0.727–0.840), but mortality in women who used CPAP increased (odds ratio, 1.320; 95% confidence interval, 1.151–1.515). In men, significant interactions were found for cerebrovascular disease, diabetes mellitus, and hypertension, but patients treated with CPAP had a lower probability of death than control subjects in all scenarios (Table 1). On the other hand, CPAP treatment was associated with lower mortality in women with cerebrovascular disease but had a neutral or detrimental effect on mortality in other scenarios (Table 1).

Overall, the population of patients treated with CPAP in Catalonia had lower mortality rates than age-, sex-, and region-matched control subjects, despite a higher prevalence of most comorbidities among patients treated with CPAP. However, this finding was driven by the 74% of men in the population, because CPAP was associated with increased mortality among women.

These population-based findings contrast with the less positive findings of the randomized SAVE study (3). In contrast, our results are consistent with recently published data from a Danish historical cohort (22,135 patients with OSA), which showed that CPAP treatment was associated with lower mortality rates in middle-aged and elderly men after adjustment for multiple comorbidities, but not in women (5). Although in our population sex differences in the results seem to be related to a high heart failure prevalence among women using CPAP, further research is needed on the potentially different characteristics of OSA, and associated comorbidities, among women and men. Finally, several potential limitations should be noted. First, the data used in this study lack information about CPAP adherence. Second, it was not possible to control for undiagnosed OSA in control subjects, and, finally, the observational design means that potential confounding from uncontrolled factors such as socioeconomic status, level of health literacy and self-care, or level of delivered health care cannot be avoided.

In conclusion, these findings suggest that CPAP treatment is associated with reductions in mortality at the population level, although only in men. Therefore, further analyses should be planned within the frame of precision medicine (6) to clarify which patients with OSA could benefit the most from CPAP treatment and whether CPAP might be detrimental in some specific patient subgroups. ■

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Galectin-3 Promotes Vascular Remodeling and Contributes to Pulmonary Hypertension

To the Editor:

Pulmonary hypertension (PH) is a debilitating and eventually fatal disease that is resistant to current therapeutics. The hyperplasia of medial pulmonary arteries (PAs) is considered a hallmark feature of PH, and progressive muscularization of low-resistance arteries leads to the narrowing and stiffening of PAs. Over time,

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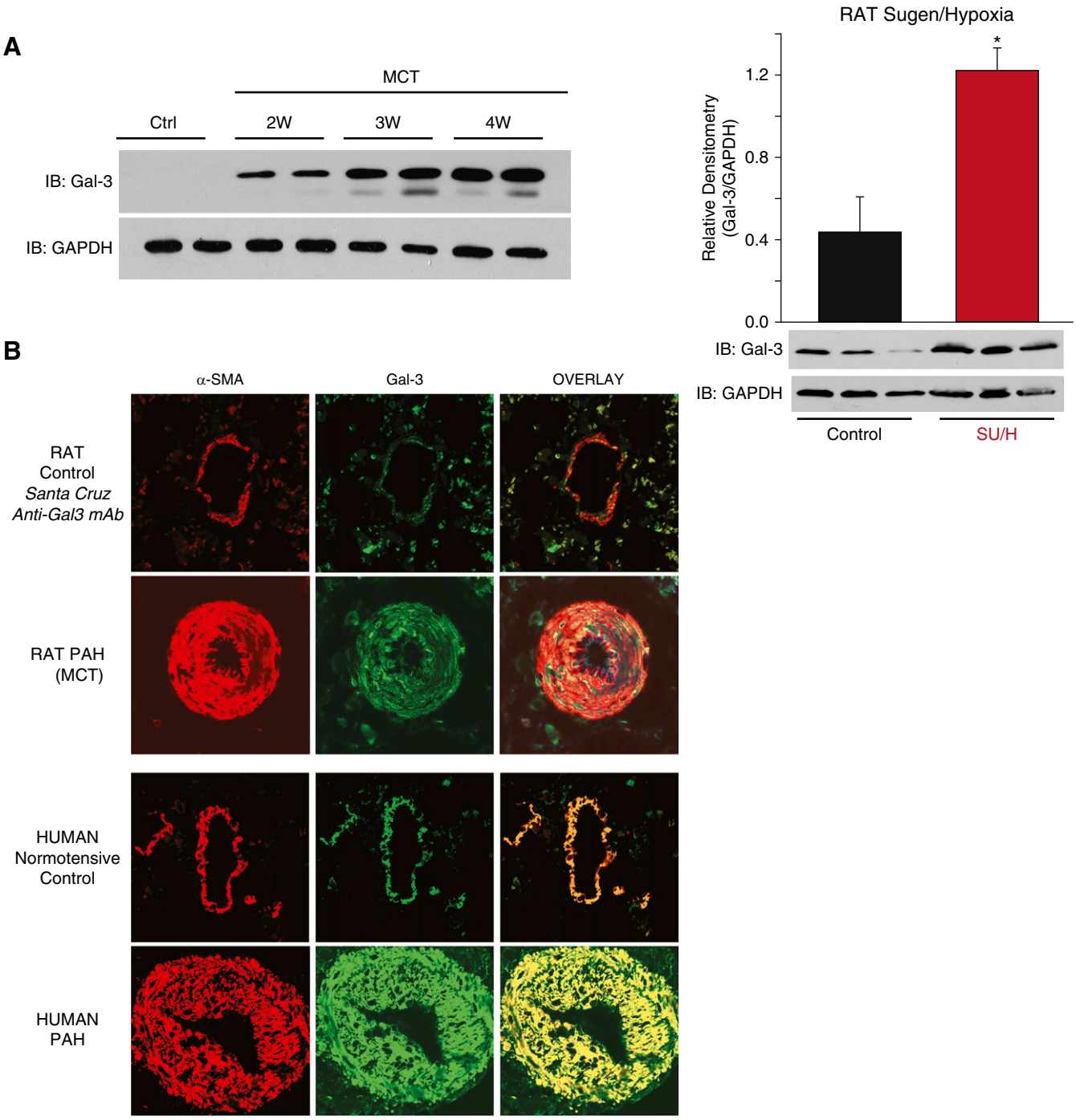

Figure 1. Galectin-3 (Gal-3) expression is increased in hypertensive pulmonary arteries in a time-dependent manner and is most abundant in the medial layer. (*A*) Increased expression of Gal-3 protein in isolated intrapulmonary pulmonary arteries (PAs) from rats with pulmonary hypertension. (Left) Time-course of increased expression in PAs from control (Sprague Dawley) and monocrotaline (MCT) rats (Weeks 1–4). (Right) Gal-3 expression in PAs from Sugen/hypoxia (SU/H) rats (SU5416, 20 mg/kg, s.c., 3 wk hypoxia [10% O_2] and 10 wk normoxia). *Different from control, $P < 0.05$ ($n = 3$ for each group). (*B*) Gal-3 is expressed primarily in the media of PAs. Confocal images of lung sections from control and experimental rat pulmonary hypertension (PH) (4 wk MCT) and control and PH patients. Sections were stained with Gal-3 and α-actin antibodies ($n = 4$). Ctrl = control; IB = immunoblot; mAb = monoclonal antibody; α-SMA = α-smooth muscle actin; PAH = pulmonary arterial hypertension; W = week.

this leads to increased vascular resistance, the elevation of pulmonary arterial blood pressure, and eventually, failure of the right ventricle. Multiple mechanisms and cell types have been shown to contribute to the altered vascular remodeling; the identification of key pathways that affect disease progression in humans remains a barrier to the development of more effective therapeutics.

Galectin-3 (Gal-3) is a β-galactoside binding lectin that regulates multiple pathways that are operational in remodeling blood vessels,

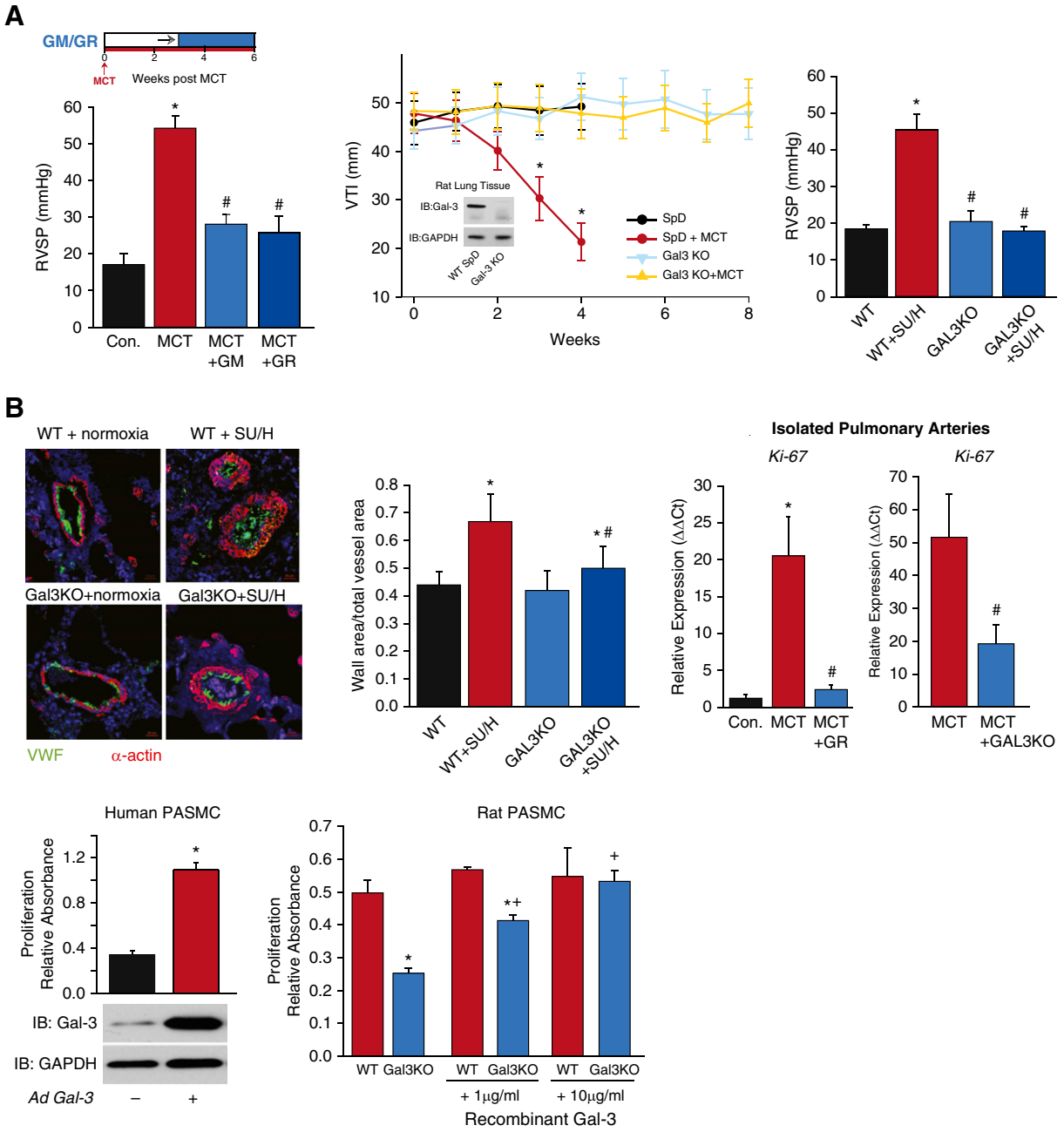

Figure 2. Galectin-3 (Gal-3) contributes to pulmonary hypertension (PH) and altered pulmonary artery (PA) remodeling via increased vascular smooth muscle cell proliferation. (*A*) Hemodynamics in rats with PH. (Left) In reversal experiments, rats with established monocrotaline (MCT)-induced PH were administered either vehicle or the Gal-3 inhibitors, GM-CT-01 (GM) and GR-MD-02 (GR), at 60 mg/kg twice weekly, starting after 3 weeks of MCT, and animals killed at Week 6 for measurements of right ventricular systolic pressure (RVSP) ($n = 5$). (Middle) Time course of PH in wild-type (WT) (Sprague Dawley [SpD]) and Gal-3 knockout (Gal-3 KO) rats treated with MCT, using weekly measurements of velocity time integral (Vevo2100) starting at Week 1 and continuing over 4–8 weeks ($n = 4$). (Inset) Western blot of Gal-3 in lung lysates of WT (SpD) and Gal-3 KO rats. (Right) RVSP in control (WT) and Gal-3 KO rats treated with and without Sugen/hypoxia (SU/H) ($n = 5$). (*B*) Vascular remodeling and pulmonary artery smooth muscle function. (Left) Confocal images of lung cross-sections from WT and Gal-3 KO rats treated with SU/H using antibodies for smooth muscle actin (red) and vWF (green). Hypertrophic remodeling was quantified by changes in the wall area relative to total vessel area ($n = 10$ vessels from three independent experiments). In isolated PAs from control, 4-week MCT WT rats ± GR and 4-week MCT Gal-3 KO rats, qRT-PCR was used to determine expression of the proliferation marker MKI67 (Ki-67) normalized to GADPH ($n = 4$). In human pulmonary artery smooth muscle cells, adenoviral delivery of Gal-3 (30 MOI) increases cell proliferation (MTT assay; $n = 3$) and proliferation (MTT assay) of smooth muscle cells isolated from the PAs of MCT-treated WT (SpD) and Gal-3 KO rats, treated with or without the indicated concentrations of recombinant Gal-3 ($n = 4$). $^{*}P < 0.05$ versus control or WT; $^{\#}P < 0.05$ versus MCT; $^{+}P < 0.05$ versus Gal-3 KO. *Ad* = adenoviral plasmid; Con. = control; Ct = threshold cycle; IB = immunoblot; KO = knockout; MOI = multiplicity of infection; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PASMC = pulmonary artery smooth muscle cell; VTI = velocity time integral; vWF = von Willebrand factor.

including cell proliferation, apoptosis, inflammation, and fibrosis (1), but its role in PH is not fully defined. Previous studies have reported that circulating levels of Gal-3 are elevated in human PH (2) and in experimental PH (3), and that the knockout (KO) of Gal-3 in mice with hypoxia-induced PH prevents right ventricle remodeling (3). The goal of the current study was to identify a functional role for Gal-3 in mediating PA remodeling and PH in the monocrotaline (MCT) and Sugen/hypoxia (SU/H) rat models that are thought to more closely resemble the pathology of human PH (4, 5).

We observed a time-dependent increase in Gal-3 expression in isolated PAs (intrapulmonary) of rat models of PH, which was found primarily within the medial smooth muscle layer of both hypertensive rat and human PAs (Figures 1A and 1B). (All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Augusta University Institutional Animal Care and Use Committee. For human samples, patient identifiers were concealed. Waiver of informed consent was approved by the Human Assurance Committee of Augusta University and by the Pulmonary Hypertension Breakthrough Initiative.) To determine whether Gal-3 plays a functional role in PH, we first employed two structurally dissimilar inhibitors of Gal-3, which have been used clinically (6), in experiments to reverse established PH. We found that both GM-CT-01 and GR-MD-02 reversed functional indices of PH in MCT-treated rats *in vivo* (Figure 2A). To confirm these findings using a genetic approach, we developed a novel Gal-3 KO rat on the Sprague Dawley (SpD) background, using CRISPR-Cas9 technology. In lung lysates, Gal-3 expression was observed in wild-type (WT) but not Gal-3 KO rats, confirming the disruption of protein coding (Figure 2A). Remodeling of PA was next assessed *in vivo* via the measurement of the velocity time integral, using high-resolution digital ultrasound (vevo2100, as shown in Reference 7) in both WT and Gal-3 KO rats treated with or without MCT, over time. MCT-treated SpD rats exhibited a time-dependent decrease in PA velocity time integral that was absent in Gal-3 KO rats. MCT-treated WT (SpD) rats exhibited advanced signs of PH by 4 weeks, whereas Gal-3 KO rats were able to survive to at least 8 weeks after MCT with velocity time integral in the normal range (Figure 2A). Similarly, right ventricular systolic pressure was significantly lower in rats exposed to SU/H treatment and not significantly different from control in Gal-3 KO rats (Figure 2A). Quantification of vascular remodeling in small PA was performed in lung sections from WT (SpD) and Gal-3 KO rats treated with SU/H. Exposure to SU/H increased medial hypertrophy of PA in WT rats, which was significantly reduced in Gal-3 KO rats (Figure 2B). RT-PCR was also performed to assess markers of mitosis in PA isolated from MCT-treated rats in which Gal-3 function was negated either through pharmacological inhibition or genetic KO. MCT treatment elicited a robust increase in Ki67 expression that was significantly reduced in animals exposed to Gal-3 inhibitors or in those lacking Gal-3 (Figure 2B). Given that the majority of Gal-3 staining was observed in the medial layer of PA, we next investigated whether Gal-3 regulates PA smooth muscle cell (PASMC) proliferation. In human PASMC, ectopic expression of Gal-3 via adenovirus-mediated gene transfer increased proliferation (Figure 2B). Conversely, in isolated PASMCs from MCT-treated rats, diminished proliferative capacity was observed in cells from Gal-3 KO rats compared with WT, and this could be fully rescued with increasing concentrations of recombinant Gal-3 (Figure 2B). Previous studies have shown that in human PH, circulating Gal-3 levels correlate with the severity of disease and predict mortality (8), and in experimental PH, the genetic loss of Gal-3 ameliorates right ventricle hypertrophy (3).

The cellular origins of increased Gal-3 expression in PH and its functional role in PA remodeling and rat models of PH were previously unknown. Novel findings of our study include increased protein expression of Gal-3 in intrapulmonary PA from multiple rat models, which was time-dependent; identification of SMC as the dominant cell type expressing Gal-3 in hypertensive PA from rats and humans; use of small-molecule Gal-3 inhibitors to reverse established PH; the development of a novel Gal-3 KO rat and its application in two distinct models of PH, which together provide additional support for a functional role of Gal-3 in the pathogenesis of experimental PH; and the identification of a major role of Gal-3 in stimulating PASMC proliferation. Genetic approaches, although important for providing increased target specificity, lack the translational significance of experiments using pharmacological approaches that prevent the development of PH. The ability of two distinct pharmacological inhibitors of Gal-3 to ameliorate established PH further suggests that this pathway may be of clinical interest. The factors responsible for elevated Gal-3 expression in hypertensive PASMC are not yet resolved; however, others have reported that PDGF (platelet-derived growth factor) (9), hypoxia (3), and transforming growth factor-β (10) can increase Gal-3 expression in naive PASMC. How Gal-3 alters PASMC function is not yet known. Gal-3 binds to substrates via specific carbohydrate motifs, and the molecules it regulates in hypertensive PA are likely to be numerous and will be important to determine in future studies.

In summary, our study reveals the effectiveness of genetic and pharmacological strategies targeting Gal-3 in halting the progression of PA remodeling and development of experimental PH. We have also found that Gal-3 regulates PASMC proliferation, which is a major feature of hypertensive PA. These data suggest that Gal-3 may be an attractive target for the treatment of PH and other related pulmonary vascular diseases. ■

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Lung Transplantation from Hepatitis C Viremic Donors to Uninfected Recipients

To the Editor:

The scarcity of donor lungs is a significant factor contributing to respiratory death while awaiting lung transplantation. The average waitlist mortality rate in the United States in 2015 was 16.5 deaths per 100 waitlist years (1). Current practice for the use of hepatitis C virus (HCV)-infected donor lungs has been to use them only for HCV-positive recipients, although this has not been broadly adopted by all centers. The recent development of direct-acting antivirals (DAAs) has now made HCV curable in virtually all those infected, leading to the consideration of using these infected organs to expand the donor pool. In addition, there are increasing numbers of HCV viremic donors associated with the opioid crisis, many of whom are young, with normal lung function, and otherwise excellent potential donors (2). A pilot trial of kidney transplants from HCV viremic donors to 10 HCV uninfected recipients demonstrated universal transmission with 100% sustained virologic response after 12 weeks of elbasvir/grazoprevir post-transplant (3). Current guidance identifies the use of HCV-infected donors as a priority for further research (4). To date, only one case of intentional transmission of HCV from a viremic donor to uninfected lung recipient has been described (5). Lung recipients receive higher-intensity immunosuppression than renal recipients, and can rarely be initiated on oral therapy immediately after transplant. Therefore, the safety of lung transplantation from infected donors to negative recipients with post-transplant DAAs requires further evaluation.

We present a case series describing outcomes of lung transplantation from five HCV viremic donors to negative recipients between November 2016 and February 2017 at the University of Alberta Hospital (University of Alberta Research Ethics approval Pro00075225) after informed consent and discussion of potential risks and benefits with patients/decision makers. All patients were rapidly deteriorating, with several being bridged to transplant with mechanical respiratory support, leaving only a small window of remaining transplant eligibility (Table 1).

All the donors were female, and four of the five were aged 40 years or younger. Of these four donors, all were known to have chronic hepatitis C infection and had never been treated with antiviral therapy. They all continued to engage in high-risk behaviors in the 12 months before their death, including intravenous drug use, high-risk sexual contact, recent incarceration, and recent unprofessional tattoos. One additional donor, age 64 years, had positive serology and nucleic acid testing at the time of transplant workup. Despite a history of blood transfusion in 1982, the donor had never been previously screened for HCV. Donor HCV RNA viral load at the time of transplant ranged from 645 IU/mL to 2.1 million IU/mL, using the Abbott RealTime Assay (Abbott Laboratories). The ischemic time for the second lung to be implanted ranged from 363 to 663 minutes, with two out of five greater than 480 minutes because of retrieval from distant regions in Canada. In two cases (patients 1 and 2), donor lungs were placed on *ex vivo* perfusion for 195 and 315 minutes, respectively. The best Pa_{O_2} in donor arterial blood ranged from 300 to 479 mm Hg.

Three patients received basiliximab induction therapy and two received no induction therapy, as they were mismatched for Epstein-Barr virus (donor positive/recipient negative), and thus were at increased risk for post-transplant lymphoproliferative disease. All received maintenance immunosuppression with tacrolimus, mycophenolate, and prednisone. HCV RNA was first detected between Days 1 to 16 after transplant (Figure 1). In the two cases in which donor lungs were receiving *ex vivo* perfusion before transplant (patients 1 and 2), HCV RNA was first detected in the recipients at 8 and 16 days, respectively. These recipients, however, went on to have the highest peak viral loads, both higher than 7 $\log_{10}$ (Table 1). Three recipients had genotype 1a, one genotype 1b, and one genotype 2 infection. The four recipients with a genotype 1 infection received sofosbuvir/ledipasvir, and the patient with genotype 2 infection received sofosbuvir/velpatasvir, all for 12 weeks. Initiation of DAA therapy after transplant ranged from

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