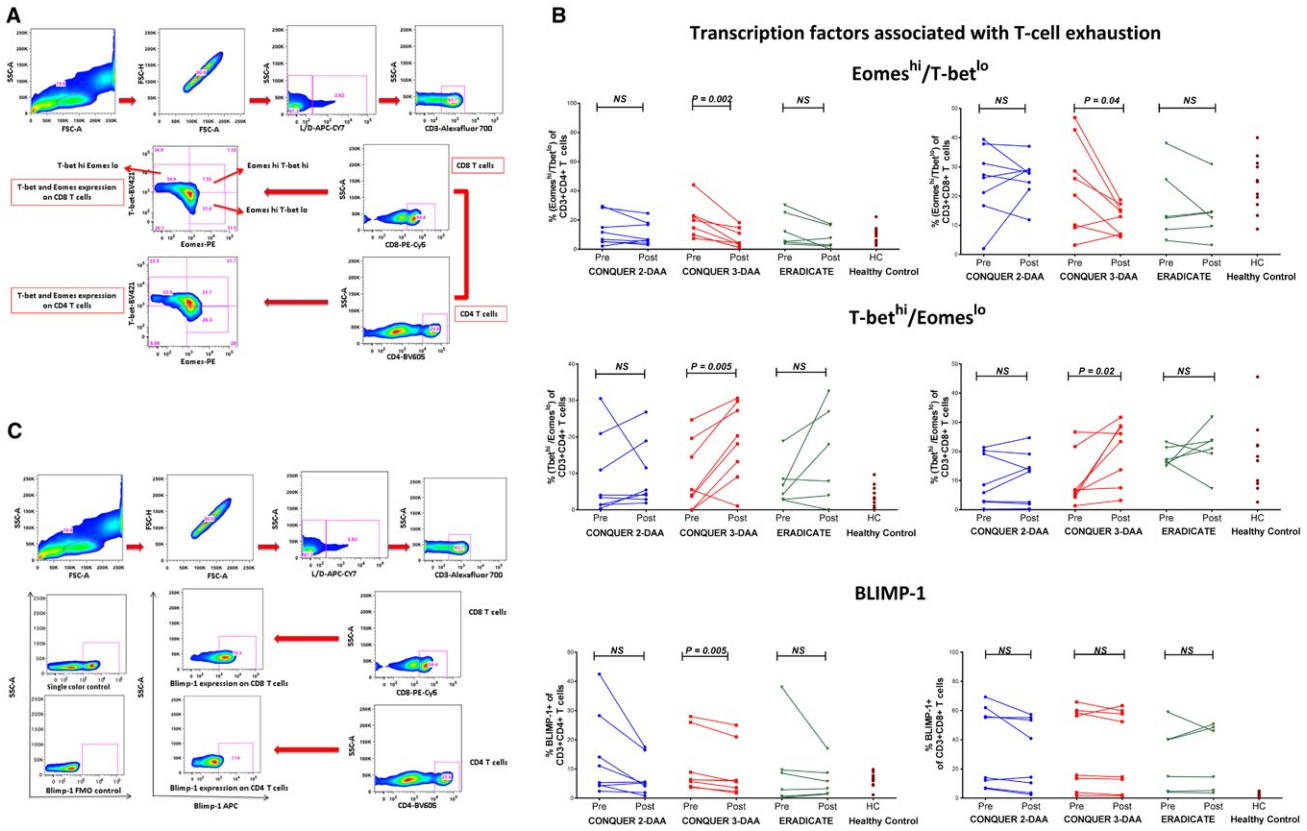


**FIG. 3.** CONQUER 3 DAA (DCV/ASV/BCV) regimen is associated with decreased expression of transcription factors BLIMP-1 and Eomes and increased expression of T-bet. T-cell immunophenotyping was performed to assess the changes in expression of transcription factors Eomes, T-bet, and BLIMP-1 on CD4 and CD8 T cells in patients coinfected with HIV/HCV successfully treated with three different combination DAA regimens in CONQUER 2 DAA, CONQUER 3 DAA, and ERADICATE studies at baseline and SVR 12. (A) Gating strategy for determining the expression of T-bet and Eomes on CD4 and CD8 T cells. (B) Line graphs showing the comparative paired changes in the frequencies of CD4 and CD8 T cells expressing different transcription factors (T-bet, Eomes, and BLIMP-1) before and after therapy. Changes in percentage of T-cell subsets expressing different markers at baseline and SVR 12 were analyzed using the paired Wilcoxon signed-rank test in GraphPad Prism version 6. $P < 0.05$ was considered significant. NS represents non significant ($P > 0.05$). Each pair represents one sample. (C) Gating strategy for determining the expression of BLIMP-1 on CD4 and CD8 T cells.

3-DAA regimen. Our results demonstrate an overall decline in markers of T-cell exhaustion, with decreased expression of the exhaustion markers PD1and TIGIT on CD4 and CD8 T cells after treatment with CONQUER 3-DAA. T-bet and Eomes are of particular interest; they represent two key transcription factors for determining CD8+ T-cell differentiation and functions. Previous studies have demonstrated that CD8 T cells with Eomes$^{hi}$ T-bet$^{lo}$ are associated with up-regulation of inhibitory receptors, impaired functional characteristics, and a transitional memory differentiation phenotype during chronic viral infections. In contrast, T-bet$^{hi}$ CD8+ T cells represent a progenitor subset with proliferative potential that give rise to Eomes$^{hi}$ CD8+ T cells, which are terminally differentiated and can no longer proliferate in response to antigen or be rescued by PD1 blockade. While both populations express PD1, Eomes$^{hi}$-exhausted cells express the highest levels of PD1.[35-40] Recent studies have implicated elevations of T-bet$^{lo}$ and Eomes$^{hi}$, along with elevated levels of BLIMP-1, with the development of T-cell exhaustion.[41-43] We found a significant decline in Eomes$^{hi}$ T-bet$^{lo}$ and an increase in T-bet$^{hi}$ Eomes$^{lo}$ populations on CD8 T cells following treatment with the 3-DAA combination, while no differences were observed with 2-DAA therapy in the CONQUER 2-DAA and ERADICATE treatment regimens. We also observed that BLIMP-1 expression was decreased

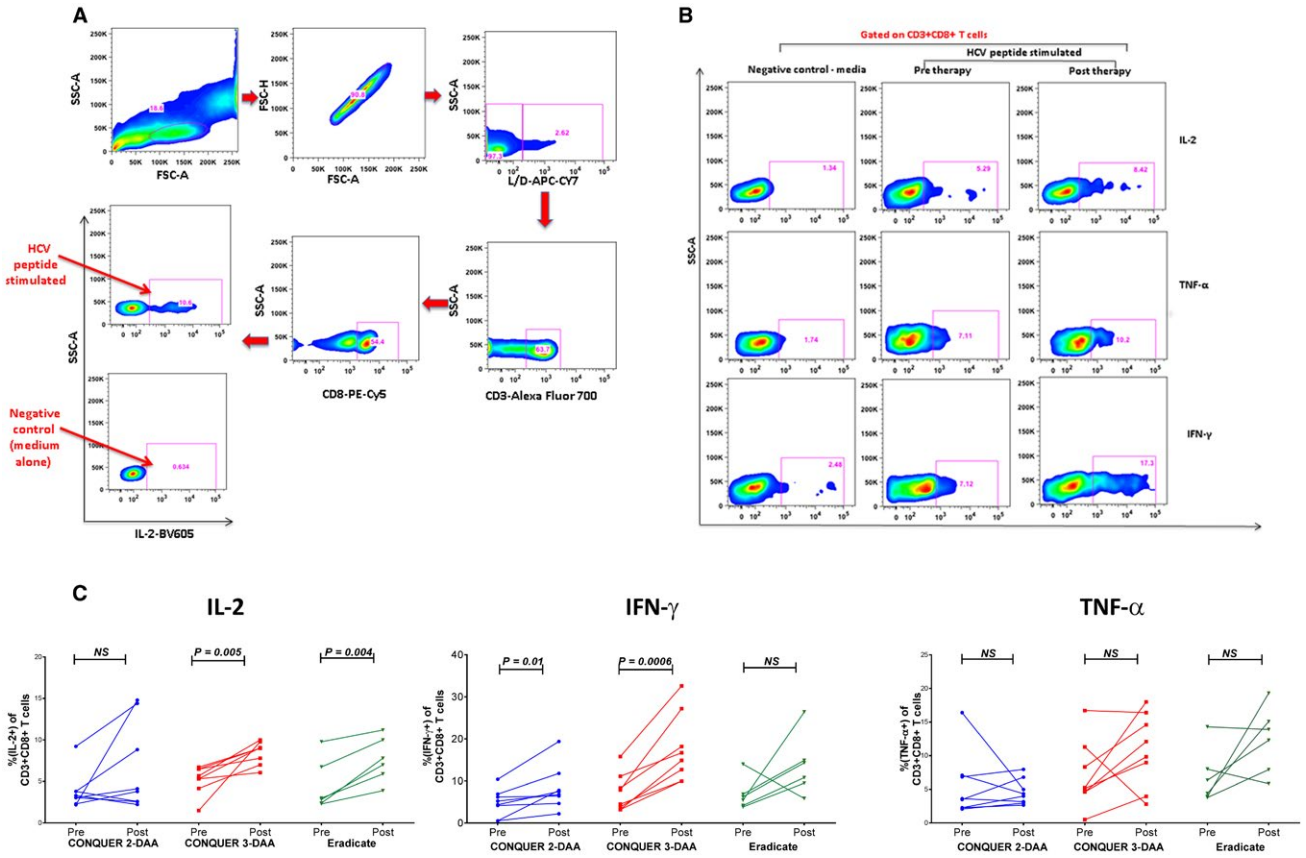


**FIG. 4.** Enhanced HCV-specific T-cell functions following treatment with CONQUER 3 DAA (DCV/ASV/BCV). Cryopreserved PBMCs were thawed to analyze the changes in HCV-specific T-cell functionality. PBMCs were stimulated *in vitro* for 4 days with a panel of HCV peptides spanning the entire HCV genome. This was followed by surface and intracellular staining for determination of different CD8 T-cell functions, such as cytokine secretion. (A) Flow gating strategy for determining the frequency of cytokine-producing HCV-specific CD8 T cells (IL-2-producing CD8 T cells). (B) Representative flow plots showing changes in frequencies of cytokine-producing HCV-specific CD8 T cells before and after therapy (IL-2, IFN-γ, and TNF-α). (C) Line graphs showing comparative paired changes in the frequencies of cytokine-producing HCV-specific CD8 T cells before and after therapy. The *P* value was evaluated by the paired Wilcoxon signed-rank test, and $P < 0.05$ was considered significant. NS represents non significant ($P > 0.05$). Each pair represents one sample.

on CD4 T cells following treatment with CONQUER 3-DAA therapy only. Thus, successful treatment with the CONQUER 3-DAA regimen was associated with decreased expression of transcription factors BLIMP-1 and Eomes (terminal subset) and increased expression of T-bet (progenitor subset).

After determining the T-cell phenotypes, we next asked whether these treatment approaches are equally effective in enhancing HCV-specific T-cell functions. Previous studies demonstrated that in patients with HIV and HCV coinfection, T-cell functions, including cytokine secretion, proliferation, and cytotoxic potential, appear to diminish gradually due to immune exhaustion. Our results demonstrate enhancement of HCV-specific T-cell functions, including an increase in cytokine production (IL-2 and IFN-γ) and an increase in polyfunctionality, as evidenced by an increase in the proportion of CD8 T cells co-expressing IFN-γ and TNF-α in patients treated with the CONQUER 3-DAA. We also observed increased CD8 T-cell cytolytic functions demonstrated by an increase in CD107A production in the CONQUER 3-DAA study and an increase in perforin secretion and granzyme B secretion in CONQUER 3-DAA patients.

In conclusion, we have shown that treatment of HCV in patients coinfected with HIV/HCV with combination DAA therapies results in decreased levels of T-cell exhaustion and chronic immune activation and an improvement in the T-cell subset profiles with an increase in the effector memory population. Our results

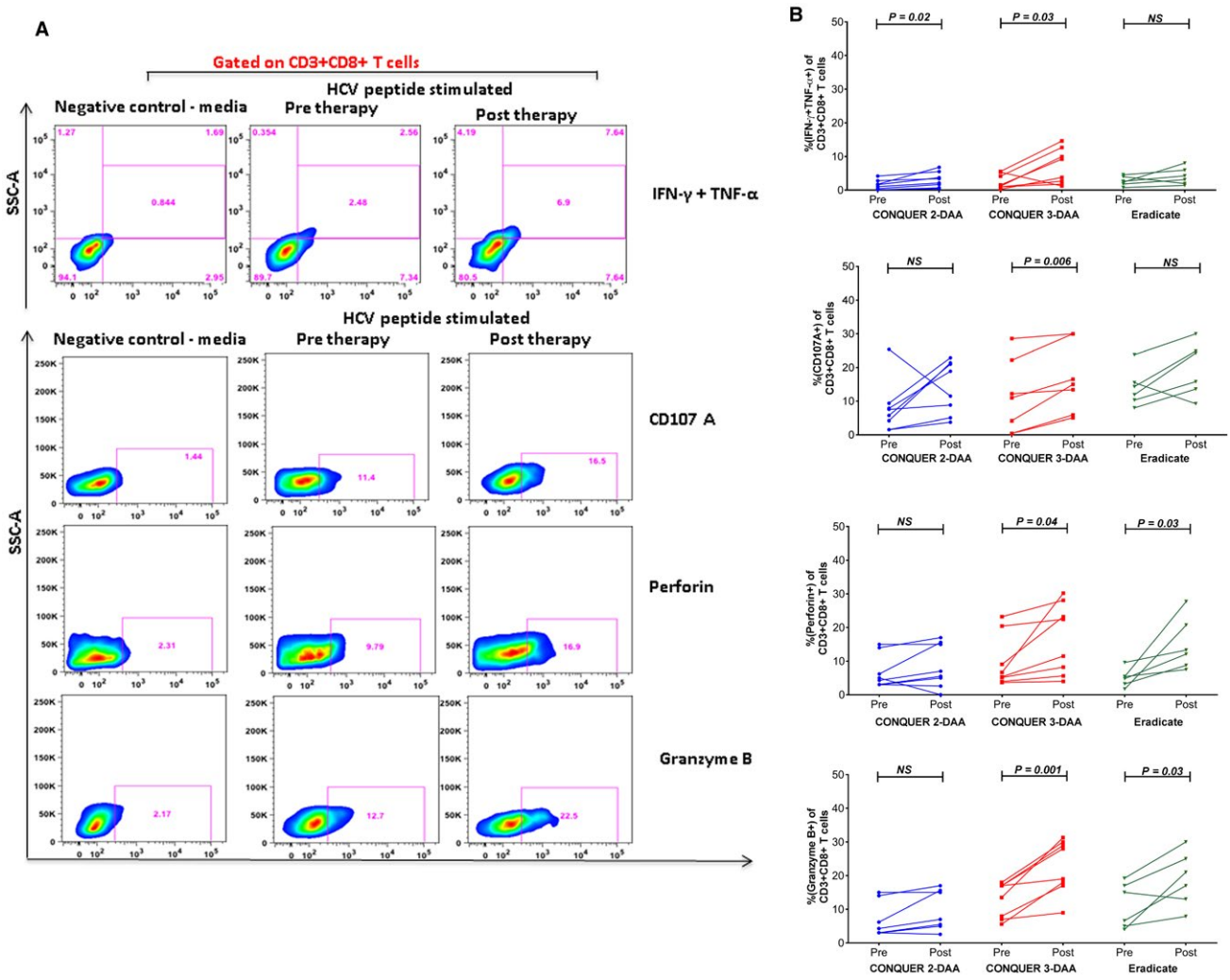


**FIG. 5.** Enhanced HCV-specific T-cell polyfunctionality (IFN-γ+ and TNF-α+), cytotoxicity (CD107A mediated), and cytolytic functions (perforin and granzyme B mediated) following treatment with CONQUER 3 DAA (DCV/ASV/BCV). (A) Representative flow plots showing changes in frequencies of polyfunctional (IFN-γ+TNF-α+)/cytotoxic (CD107A+) and cytolytic (perforin+ and granzyme B+) HCV-specific CD8 T cells before and after therapy. (B) Line graphs showing comparative paired changes in the frequencies of IFN-γ+TNF-α+/CD107A/perforin and granzyme B produced by HCV-specific CD8 T cells before and after therapy. The *P* value was evaluated by the paired Wilcoxon signed rank test, and $P < 0.05$ was considered significant. NS represents non significant ($P > 0.05$). Each pair represents one sample.

not only demonstrate improvement in immune phenotypes following DAA treatment but also demonstrate augmented HCV-specific T-cell function, including cytokine production, polyfunctionality, and cytolytic capacity, with the CONQUER 3-DAA (DCV/ASV/BCV) treatment group (Fig. 7). Thus, the most profound restoration of HCV-specific immune responses was observed in the group of patients coinfected with HIV/HCV treated with a regimen that inhibits three distinct stages of the HCV life cycle. Whether this is due to more potent suppression of HCV *in vivo* or an independent effect on the immune system is unknown. It is possible that the antiviral augmentation of host immunity may have a significant impact on HCV clearance and may play a role in achieving SVR, but treatment of 12 weeks or longer may overcome host immune factors in achieving successful viral clearance. Although patients coinfected with HIV and HCV have similar success rates to HCV treatment as patients monoinfected with HCV, the selection of HCV treatment regimens is complicated in patients coinfected with HIV by both the consideration of drug–drug

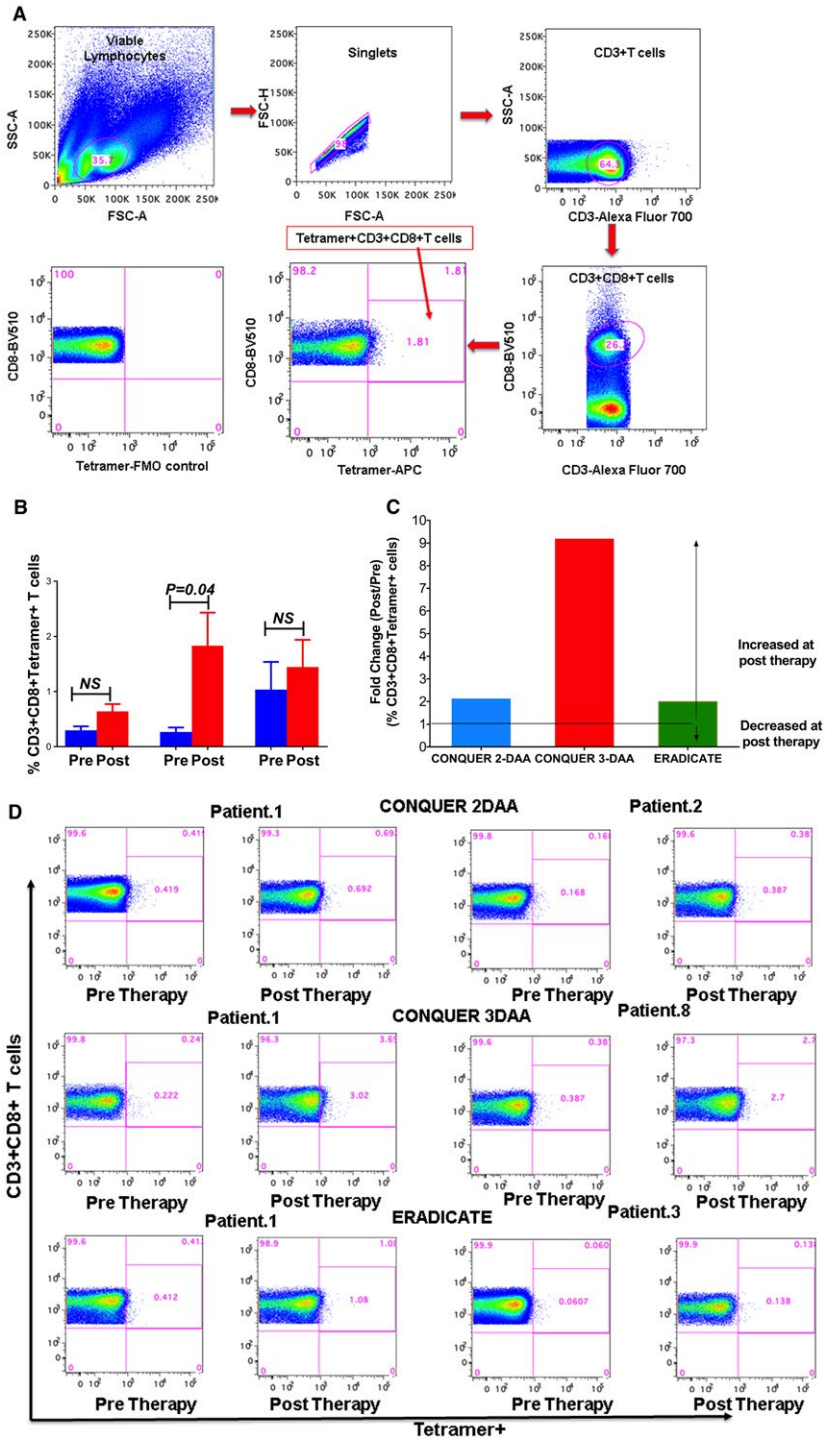


**FIG. 6.** Increased HCV tetramer-specific CD 8 T-cell response following CONQUER 3-DAA therapy. (A) Flow gating strategy used for determining the frequency of tetramer-positive HCV-specific CD8 T cells in patients positive for HLA-A*02. (B) Bar graphs showing changes in the frequencies of tetramer-positive HCV-specific CD8 T cells before and after therapy. The *P* value was evaluated by the paired Wilcoxon signed-rank test, and $P < 0.05$ was considered significant. (Data represents mean ± SEM), NS represents non significant ($P > 0.05$). (C) Graph showing fold changes in the frequencies of tetramer-positive HCV-specific CD8 T cells after therapy versus before therapy. (D) Representative plots of two patients in each group are shown. HCV-specific CD8+ T cells were detected by positive-tetramer binding. Abbreviation: Pt, patient.

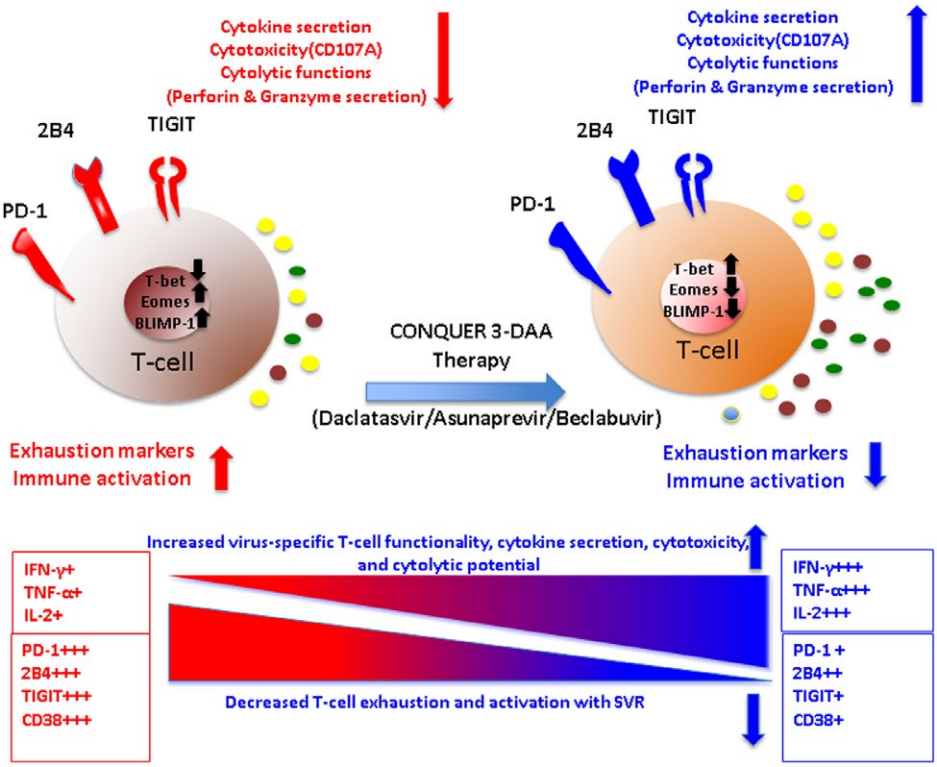


**FIG. 7.** Multitarget DAA therapy is associated with superior immunologic recovery in patients coinfected with HIV/HCV. CONQUER 3-DAA combination therapy is associated with superior immunologic recovery evidenced by decreased levels of T-cell exhaustion (PD1, TIGIT, T-bet, and Eomes) and chronic immune activation (CD38) and an improvement in the T-cell subset profiles, with an increase in the effector memory population. It also resulted in augmentation of HCV-specific T-cell functions, including cytokine production (IFN-γ, TNF-α, and IL-2) and cytolytic capacity with HCV clearance at SVR12 in the patients coinfected with HIV/HCV. (+ indicates significantly decreased expression, ++ no significant changes in expression, +++ indicates significantly increased expression)

interactions and formulary preferences. Therefore, the significance of our study is that we show that coinfected patients, a population that has previously been shown to have accelerated fibrosis progression and lower treatment responses, may benefit differentially from HCV treatment regimens in addition to attaining SVR; this information may be useful when providers select regimens for patients coinfected with HIV/HCV. While ASV and BCV are not approved in the United States (although ASV is approved in Japan and Russia), other medications from these classes are available, and their effects in patients coinfected with HIV/HCV may warrant further study.

Our study has certain limitations that include a small number of participants in each study group. However, our samples were taken from clinical trials, and so the number enrolled and those with remaining samples limited our sample size. Nevertheless, our findings require further confirmation in larger studies with comparable baseline demographics. We hope that our results are hypothesis generating and can be further confirmed in larger studies. A second limitation is that our study only explored improvements in T-cell phenotype and functions in peripheral blood. Although changes in intrahepatic T-cell profiles would be very important for studying the effect of DAA therapy, the difficulty and potential morbidity associated in obtaining this tissue limited our study. To the best of our knowledge, we have demonstrated for the first time that triple-DAA-based therapy, coupling NS3 inhibition with potent NS5A and NS5B inhibition, may have a profound effect on reducing immune activation and exhaustion and restoring immune function in patients coinfected with HIV/HCV.

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## REFERENCES


1) Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. Hepatology 1999;30:1054-1058.
2) Poynard T, Mathurin P, Lai CL, Guyader D, Poupon R, Tainturier MH, et al. PANFIBROSIS Group. A comparison of fibrosis progression in chronic liver diseases. J Hepatol 2003;38:257-265.
3) Chen JY, Feeney ER, Chung RT. HCV and HIV co-infection: mechanisms and management. Nat Rev Gastroenterol Hepatol 2014;11:362-371.
4) Brau N, Salvatore M, Rios-Bedoya CF, Fernandez-Carbia A, Paronetto F, Rodriguez-Orengo JF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. J Hepatol 2006;44:47-55.
5) Habib S, Meister E, Habib S, Murakami T, Walker C, Rana A, et al. Slower fibrosis progression among liver transplant recipients with sustained virological response after hepatitis C treatment. Gastroenterology Res 2015;8:237-246.
6) Chung RT, Andersen J, Volberding P, Robbins GK, Liu T, Sherman KE, et al; AIDS Clinical Trials Group A5071 Study Team. Peginterferon Alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. N Engl J Med 2004;351:451-459.
7) Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.
8) Abdel-Hakeem MS, Bedard N, Badr G, Ostrowski M, Sekaly RP, Bruneau J, et al. Comparison of immune restoration in early versus late alpha interferon therapy against hepatitis C virus. J Virol 2010;84:10429-10435.
9) Badr G, Bedard N, Abdel-Hakeem MS, Trautmann L, Willems B, Villeneuve JP, et al. Early interferon therapy for hepatitis C virus infection rescues polyfunctional, long-lived CD8+ memory T cells. J Virol 2008;82:10017-10031.
10) Missale G, Pilli M, Zerbini A, Penna A, Ravanetti L, Barili V, et al. Lack of full CD8 functional restoration after antiviral treatment for acute and chronic hepatitis C virus infection. Gut 2012;61:1076-1084.
11) Seigel B, Bengsch B, Lohmann V, Bartenschlager R, Blum HE, Thimme R. Factors that determine the antiviral efficacy of HCV-specific CD8(+) T cells ex vivo. Gastroenterology 2013;144:426-436.
12) Martin B, Hennecke N, Lohmann V, Kayser A, Neumann-Haefelin C, Kukolj G, et al. Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. J Hepatol 2014;61:538-543.
13) Shrivastava S, Wilson E, Poonia B, Tang L, Osinusi A, Kohli A, et al. Augmentation of hepatitis C virus-specific immunity and sustained virologic response. J Viral Hepat 2017;24:742-749.
14) Osinusi A, Townsend K, Kohli A, Nelson A, Seamon C, Meissner EG, et al. Virologic response following combined ledipasvir and sofosbuvir administration in patients with HCV genotype 1 and HIV co-infection. JAMA 2015;313:1232-1239.
15) Rockstroh JK, Nelson M, Katlama C, Lalezari J, Mallolas J, Bloch M, et al. Efficacy and safety of grazoprevir (MK-5172) and elbasvir (MK-8742) in patients with hepatitis C virus and HIV co-infection (C-EDGE CO-INFECTION): a non-randomised, open-label trial. Lancet HIV 2015;2:e319-327.
16) Rosenthal ES, Howard L, Purdy J, McLaughlin M, Kattakuzhy S, Kohli A, et al. Virologic response following asunaprevir/daclatasvir with or without beclabuvir for treatment of HCV genotype 1 in patients co-infected with HIV [Abstract]. J Hepatol 2016;64(Suppl.):S760-S761.
17) Khaitan A, Unutmaz D. Revisiting immune exhaustion during HIV infection. Curr HIV/AIDS Rep 2011;8:4-11.
18) Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, Jacobson LP, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 1999;179:859-870.
19) Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K. Detels R. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr 1993;6:904-912.
20) Rosenblum MD, Way SS, Abbas AK. Regulatory T cell memory. Nat Rev Immunol 2016;16:90-101.
21) **Golubovskaya V, Wu L**. Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. Cancers (Basel) 2016;8.pii:E36.
22) Wherry EJ. T cell exhaustion. Nat Immunol 2011;12:492-499.
23) Goepfert PA, Bansal A, Edwards BH, Ritter GD Jr, Tellez I, McPherson SA, et al. A significant number of human immunodeficiency virus epitope-specific cytotoxic T lymphocytes detected by tetramer binding do not produce gamma interferon. J Virol 2000;74:10249-10255.
24) Kostense S, Ogg GS, Manting EH, Gillespie G, Joling J, Vandenberghe K, et al. High viral burden in the presence of major HIV-specific CD8(+) T cell expansions: evidence for impaired CTL effector function. Eur J Immunol 2001;31:677-686.
25) Shankar P, Russo M, Harnisch B, Patterson M, Skolnik P, Lieberman J. Impaired function of circulating HIV-specific CD8(+) T cells in chronic human immunodeficiency virus infection. Blood 2000;96:3094-3101.
26) Gruener NH, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, et al. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. J Virol 2001;75:5550-5558.
27) Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000;191:1499-1512.
28) Reignat S, Webster GJ, Brown D, Ogg GS, King A, Seneviratne SL, et al. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. J Exp Med 2002;195:1089-1101.
29) Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682-687.
30) **Lee PP, Yee C**, Savage PA, Fong L, Brockstedt D, Weber JS, et al. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. Nat Med 1999;5:677-685.
31) Kurtulus S, Sakuishi K, Ngiow SF, Joller N, Tan DJ, Teng MW, et al. TIGIT predominantly regulates the immune response via regulatory T cells. J Clin Invest 2015;125:4053-4062.

32) Joller N, Hafler JP, Brynedal B, Kassam N, Spoerl S, Levin SD, et al. Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. J Immunol 2011;186:1338-1342.
33) Levin SD, Taft DW, Brandt CS, Bucher C, Howard ED, Chadwick EM, et al. Vstm3 is a member of the CD28 family and an important modulator of T-cell function. Eur J Immunol 2011;41:902-915.
34) Hwang S, Cobb DA, Bhadra R, Youngblood B, Khan IA. Blimp-1-mediated CD4 T cell exhaustion causes CD8 T cell dysfunction during chronic toxoplasmosis. J Exp Med 2016;213:1799-1818.
35) Buggert M, Tauriainen J, Yamamoto T, Frederiksen J, Ivarsson MA, Michaelsson J, et al. T-bet and Eomes are differentially linked to the exhausted phenotype of CD8+ T cells in HIV infection. PLoS Pathog 2014;10:e1004251.
36) Pearce EL, Mullen AC, Martins GA, Krawczyk CM, Hutchins AS, Zediak VP, et al. Control of effector CD8+ T cell function by the transcription factor eomesodermin. Science 2003;302:1041-1043.
37) Banerjee A, Gordon SM, Intlekofer AM, Paley MA, Mooney EC, Lindsten T, et al. Cutting edge: the transcription factor eomesodermin enables CD8+ T cells to compete for the memory cell niche. J Immunol 2010;185:4988-4992.
38) Joshi NS, Cui W, Chandele A, Lee HK, Urso DR, Hagman J, et al. Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity 2007;27:281-295.
39) Joshi NS, Cui W, Dominguez CX, Chen JH, Hand TW, Kaech SM. Increased numbers of preexisting memory CD8 T cells and decreased T-bet expression can restrain terminal differentiation of secondary effector and memory CD8 T cells. J Immunol 2011;187:4068-4076.
40) Zhou X, Yu S, Zhao DM, Harty JT, Badovinac VP, Xue HH. Differentiation and persistence of memory CD8(+) T cells depend on T cell factor 1. Immunity 2010;33:229-240.
41) Shin H, Blackburn SD, Intlekofer AM, Kao C, Angelosanto JM, Reiner SL, et al. A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. Immunity 2009;31:309-320.
42) Kao C, Oestreich KJ, Paley MA, Crawford A, Angelosanto JM, Ali MA, et al. Transcription factor T-bet represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8+ T cell responses during chronic infection. Nat Immunol 2011;12:663-671.
43) Paley MA, Kroy DC, Odorizzi PM, Johnnidis JB, Dolfi DV, Barnett BE, et al. Progenitor and terminal subsets of CD8+ T cells cooperate to contain chronic viral infection. Science 2012;338:1220-1225.

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# Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1258/suppinfo.