

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

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### **Abbreviations**

MYTHIC, multicenter study to transplant HCV-infected kidneys; HCV, hepatitis C virus; DAAs, direct acting antivirals; SVR, sustained viral response; BKPyV, BK Polyomavirus, G/P, glecaprevir and pibrentasvir

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## **Abstract**

Our previous multicenter trial to transplant HCV-infected kidneys (MYTHIC) observed that 100% of 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 9 patients with HCV viremia at day 3 post-kidney transplant (post-KT D3), IP-10, IL-10, MIP-1 $\beta$  and IL-8 were significantly elevated from baseline. However, over the subsequent visits there was a rapid, dramatic reduction back to baseline levels. Among 7 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.

## **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<sup>1</sup>. In the past, kidneys from donors with HCV infection had been underused in the past because of the risk for 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 past five years has shown that nearly all recipients transplanted with HCV viremic kidneys have achieved sustained virologic response (SVR) with good 1- and 5-year allograft outcomes<sup>2,3</sup>.

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 infection<sup>4,5</sup>. An altered inflammatory milieu can affect the long-term outcome of kidney transplant recipients<sup>6</sup>, for example, by triggering BK Polyomavirus (BKPyV) viremia and nephropathy or leading to an increased risk of cytomegalovirus infection<sup>7,8</sup>, 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<sup>9</sup>.

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 to 5 post-transplant, and all achieved SVR<sup>10</sup>. While the therapy was well tolerated and allograft function was preserved at one 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<sup>10</sup>. 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 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 1yr). 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-1 $\beta$ , IL-8, IL-6, eotaxin, MCP-3, MCP-1, IFN- $\gamma$ , IL-1 $\beta$ , IL-4, IL-17<sup>4,5</sup>. 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 <0.05 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-1 $\beta$  and IL-8 are induced upon HCV infection but gradually normalized during DAA therapy<sup>4,5</sup>. Similarly, IP-10, IL-10, MIP-1 $\beta$  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<sup>4,5</sup>. In this study, none of them were significantly induced at post-KT D3 or beyond regardless of HCV viremia status (**Figure 3A and B**).

In addition, IFN- $\gamma$ , IL-1 $\beta$ , IL-4, and IL-17 have been reported to be downregulated upon HCV infection and constantly downregulated even after HCV eradication<sup>4,5</sup>. However, these cytokines were not significantly downregulated at post-KT D3 or beyond regardless of HCV viremia (**Figure 4A and B**).

#### 4. Discussion

Chronic and acute HCV infection alter the inflammatory milieu; recent studies have indicated that these perturbations persist even after viral clearance with DAAs<sup>4,5</sup>. 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<sup>8</sup>, suggesting the immune system of the HCV viremic kidney recipients is less able to control BKPyV. IFN- $\gamma$ <sup>11</sup>, IL-1 $\beta$ <sup>12,13</sup>, IL-4<sup>14</sup>, and IL-17<sup>15</sup> play important roles in regulating viral infection and it has been reported that the reduced IFN- $\gamma$  production by T cells and the decreased urinary IL-17 level are associated with BKPyV viremia<sup>16-18</sup>. We found that plasma IFN- $\gamma$ , IL-1 $\beta$ , 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 1yr after the oral immunosuppressant dose was reduced<sup>10</sup>. Plasma IFN- $\gamma$ , IL-1 $\beta$ , IL-4, and IL-17 levels of patients with BKPyV viremia appeared to be suppressed at post-Tx W12 and then recovered (**Supplementary Figure 1**), likely reflecting the effect of immunosuppressants. In addition, 3 out of 4 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<sup>19,20</sup>. Several plasma cytokines were transiently induced after HCV viremic kidney transplant and appeared to correlate with plasma HCV RNA (**Supplementary Figure 2**), 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 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<sup>21</sup>. 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 been rapidly accumulated<sup>21</sup>. 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<sup>22,23</sup>. 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**

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### **Disclosure**



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### 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.

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**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 yr at time of consent: median (IQR)	57.5 (56 to 64)	59 (51 to 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 <sup>2</sup> : median (IQR)	25.1 (23.0 to 32.1)	33.2 (26.9 to 34.8)
Weeks on waitlist before consent: median (IQR)	124.9 (70.6 to 135.0)	99.1 (57.1 to 145.3)
Weeks from consent to transplant: median (IQR)	3.1 (0.1 to 6.0)	9.4 (7.9 to 15.4)

Figure 1. Plasma HCV RNA levels.

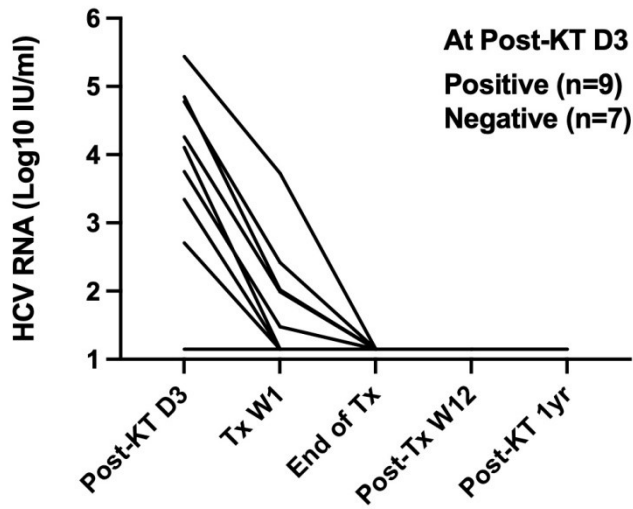


Figure 2. Plasma levels of the cytokines reported to be induced upon HCV infection and to be normalized during direct-acting antiviral therapy.

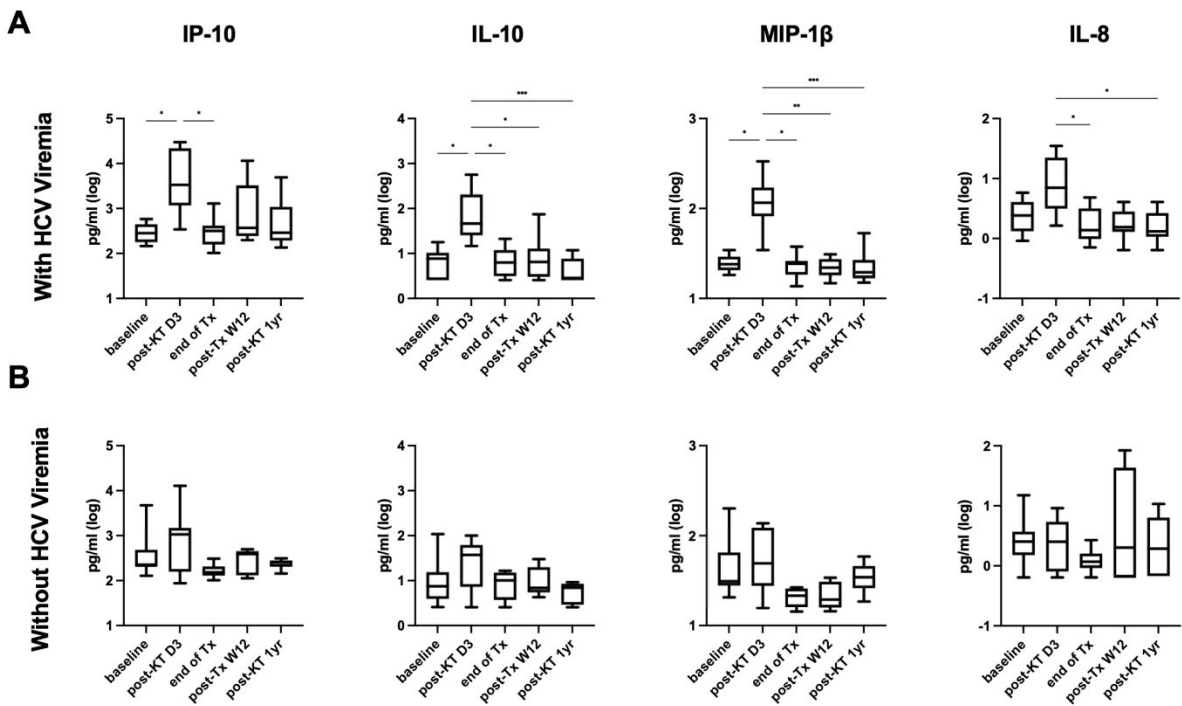


Figure 3. Plasma levels of the cytokines reported to be induced upon HCV infection and to remain upregulated even after HCV eradication with direct-acting antiviral therapy

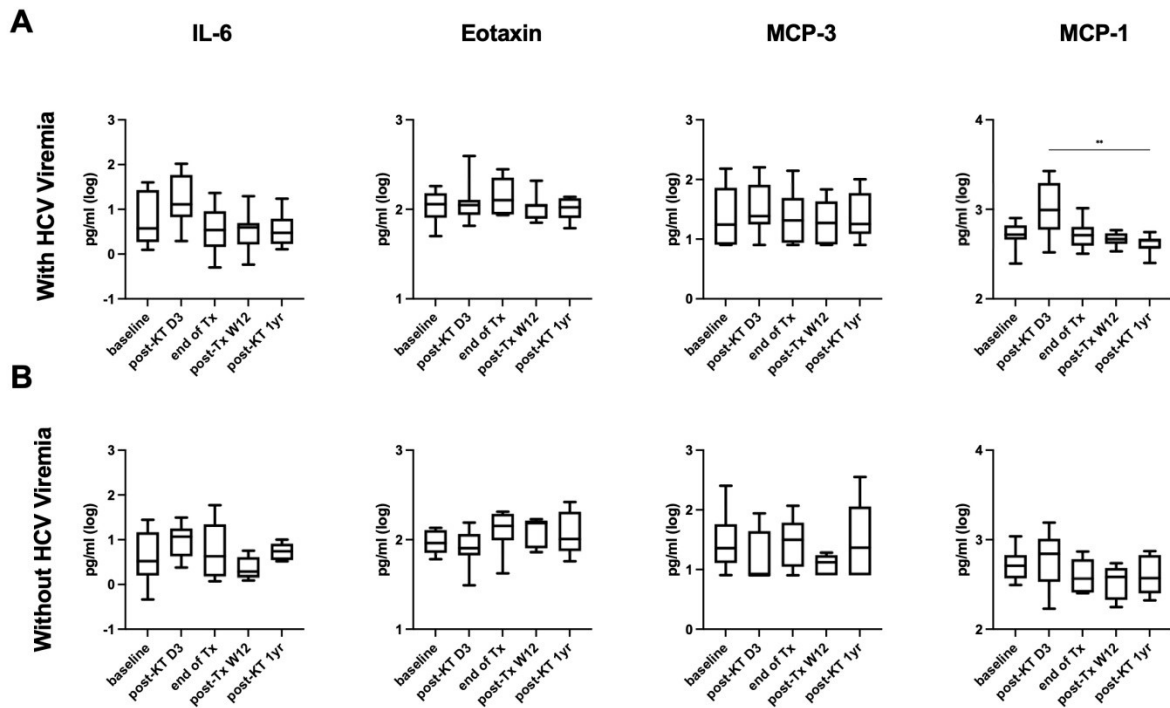


Figure 4. Plasma levels of the cytokines reported to be downregulated upon HCV infection and to remain downregulated even after HCV eradication with direct-acting antiviral therapy

