Received: 19 December 2022 | Revised: 4 January 2023 | Accepted: 26 January 2023

DOI: 10.1111/ctr.14926

Clinical TRANSPLANTATION
The Journal of Clinical and Translational Research
WILEY

BRIEF COMMUNICATION

Early initiation of glecaprevir/pibrentasvir after transplantation of HCV-viremic kidneys into HCV-negative recipients is associated with normalization in the altered inflammatory milieu

Myung-Ho Kim[1] | Meghan E. Sise[2] | Min Xu[1] | David S. Goldberg[3] | Robert J. Fontana[4] | Jens J. Kort[5] | Rita R. Alloway[6] | Christine M. Durand[7] | Robert S. Brown Jr[8] | Josh Levitsky[9] | Jenna L. Gustafson[1] | Peter P. Reese[10,11] | Raymond T. Chung[1]

[1]Liver Center, Gastrointestinal Division, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

[2]Nephrology Division, Massachusetts General Hospital, Boston, Massachusetts, USA

[3]Division of Digestive Health & Liver Diseases, University of Miami Miller School of Medicine, Miami, Florida, USA

[4]Division of Gastroenterology & Hepatology, University of Michigan Medical School, Ann Arbor, Michigan, USA

[5]Global Medical Affairs Research & Development, AbbVie Inc., North Chicago, Illinois, USA

[6]Division of Nephrology, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

[7]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[8]Division of Gastroenterology & Hepatology, Weill Cornell Medicine, New York, New York, USA

[9]Comprehensive Transplant Center, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

[10]Department of Biostatistics, Epidemiology and Informatics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA

[11]Renal-Electrolyte and Hypertension Division, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence
Raymond T. Chung.
Email: Chung.Raymond@mgh.harvard.edu

Abstract

Our previous Multicenter Trial to Transplant HCV-infected Kidneys (MYTHIC) observed that 100% of hepatitis C virus (HCV)-uninfected patients who received a kidney from an HCV-infected deceased donor were cured of HCV with an 8-week regimen of glecaprevir and pibrentasvir (G/P) initiated 2–5 days after transplantation. Following acute and chronic infection with HCV, immune system perturbations have been reported to persist even after viral clearance. The aim of this study was to determine whether HCV viremic kidney recipients in the MYTHIC study experience sustained changes in the soluble inflammatory milieu associated with HCV infection. Among nine patients with HCV viremia at day 3 post-kidney transplant (post-KT D3), IP-10, IL-10, MIP-1β, and IL-8 were significantly elevated from baseline. However, over the subsequent visits, there was a rapid, dramatic reduction back to baseline levels. Among seven patients who were not HCV viremic at post-KT D3, the cytokine levels did not significantly change. HCV-uninfected patients who received a kidney from an HCV-viremic

deceased donor and were treated with early G/P experienced only transient alterations in the soluble inflammatory milieu. These data provide reassuring evidence that there appear to be no persistent cytokine disturbances with transient HCV viremia accompanying HCV donor positive/recipient negative kidney transplant.

KEYWORDS
biomarker, immune regulation, infection and infectious agents, viral: hepatitis C

1 | INTRODUCTION

According to data from the Organ Procurement Transplantation Network, there were 89 896 patients on the waiting list for kidney transplantation as of August 22, 2022, in the United States (US), and only 24 670 patients underwent kidney transplantation in 2021.[1] In the past, kidneys from donors with hepatitis C virus (HCV) infection had been underused in the past because of the risk of HCV transmission to recipients; however, because direct-acting antivirals (DAAs) are so effective at curing HCV infection, there is tremendous interest in transplanting organs harvested from HCV-infected donors into uninfected recipients. Indeed, data over the past 5 years have shown that nearly all recipients transplanted with HCV viremic kidneys have achieved sustained virologic response (SVR) with good 1- and 5-year allograft outcomes.[2,3]

However, acute or persistent HCV infection can cause alterations in the frequency, phenotype, and function of innate and adaptive immune cells. Furthermore, prior studies have shown that HCV eradication with DAA therapy does not completely reverse the alterations in the inflammatory milieu in both acute and chronic HCV infections.[4,5] An altered inflammatory milieu can affect the long-term outcome of kidney transplant recipients,[6] for example, by triggering BK polyomavirus (BKPyV) viremia and nephropathy or leading to an increased risk of cytomegalovirus infection,[7,8] both of which have occurred at higher than expected incidence in a prior report of HCV donor positive/recipient negative kidney transplantation with delayed DAA therapy.[9]

In a previous study, we conducted a multicenter trial of uninfected kidney recipients who were transplanted with HCV viremic kidneys. All 30 recipients were treated with an 8-week course of glecaprevir and pibrentasvir (G/P) beginning day 2–5 post transplant, and all achieved SVR.[10] While the therapy was well tolerated and allograft function was preserved at 1 year of followup, given the potential safety concerns of an intentional donor-derived infection, we sought to determine whether even transient HCV viremia following HCV viremic kidney transplantation causes alterations in the soluble inflammatory milieu of the recipients. Thus, we measured changes in plasma cytokine levels in the recipients transplanted with HCV viremic kidneys.

2 | MATERIALS AND METHODS

This study utilized samples from our previously published study: Multicenter Study to Transplant Hepatitis C-infected Kidneys (MYTHIC), in which the recipients were transplanted with kidneys from deceased donors with HCV infection and treated with G/P for 8 weeks.[10] The trial protocol was approved by the institutional review boards at the clinical sites: Massachusetts General Hospital, University of Pennsylvania, University of Cincinnati, Johns Hopkins University, University of Michigan, Northwestern Medical Center, and Weil Cornell Medical Center. The trial was conducted in accordance with the protocol and followed the International Conference on Harmonization guidelines, applicable regulations and guidelines governing clinical study conduct, and the ethical principles that have their origin in the Declaration of Helsinki. Initiation of G/P therapy occurred between days 2 and 5 following the transplant. Plasma samples were available from 16 recipients of HCV-infected kidneys, and collected at baseline (prior to the transplant), day 3 post-kidney transplant (post-KT D3), the end of G/P therapy (end of Tx), 12 weeks after completing G/P (post-Tx W12), and 1-year post-kidney transplant (post-KT 1 year). We evaluated the levels of plasma cytokines that have been reported to be induced or downregulated upon HCV infection, including IP-10, IL-10, MIP-1β, IL-8, IL-6, eotaxin, MCP-3, MCP-1, IFN-γ, IL-1β, IL-4, and IL-17.[4,5] All cytokines were measured using the MILLIPLEX® Human Cytokine/Chemokine/Growth Factor Panel A (HCYTA-60K, Merck Millipore, Billerica, MA). All analyses were performed with Prism 9.0 (GraphPad Software Inc, San Diego, CA). A p-value of $<.05$ was considered to indicate statistical significance.

3 | RESULTS

All 16 patients began G/P therapy between days 2 and 5 post transplant. At post-KT D3, 9/16 patients had detectable plasma HCV RNA while the other 7/16 had undetectable HCV RNA. All patients with HCV viremia cleared viremia by the end of Tx (Table 1 and Figure 1) and all patients experienced SVR. Previous studies demonstrated that IP-10, IL-10, MIP-1β, and IL-8 are induced upon HCV infection but gradually normalized during DAA therapy.[4,5] Similarly, IP-10, IL-10, MIP-1β, and IL-8 were significantly elevated from baseline in patients with HCV viremia at post-KT D3. However, over the 8-week G/P therapy, there was a rapid reduction back to baseline levels (Figure 2A). In contrast, in those without HCV viremia, these cytokine levels remained unchanged immediately after transplant and beyond (Figure 2B). It has been reported that IL-6, eotaxin, MCP-3, and MCP-1 are induced upon HCV infection and remain upregulated even after HCV eradication with DAAs.[4,5] In this study, none of them were significantly

TABLE 1 Baseline demographic and clinical characteristics of patients with and without HCV viremia at post-KT D3.

Characteristic	Without HCV viremia ($n = 7$)	With HCV viremia ($n = 9$)
Age in year at time of consent: median (IQR)	57.5 (56–64)	59 (51–62)
Women: count	3 (42.9%)	3 (33.3%)
Race/ethnicity: count		
White, not Hispanic	3 (42.9%)	6 (66.7%)
Hispanic	0	1 (11.1%)
Black	3 (42.9%)	2 (22.2%)
Other	1 (14.3%)	0
ESKD cause: count		
Congenital/Genetic	1 (14.3%)	1 (11.1%)
Diabetic nephropathy	2 (28.6%)	5 (55.6%)
Hypertension	2 (28.6%)	1 (11.1%)
Polycystic kidney disease	1 (14.3%)	2 (22.2%)
Other GN	1 (14.3%)	0
On dialysis at consent	6 (85.7%)	7 (77.8%)
BMI in kg/m^2: median (IQR)	25.1 (23.0–32.1)	33.2 (26.9–34.8)
Weeks on waitlist before consent: median (IQR)	124.9 (70.6–135.0)	99.1 (57.1–145.3)
Weeks from consent to transplant: median (IQR)	3.1 (.1–6.0)	9.4 (7.9–15.4)

Abbreviation: HCV, hepatitis C virus.

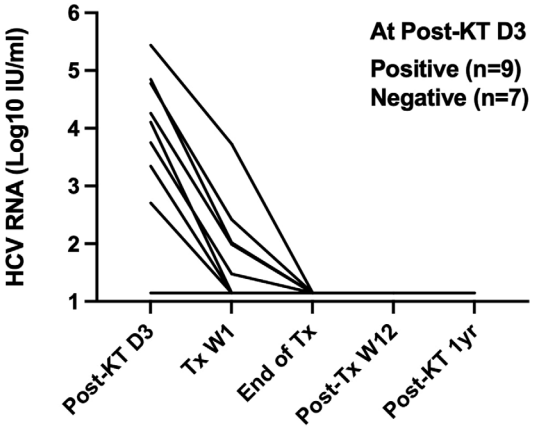

FIGURE 1 Plasma HCV RNA levels. HCV, hepatitis C virus.

induced at post-KT D3 or beyond regardless of HCV viremia status (Figure 3A,B).

In addition, IFN-γ, IL-1β, IL-4, and IL-17 have been reported to be downregulated upon HCV infection and constantly downregulated even after HCV eradication.[4,5] However, these cytokines were not significantly downregulated at post-KT D3 or beyond regardless of HCV viremia (Figure 4A,B).

4 | DISCUSSION

Chronic and acute HCV infection alters the inflammatory milieu; recent studies have indicated that these perturbations persist even after viral clearance with DAAs.[4,5] It remains unclear how the immune system is affected by HCV viremia kidney transplant.

In this study, we found that recipients transplanted with HCV viremic kidneys and treated with early G/P experienced only transient alterations in the soluble inflammatory milieu.

It has been reported that severe BKPyV viremia (>10 000 copies/mL) after kidney transplant is associated with donor HCV infection status,[8] suggesting the immune system of the HCV viremic kidney recipients is less able to control BKPyV. IFN-γ,[11] IL-1β,[12,13] IL-4,[14] and IL-17[15] play important roles in regulating viral infection and it has been reported that the reduced IFN-γ production by T cells and the decreased urinary IL-17 level are associated with BKPyV viremia.[16–18] We found that plasma IFN-γ, IL-1β, IL-4, and IL-17 levels were not decreased upon HCV viremic kidney transplant or beyond, suggesting a low likelihood of BKPyV viremia. In fact, 4 out of 16 patients had BKPyV viremia at post-Tx W12 but they eventually became BKPyV negative at post-KT 1 year after the oral immunosuppressant dose was reduced.[10] Plasma IFN-γ, IL-1β, IL-4, and IL-17 levels of patients with BKPyV viremia appeared to be suppressed at post-Tx W12 and then recovered (Figure S1), likely reflecting the effect of immunosuppressants. In addition, three out of four patients who experienced BKPyV viremia events were HCV negative at Post-KT D3, indicating that HCV viremia following transplantation might not be a contributing factor for BKPyV viremia.

Prior studies have shown that HCV infection can leave epigenetic changes in the host immune system that persist post-SVR following DAA therapy.[19,20] Several plasma cytokines were transiently induced after HCV viremic kidney transplant and appeared to correlate with plasma HCV RNA (Figure S2), suggesting the response to transient HCV viremia after HCV viremic kidney transplantation. In addition, cytokines known to be persistently upregulated or downregulated in patients who had experienced HCV infection were not altered by HCV

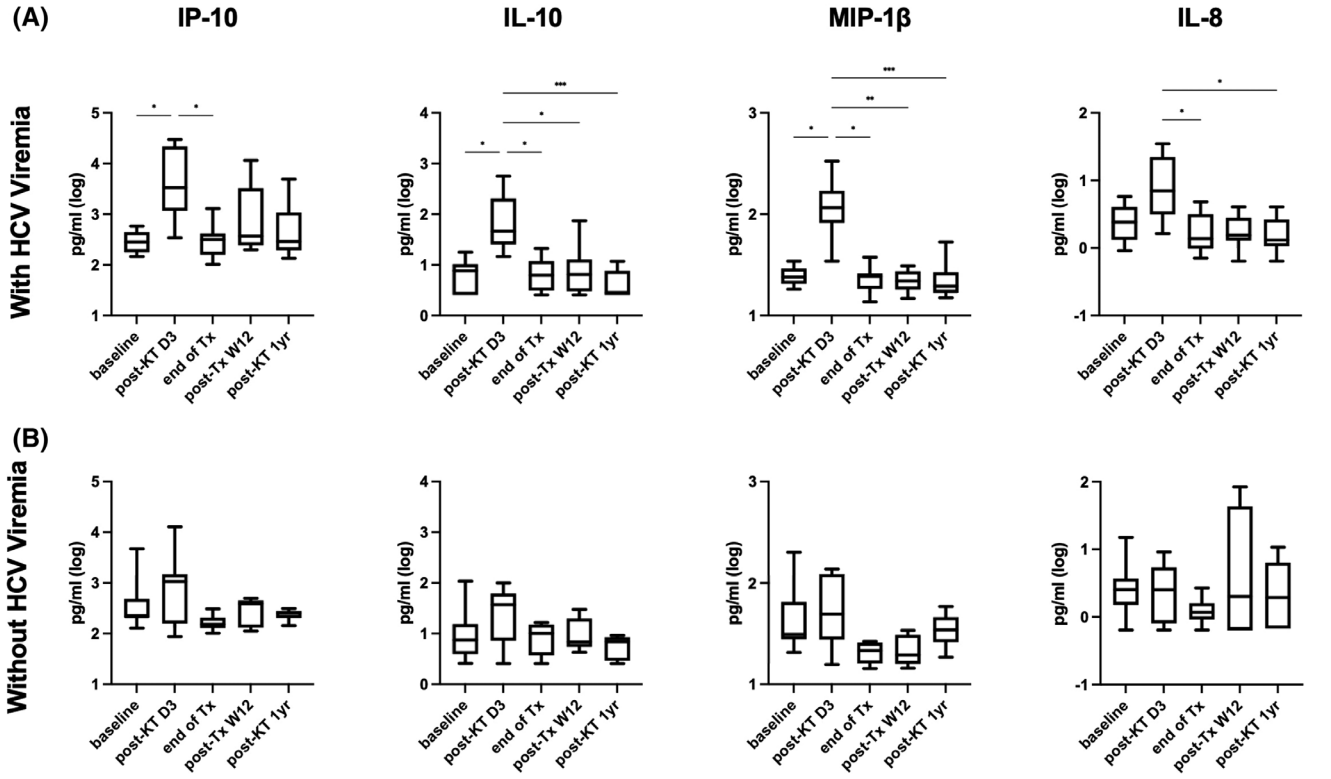

FIGURE 2 Plasma levels of the cytokines reported to be induced upon HCV infection and to be normalized during direct-acting antivirals therapy. HCV, hepatitis C virus.

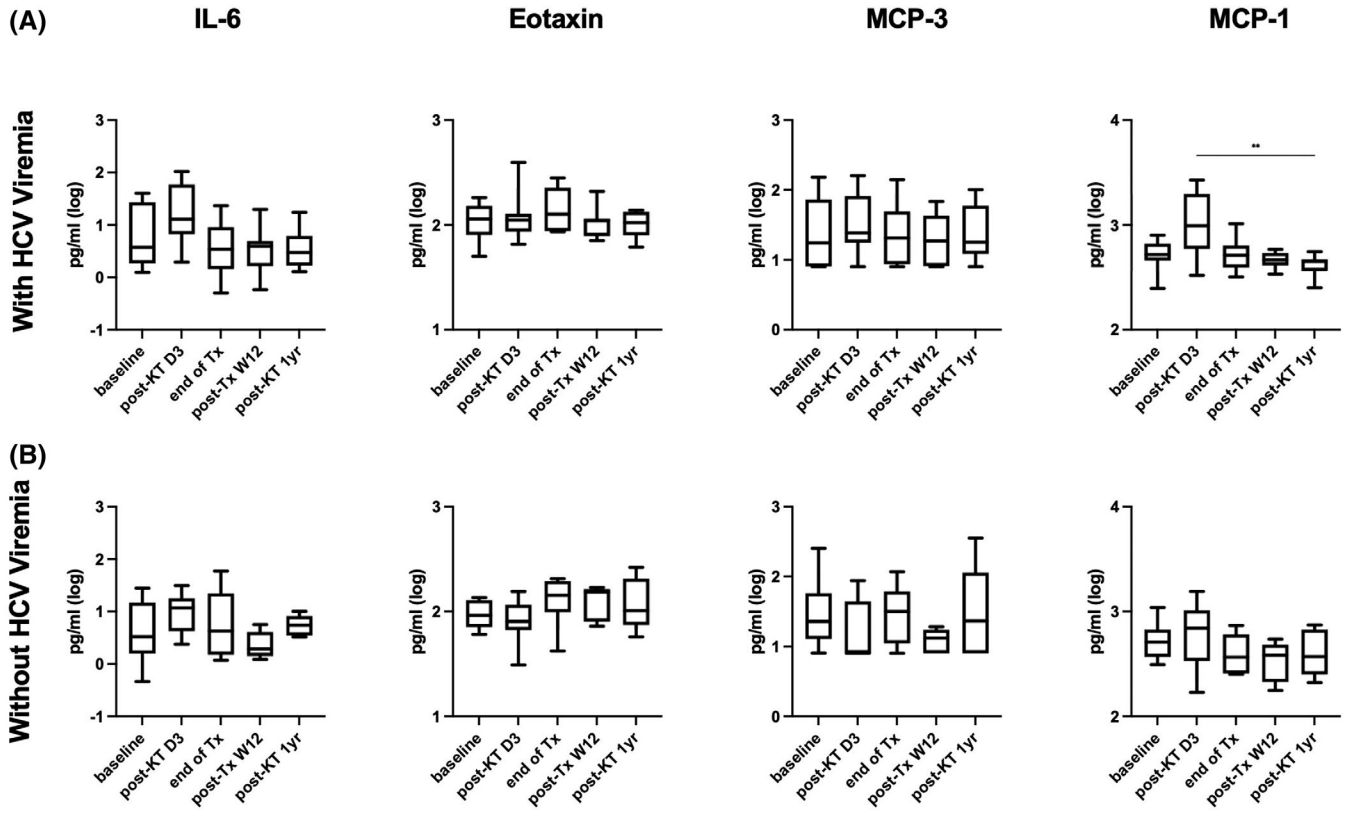

FIGURE 3 Plasma levels of the cytokines reported to be induced upon HCV infection and to remain upregulated even after HCV eradication with direct-acting antivirals therapy. HCV, hepatitis C virus.

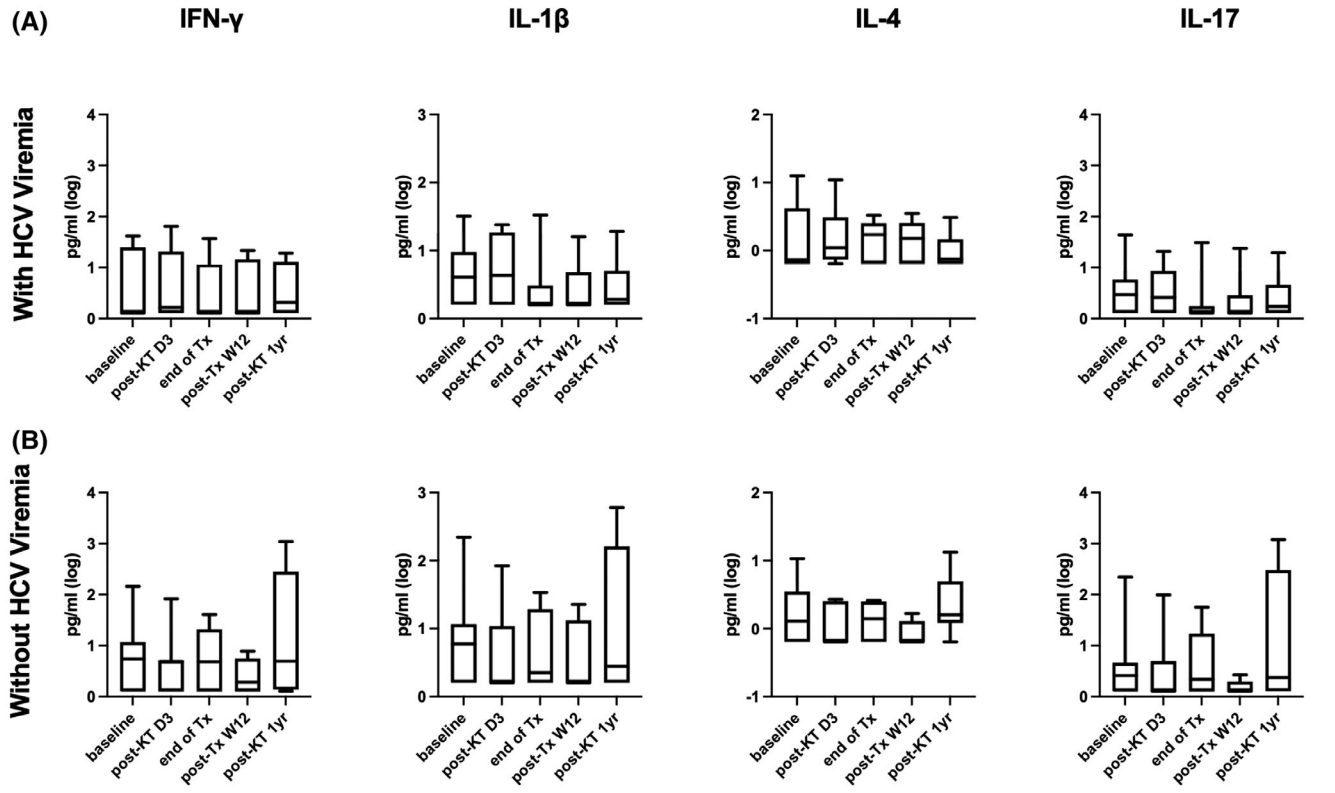

FIGURE 4 Plasma levels of the cytokines reported to be downregulated upon HCV infection and to remain downregulated even after HCV eradication with direct-acting antivirals therapy. HCV, hepatitis C virus.

viremic kidney transplant. Thus, HCV viremic kidney transplant and subsequent transient HCV viremia appear not to induce epigenetic changes in the recipients when offered early treatment with DAAs. On the other hand, the importation of donor-derived immune cells could affect the recipient's inflammatory milieu, but even so, the effect was short-lived.

Among centers reporting their experiences, the timing of DAA therapy has varied from hours before transplant to a median of 76 days after transplant.[21] Aside from the clinical complications of untreated HCV infection, including acute hepatitis and membranoproliferative glomerulonephritis, delayed DAA therapy to HCV viremic kidney recipients also introduces the possibility of epigenetic changes and sustained alterations in the inflammatory milieu. For these reasons, our study, in an effort to minimize recipient risk and exposure, offered early therapy that appears to abrogate these effects.

We acknowledge several important limitations. First, we only studied 16 subjects due to the limited number of participants with serial blood samples available, which may have impacted our ability to see large changes over time; nevertheless, we were able to confirm the only transient alterations in the inflammatory milieu of the HCV viremic recipients. Our findings can be reliably verified using a larger dataset as cases of HCV viremic kidney transplant into HCV-uninfected recipients have rapidly accumulated.[21] Second, this study did not include HCV-negative kidney transplant recipients as a standard control in evaluating the plasma cytokine levels because blood samples stored at the corresponding time points, especially at post-KT D3, were rarely available. In prior studies of the recipients who received HCV-negative kidneys from living donors and had good allograft function, it has been shown that many pro-inflammatory cytokines remain stable while regulatory cytokines such as IL-4, IL-5, and IL-10 are induced within 2 years of transplant.[22,23] Similarly, the recipients without HCV viremia exhibited stable cytokine levels throughout the time points suggesting this group as an aviremic (or less overtly viremic) control. Thus, our findings might not be compromised by the absence of the standard control.

In conclusion, this study demonstrated that HCV-uninfected recipients transplanted with kidneys from HCV-infected deceased donors and treated with early G/P experienced only transient alterations in the soluble inflammatory milieu. These data provide reassuring evidence that there appear to be no persistent cytokine disturbances with transient HCV viremia accompanying HCV donor positive/recipient negative kidney transplant. Further studies are warranted, but this study provides strong support for the very early DAA treatment approach following solid organ transplantation.

ACKNOWLEDGMENTS

AbbVie funded the study and provided G/P through a collaboration study agreement with the Massachusetts General Hospital. The Massachusetts General Hospital was the sponsor of the study. Glecaprevir was identified by AbbVie and Enanta. Raymond T. Chung was supported by the MGH Research Scholars Program

CONFLICT OF INTEREST STATEMENT

M.E.S. has received research grants from Abbvie, Gilead, Merck, EMD-Serono, and Angion. M.E.S. has been a scientific advisory board member for Travere, Mallinckrodt, and Novartis. M.E.S. was supported by NIH K23DK117014. D.S.G. has received research funding to his institution from AbbVie and Gilead. R.J.F. has received research funding to his institution from Abbvie and Gilead. J.J.K. is an employee of AbbVie Inc. J.J.K. may hold AbbVie Inc. stocks and holds stock options (shareholder). J.J.K. has a patent application US2017333428 issued to AbbVie Inc. R.R.A. has been board member for Sanofi-Speakers Bureau and Advisory Board, Veloxis-Speakers Bureau and Advisory Board. R.R.A has received research funding to her institution from Bristol Myers Squibb and Nobelpharma. C.M.D. received honoraria from Gilead Sciences for serving on a grant review committee. R.S.B. has received research funding to his institution from and consults for AbbVie and Gilead. J.L. is an advisor for Eurofins and Mallinckrodt. P.P.R. was supported by Abbvie, through a subcontract to the University of Pennsylvania for this study. P.P.R. has received investigator-initiated grants from Merck and Gilead to support research in the transplantation of organs from donors with HCV infection. P.P.R. is an Associate Editor for the American Journal of Kidney Diseases and an unpaid Advisor to eGenesis related to xenotransplantation. R.T.C. received research grants to his institution from Abbvie, Gilead, Merck, BMS, Boehringer and Janssen.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data requests will be reviewed by representatives of the Coauthor consortium.

ORCID

Myung-Ho Kim https://orcid.org/0000-0003-2320-1633

REFERENCES

1. US Department of Health and Human Services. Health Resources and Services Administration (HRSA) organ procurement transplant network. National data. Accessed August 22, 2022. https://optn.transplant.hrsa.gov/data/
2. Roth D, Ladino M. Transplantation of kidneys from HCV-positive donors: how to best use a scarce resource. *J Am Soc Nephrol*. 2017;28(11):3139-3141.
3. Schaubel DE, Tran AH, Abt PL, Potluri VS, Goldberg DS, Reese PP. Five-year allograft survival for recipients of kidney transplants from hepatitis C virus infected vs uninfected deceased donors in the direct-acting antiviral therapy era. *JAMA*. 2022;328(11):1102-1104.
4. Khera T, Du Y, Todt D, et al. Long-lasting imprint in the soluble inflammatory milieu despite early treatment of acute symptomatic hepatitis C. *J Infect Dis*. 2021;226:441-452.
5. Hengst J, Falk CS, Schlaphoff V, et al. Direct-acting antiviral-induced hepatitis C virus clearance does not completely restore the altered cytokine and chemokine milieu in patients with chronic hepatitis C. *J Infect Dis*. 2016;214(12):1965-1974.
6. Seifert ME, Agarwal G, Bernard M, et al. Impact of subclinical borderline inflammation on kidney transplant outcomes. *Transplant Direct*. 2021;7(2):e663.
7. Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R. BK polyomavirus: clinical aspects, immune regulation, and emerging therapies. *Clin Microbiol Rev*. 2017;30(2):503-528.
8. Molnar MZ, Potluri VS, Schaubel DE, et al. Association of donor hepatitis C virus infection status and risk of BK polyomavirus viremia after kidney transplantation. *Am J Transplant*. 2022;22(2):599-609.
9. Molnar MZ, Nair S, Csepreka O, et al. Transplantation of kidneys from hepatitis C-infected donors to hepatitis C-negative recipients: single center experience. *Am J Transplant*. 2019;19(11):3046-3057.
10. Sise ME, Goldberg DS, Kort JJ, et al. Multicenter Study to Transplant Hepatitis C-Infected Kidneys (MYTHIC): an open-label study of combined Glecaprevir and Pibrentasvir to treat recipients of transplanted kidneys from deceased donors with hepatitis C virus infection. *J Am Soc Nephrol*. 2020;31(11):2678-2687.
11. Kang S, Brown HM, Hwang S. Direct antiviral mechanisms of interferon-gamma. *Immune Netw*. 2018;18(5):e33.
12. Aarreberg LD, Wilkins C, Ramos HJ, et al. Interleukin-1β signaling in dendritic cells induces antiviral interferon responses. *mBio*. 2018;9(2):e00342-18.
13. Orzalli MH, Smith A, Jurado KA, Iwasaki A, Garlick JA, Kagan JC. An antiviral branch of the IL-1 signaling pathway restricts immune-evasive virus replication. *Mol Cell*. 2018;71(5):825-840.
14. Lee A, Park SP, Park CH, et al. IL-4 induced innate CD8+ T cells control persistent viral infection. *PLoS Pathog*. 2015;11(10):e1005193.
15. Sahu U, Biswas D, Prajapati VK, Singh AK, Samant M, Khare P. Interleukin-17—a multifaceted cytokine in viral infections. *J Cell Physiol*. 2021;236(12):8000-8019.
16. Schachtner T, Stein M, Babel N, Reinke P. The loss of BKV-specific immunity from pretransplantation to posttransplantation identifies kidney transplant recipients at increased risk of BKV replication. *Am J Transplant*. 2015;15(8):2159-2169.
17. Abend JR, Low JA, Imperiale MJ. Inhibitory effect of gamma interferon on BK virus gene expression and replication. *J Virol*. 2007;81(1):272-279.
18. Sadeghi M, Daniel V, Schnitzler P, et al. Urinary proinflammatory cytokine response in renal transplant recipients with polyomavirus BK viruria. *Transplantation*. 2009;88(9):1109-1116.
19. Perez S, Kaspi A, Domovitz T, et al. Hepatitis C virus leaves an epigenetic signature post cure of infection by direct-acting antivirals. *PLoS Genet*. 2019;15(6):e1008181.
20. Hlady RA, Zhao X, El Khoury LY, et al. Interferon drives HCV scarring of the epigenome and creates targetable vulnerabilities following viral clearance. *Hepatology*. 2022;75(4):983-996.
21. Fabrizi F, Cerutti R, Alfieri CM, Messa P. Updated view on kidney transplant from HCV-infected donors and DAAs. *Pharmaceutics*. 2021;13(4):496.
22. Mota AP, Vilaça SS. Cytokines signatures in short and long-term stable renal transplanted patients. *Cytokine*. 2013;62(2):302-309.
23. Ito A, Sugimura J, Matsuura T, Abe T, Obara W. Plasma cytokine levels before and 1 year after successful living-donor renal transplantation. *Renal Replace Ther*. 2020;6(1):48.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kim MH, Sise ME, Xu M, et al. Early initiation of glecaprevir/pibrentasvir after transplantation of HCV-viremic kidneys into HCV-negative recipients is associated with normalization in the altered inflammatory milieu. *Clin Transplant*. 2023;37:e14926. https://doi.org/10.1111/ctr.14926