



## Infectious Disease

# Risk Factors for Parainfluenza Virus Lower Respiratory Tract Disease after Hematopoietic Cell Transplantation

Sachiko Seo<sup>1,2</sup>, Hu Xie<sup>3</sup>, Wendy M. Leisenring<sup>3</sup>, Jane M. Kuypers<sup>4</sup>, Farah T. Sahoo<sup>1,5</sup>, Sonia Goyal<sup>1</sup>, Louise E. Kimball<sup>1</sup>, Angela P. Campbell<sup>1,†</sup>, Keith R. Jerome<sup>1,4</sup>, Janet A. Englund<sup>6,7</sup>, Michael Boeckh<sup>1,3,8,\*</sup>

<sup>1</sup> Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington

<sup>2</sup> Department of Hematology & Oncology, National Cancer Research Center East, Chiba, Japan

<sup>3</sup> Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington

<sup>4</sup> Department of Laboratory Medicine, University of Washington, Seattle, Washington

<sup>5</sup> Department of Health Services, University of Washington, Seattle, Washington

<sup>6</sup> Department of Pediatrics, University of Washington, Seattle, Washington

<sup>7</sup> Pediatric Infectious Diseases Division, Seattle Children's Hospital, Seattle, Washington

<sup>8</sup> Department of Medicine, University of Washington, Seattle, Washington

### Article history:

Received 25 June 2018

Accepted 20 August 2018

### Key Words:

Parainfluenza virus  
Hematopoietic cell transplantation  
Lower respiratory tract disease  
Progression  
Ribavirin

### A B S T R A C T

Parainfluenza virus (PIV) infection can progress from upper respiratory tract infection (URTI) to lower respiratory tract disease (LRTD) in immunocompromised hosts. Risk factors for progression to LRTD and presentation with LRTD without prior URTI are poorly defined. Hematopoietic cell transplant (HCT) recipients with PIV infection were retrospectively analyzed using standardized definitions of LRTD. PIV was detected in 540 HCT recipients; 343 had URTI alone and 197 (36%) had LRTD (possible, 76; probable, 19; proven, 102). Among 476 patients with positive nasopharyngeal samples, the cumulative incidence of progression to probable/proven LRTD by day 40 was 12%, with a median time to progression of 7 days (range, 2 to 40). In multivariable analysis monocytopenia (hazard ratio, 2.22;  $P = .011$ ), steroid use  $\geq 1\text{mg/kg}$  prior to diagnosis (hazard ratio, 1.89;  $P = .018$ ), co-pathogen detection in blood (hazard ratio, 3.21;  $P = .027$ ), and PIV type 3 (hazard ratio, 3.57;  $P = .032$ ) were associated with increased progression risk. In the absence of all 4 risk factors no patients progressed to LRTD, whereas progression risk increased to  $>30\%$  if 3 or more risk factors were present. Viral load or ribavirin use appeared to have no effect on progression. Among 121 patients with probable/proven LRTD, 64 (53%) presented LRTD without prior URTI, and decreased lung function before infection and lower respiratory co-pathogens were risk factors for this presentation. Mortality was unaffected by the absence of prior URTI. We conclude that the risk of progression to probable/proven LRTD exceeded 30% with  $\geq 3$  risk factors. To detect all cases of LRTD, virologic testing of lower respiratory samples is required regardless of URTI symptoms.

© 2018 American Society for Blood and Marrow Transplantation.

## INTRODUCTION

Parainfluenza virus (PIV) is a respiratory virus commonly detected after hematopoietic cell transplantation (HCT) [1–3]. Human PIV has 4 subtypes, numbered 1 through 4 [4,5]. PIV type 3 (PIV-3) is the most common subtype and a frequent cause of outbreaks among HCT recipients [6–8]. PIV can progress from upper respiratory tract infection (URTI) to lower respiratory tract disease (LRTD), resulting in mortality as high

as 40% to 50% [3,9–11]. Identification of risk factors for progression to LRTD and their relative importance is critical in clinical practice and designing clinical trials of novel drugs and immunotherapies.

Progression to LRTD in respiratory viral infections generally indicates progression from URTI to LRTD. However, PIV LRTD is occasionally diagnosed without evidence of prior URTI in HCT recipients [9,11–13]. Although a better understanding of this phenomenon is critical to manage viral infections after HCT and to develop comprehensive prevention strategies, little is known about the biologic mechanism of or risk factors for PIV LRTD without prior URTI.

We previously proposed a classification of LRTD into 3 groups, possible, probable, and proven, using PIV infection [14]. Survival after probable or proven LRTD was significantly

*Financial disclosure:* See Acknowledgments on page 170.

\* Correspondence and reprint requests: Michael Boeckh, MD, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109. E-mail: [mboeckh@fredhutch.org](mailto:mboeckh@fredhutch.org) (M. Boeckh).

† Current address: Centers for Disease Control and Prevention, Atlanta, Georgia.

worse than that after possible LRTD, which was similar to survival after URTI.

The aim of this study was to evaluate the progression rate from URTI to LRTD in HCT recipients with PIV infection, the risk factors for progression to LRTD, and the effect of ribavirin on progression based on stringent definitions. Moreover, because PIV LRTD frequently occurred without prior URTI, we identified factors associated with LRTD as an initial manifestation of infection.

## METHODS

### Study Design

This retrospective cohort study evaluated patients who were transplanted between 1990 and 2011 at the Fred Hutchinson Cancer Research Center and were demonstrated to have laboratory-documented PIV infection after transplantation; a subset of patients were analyzed in a previously published article on characteristics of PIV LRTD [14]. Only an individual's first episode of PIV infection was analyzed. Patients' demographic data and transplant information were retrieved from the Fred Hutchinson Cancer Research Center database, and other data related to the clinical course of PIV infections were collected by medical chart reviews. The study was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center.

### Laboratory Testing

PIV was examined in HCT recipients with upper respiratory tract symptoms or bronchoscopic examination using conventional culture, direct fluorescent antibody staining, and/or reverse transcription PCR assay in respiratory samples. The routine test for 17 respiratory viruses using reverse transcription PCR started in 2007 at our center. We generally performed examination for respiratory viruses using a nasopharyngeal sample when patients had URTI symptoms and bronchoscopy when patients had lower respiratory tract symptoms and radiographic abnormalities. Viral load was determined by quantitative reverse transcription PCR using nasopharyngeal samples at diagnosis of URTI [15].

### Definitions

PIV detection in a nasopharyngeal sample was defined as URTI; virologic evaluation was performed when patients presented with URTI symptoms [14]. Patients with radiographic signs of LRTD with or without clinical symptoms were evaluated by bronchoalveolar lavage (BAL) according to the attending physician's discretion. PIV LRTD categories were designated as previously described [14]: possible referring to PIV detection in the upper respiratory tract with new pulmonary infiltrates, probable as PIV detection in the lower respiratory tract (eg, a BAL or lung biopsy sample) with lower respiratory tract symptoms and no new pulmonary infiltrates, and proven LRTD based on PIV detection in the lower respiratory tract with new pulmonary infiltrates [14]. Progression to LRTD occurred when patients were diagnosed with LRTD  $\geq 2$  days after URTI diagnosis [16]. A co-pathogen was defined as a significant pathogen (bacterium, fungus, virus) detected in a concurrent

respiratory sample or in a blood sample obtained within 2 days of diagnosis of PIV infection. Coagulase-negative staphylococcal bacteremia and asymptomatic cytomegalovirus DNAemia or antigenemia were not considered significant co-pathogens. Cell counts closest to the day of infection diagnosis within 1 week were recorded. Peak steroid doses at PIV diagnosis and after diagnosis were recorded during a period of 2 weeks before and after diagnosis, respectively [14].

### Statistical Analysis

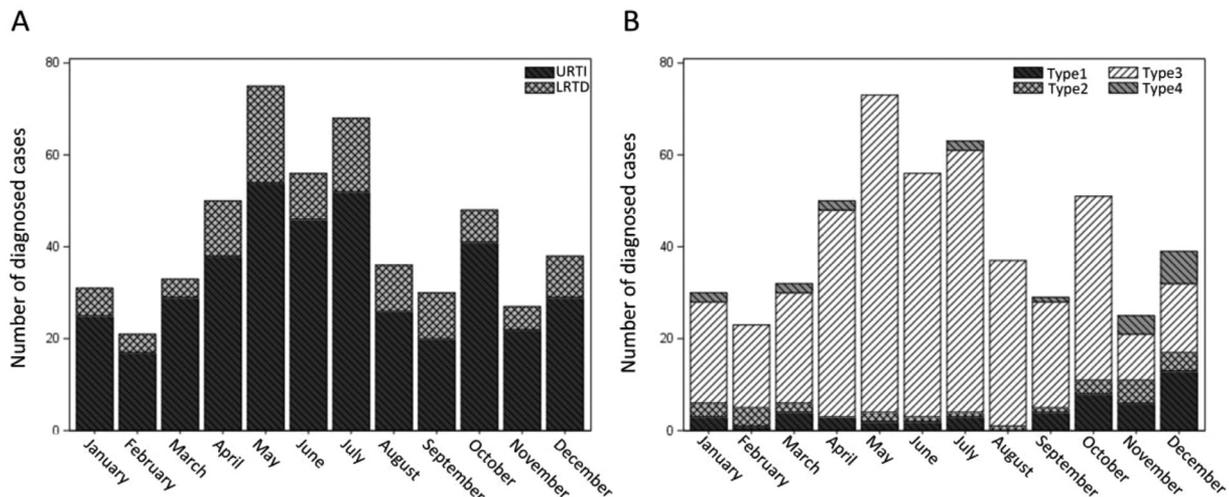
The probabilities of progression to LRTD among patients who presented with URTI were estimated by cumulative incidence curves, treating death as a competing risk. Cox proportional hazards models were used to evaluate unadjusted and adjusted hazard ratios (aHRs) for progression from URTI or possible LRTD to probable or proven LRTD. Logistic regression models were used to evaluate cross-sectional associations between each risk factor and occurrence of LRTD without prior PIV detection in upper respiratory tract samples among patients with probable/proven LRTD. Several models were evaluated for each endpoint when the number of events did not permit inclusion of all variables into 1 model. The log-rank test was used to compare univariate hazards of time-to-event outcomes. Two-sided  $P < .05$  was considered statistically significant. All statistical analyses were performed using SAS 9.4 (TS1M3) for Windows (SAS Institute, Inc., Cary, NC).

## RESULTS

### Incidence of PIV Infections and Patient Characteristics

PIV was detected in 540 HCT recipients altogether; 343 (64%) had URTI alone and 197 (36%) had LRTD. The median time to PIV infection after HCT was 70.5 days (range, 0 to 3140), and 80% of PIV infections were due to PIV-3. The number of PIV infections by month for 20 years between January 1992 and December 2011 is shown in Figure 1A. PIV infection was more frequently detected between April and July than throughout the remainder of the year. The incidence by each PIV subtype is shown in Figure 1B. PIV-3 infections commonly occurred in spring and summer, whereas the other types were detected more in autumn and winter.

Characteristics of patients with PIV infection are shown in Table 1. Among 540 patients with PIV infection, median age at diagnosis was 42.3 (range, 0 to 73). Among 197 LRTD cases, possible, probable, and proven LRTD cases occurred in 76 (39%), 19 (10%), and 102 (52%), respectively. Nosocomial infection, co-pathogen in blood, and low WBC counts at diagnosis were more frequently observed in LRTD cases than in URTI alone cases. The median viral load in nasal wash samples at diagnosis was 6.5 log<sub>10</sub> copies/mL (range, 2.8 to 9.7).



**Figure 1.** Monthly distribution of PIV infection. (A) The number of URTI or LRTD cases with PIV infection by month between January 1992 and December 2011. (B) The number of PIV infection cases by each type between January 1992 and December 2011. PCR testing for PIV-4 started in January 2007, and the cases with unknown PIV type were excluded.

**Table 1**  
Characteristics of All Patients with PIV Infection

Characteristics	Total (n = 540)	URTI Alone (n = 343)	LRTD		
			Possible (n = 76)	Probable (n = 19)	Proven (n = 102)
Sex					
Male	318 (59)	200 (58)	39 (51)	15 (79)	64 (63)
Female	222 (41)	143 (42)	37 (49)	4 (21)	38 (37)
Age at PIV infection, yr					
≤20	101 (19)	71 (21)	17 (22)	2 (11)	11 (11)
21–60	360 (67)	222 (65)	44 (58)	17 (89)	77 (75)
>60	79 (15)	50 (15)	15 (20)	0 (0)	14 (14)
Disease risk					
Standard	344 (64)	223 (65)	51 (67)	14 (74)	56 (55)
High	196 (36)	120 (35)	25 (33)	5 (26)	46 (45)
Transplant year					
1990–2000	270 (50)	176 (51)	25 (33)	15 (79)	54 (53)
2001–2011	270 (50)	167 (49)	51 (67)	4 (21)	48 (47)
Number of transplantations					
1	489 (91)	312 (91)	68 (89)	19 (100)	90 (88)
2	49 (9)	29 (8)	8 (11)	0 (0)	12 (12)
3	2 (0)	2 (1)	0 (0)	0 (0)	0 (0)
Cell source					
Bone marrow	252 (47)	169 (49)	22 (29)	16 (84)	45 (44)
Peripheral blood stem cell	264 (49)	164 (48)	45 (59)	3 (16)	52 (51)
Cord blood	24 (4)	10 (3)	9 (12)	0 (0)	5 (5)
Donor type					
Autologous	103 (19)	70 (20)	16 (21)	0 (0)	17 (17)
Related	200 (37)	135 (39)	22 (29)	8 (42)	35 (34)
Unrelated	237 (44)	138 (40)	38 (50)	11 (58)	50 (49)
Conditioning regimen					
MA including high-dose TBI (≥12 Gy)	209 (39)	127 (37)	28 (37)	11 (58)	43 (42)
MA ± low-dose TBI (≤2 Gy)	240 (44)	155 (45)	34 (45)	6 (62)	45 (44)
Reduced intensity	91 (17)	61 (18)	14 (18)	2 (11)	14 (14)
GVHD prophylaxis					
CNI+MTX	276 (51)	127 (37)	33 (43)	15 (79)	43 (42)
CNI+MMF	110 (20)	155 (45)	24 (32)	2 (11)	45 (44)
Others	51 (9)	61 (18)	3 (4)	2 (11)	14 (14)
ATG use					
No	474 (88)	306 (89)	67 (88)	13 (68)	88 (86)
≥6 mo before diagnosis	12 (2)	7 (2)	4 (5)	0 (0)	1 (1)
Within 6 mo before diagnosis	46 (9)	25 (7)	5 (7)	5 (26)	11 (11)
After diagnosis	8 (1)	5 (1)	0 (0)	1 (5)	2 (2)
%FEV <sub>1</sub> /FVC before PIV infection					
≥70	377 (70)	247 (72)	49 (64)	13 (68)	68 (67)
<70	93 (17)	53 (15)	14 (18)	4 (21)	22 (22)
Missing	70 (13)	43 (13)	13 (17)	2 (11)	12 (12)
%TLC before PIV infection					
≥80	386 (71)	251 (73)	53 (70)	14 (74)	68 (67)
<80	61 (11)	33 (10)	9 (12)	2 (11)	17 (17)
Missing	93 (17)	59 (17)	14 (18)	3 (16)	17 (17)
Days between transplantation and PIV infection					
≤30	119 (22)	66 (19)	22 (28)	3 (16)	27 (26)
31–100	349 (64)	238 (69)	40 (51)	10 (53)	39 (38)
>100	76 (14)	41 (12)	16 (21)	6 (32)	36 (35)
Nosocomial infection					
No	405 (75)	280 (82)	51 (67)	13 (68)	61 (60)
Yes	132 (24)	60 (17)	25 (33)	6 (32)	41 (40)
Missing	3 (1)	3 (1)	0 (0)	0 (0)	0 (0)
PIV type					
PIV-1	51 (9)	25 (7)	16 (21)	1 (5)	9 (9)
PIV-2	29 (5)	23 (7)	3 (4)	0 (0)	3 (3)
PIV-3	432 (80)	275 (80)	51 (67)	17 (89)	89 (87)
PIV-4	22 (4)	16 (5)	5 (7)	1 (5)	0 (0)
Unclassified	6 (1)	4 (1)	1 (1)	0 (0)	1 (1)
Diagnostic test of URTI					
CU/direct fluorescent antibody	367 (68)	258 (75)	50 (66)	12 (63)	47 (46)
PCR	112 (21)	76 (22)	20 (26)	1 (5)	15 (15)
Both	13 (2)	4 (1)	6 (8)	0 (0)	3 (3)
Missing	48 (9)	5 (1)	0 (0)	–	–
Co-pathogen					
No	477 (88)	308 (90)	65 (86)	18 (95)	86 (84)
Upper respiratory tract	43 (8)	29 (8)	8 (11)	1 (5)	5 (5)
Blood	20 (4)	6 (2)	3 (4)	0 (0)	11 (11)
WBC count					

(continued)

**Table 1** (Continued)

Characteristics	Total (n = 540)	URTI Alone (n = 343)	LRTD		
			Possible (n = 76)	Probable (n = 19)	Proven (n = 102)
>1000/ $\mu$ L	444 (82)	297 (87)	57 (75)	19 (10)	71 (70)
$\leq$ 1000/ $\mu$ L	81 (15)	32 (9)	19 (25)	0 (0)	30 (29)
Missing	15 (3)	14 (4)	0 (0)	0 (0)	1 (1)
Neutrophil count					
>500/ $\mu$ L	441 (82)	296 (86)	56 (74)	18 (95)	71 (70)
$\leq$ 500/ $\mu$ L	81 (15)	33 (10)	19 (25)	1 (5)	29 (28)
Missing	15 (3)	14 (4)	1 (1)	0 (0)	2 (2)
Lymphocyte count					
>300/ $\mu$ L	328 (61)	221 (64)	42 (55)	11 (58)	54 (53)
100-300/ $\mu$ L	124 (23)	79 (23)	21 (28)	1 (5)	23 (23)
<100/ $\mu$ L	67 (12)	26 (8)	11 (14)	7 (37)	23 (23)
Missing	21 (4)	17 (5)	2 (3)	0 (0)	2 (2)
Monocyte count					
>300/ $\mu$ L	237 (44)	167 (49)	34 (45)	4 (21)	32 (31)
100-300/ $\mu$ L	147 (27)	97 (28)	18 (24)	9 (47)	23 (23)
<100/ $\mu$ L	134 (25)	61 (18)	22 (29)	6 (32)	45 (44)
Missing	22 (4)	18 (5)	2 (3)	0 (0)	2 (2)
Steroid dose before diagnosis					
No	238 (44)	157 (46)	40 (53)	5 (26)	36 (35)
<1 mg/kg	146 (27)	88 (26)	25 (33)	4 (21)	29 (28)
1-2 mg/kg	143 (26)	93 (27)	10 (13)	7 (37)	33 (32)
>2 mg/kg	13 (2)	5 (1)	1 (1)	3 (16)	4 (4)

All values are indicated as the number (%).

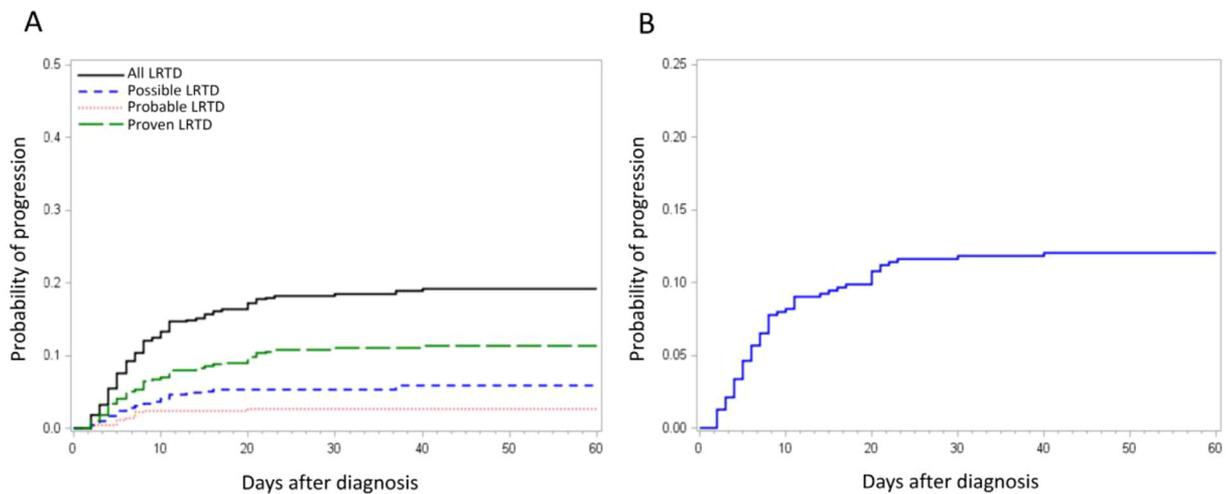
MA indicates myeloablative; TBI, total body irradiation; GVHD, graft versus host disease; CN1, calcineurin inhibitor; MTX, methotrexate; MMF, mycophenolate mofetil; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; ATG, antithymocyte globulin; CU, culture; TLC, total lung capacity.

### Progression to LRTD and Risk Stratification

Among 540 patients with PIV infection, 424 (79%) originally presented with URTI as an initial manifestation of their PIV infection. Among these 424 patients, 81 (19%) progressed to LRTD in a median of 7 days (range, 2 to 40) after the diagnosis of URTI (black line in Figure 2A). The probability of progression from URTI to each LRTD group is also shown in Figure 2A. Among 476 patients with positive nasopharyngeal samples (ie, URTI and possible LRTD [n = 52] as an initial manifestation), the probability of progression to probable/proven LRTD with PIV detection in the lung within 40 days was 12% (Figure 2B).

Because survival after possible LRTD has been documented to be similar to that after URTI and much higher than that after probable or proven LRTD [14], we analyzed the risk factors for progression from URTI/possible LRTD to probable/proven

LRTD. In univariable Cox models low blood cell counts were selected as important risk factors (Table 2). Patients with a lymphocyte count < 100 cells/ $\mu$ L had a high progression rate of approximately 30%, whereas patients with lymphocyte counts  $\geq$  100 cells/ $\mu$ L had similar progression rates of 9% to 12%, independent of the lymphocyte level (Figure 3A). Similar patterns were seen for monocyte counts with levels < 100 cells/ $\mu$ L having higher progression rates (Figure 3B). No completely protective level could be identified for either lymphocyte or monocyte counts. The diagnostic methods had no correlation with the progression rate (PCR alone versus conventional culture and/or direct fluorescent antibody; HR, .71; 95% confidence interval [CI], .36 to 1.40;  $P = .32$ ). In a multivariable model of risk factors for progression, PIV-3, co-pathogen in blood, low monocyte counts, and steroid use of  $\geq$ 1 mg/kg



**Figure 2.** Probability of progression from URTI to LRTD. (A) The probability of progression from URTI to final LRTD status. Black line, progression from URTI to any type of LRTD; blue line, progression from URTI to possible LRTD; red line, progression from URTI to probable LRTD; green line, progression from URTI to proven LRTD. (B) The probability of progression from URTI or possible LRTD to probable or proven LRTD.

**Table 2**  
Risk Factors for Progression from URTI/Possible LRTD to Probable/Proven LRTD (n = 476)

	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	P	HR	95% CI	P
Transplant year						
1990–2000	1.00					
2001–2011	.55	.3–.9	.028			
Cell source						
PBSC	1.00					
BM or CB	1.93	1.1–3.4	.019			
ATG use before PIV infection						
No/> 6 mo	1.00			1.00		
Within 6 mo	2.27	1.1–4.5	.025	1.89	.9–3.8	.09
PIV type						
PIV-1, 2, 4	1.00			1.00		
PIV-3	4.55	.3–14.3	.011	3.57	1.1–11.1	.032
Co-pathogen						
No	1.00			1.00		
Upper respiratory tract	.85	.3–2.4	.76			
Blood	3.00	1.1–8.3	.035	3.21	1.1–9.0	.027
WBC count						
>1000/ $\mu$ L	1.00					
$\leq$ 1000/ $\mu$ L	2.29	1.3–4.1	.006			
Neutrophil count						
>500/ $\mu$ L	1.00					
$\leq$ 500/ $\mu$ L	2.05	1.1–3.8	.02			
Lymphocyte count						
>300/ $\mu$ L	1.00			1.00		
100–300/ $\mu$ L	1.16	1.7–5.6	.66			
<100/ $\mu$ L	3.25	1.7–6.1	<.001	1.79	.9–3.6	.10
Monocyte count						
>300/ $\mu$ L	1.00			1.00		
100–300/ $\mu$ L	1.68	.8–3.4	.16			
<100/ $\mu$ L	3.96	2.1–7.5	<.001	2.22	1.2–4.2	.011
Steroid dose before diagnosis						
No	1.00			1.00		
<1 mg/kg	.94	.4–2.1	.89			
1–2 mg/kg	2.53	1.4–4.6	.002	1.89	1.1–3.2	.018
>2 mg/kg	4.20	1.0–18.1	.054			
Steroid dose after diagnosis*						
No	1.00					
<.5 mg/kg	.60	.3–1.4	.24			
.5–1 mg/kg	.67	.3–1.6	.36			
>1 mg/kg	2.35	.7–7.6	.15			
Ribavirin use*						
No	1.00					
Yes	2.29	.7–7.4	.16			
Viral load in NP sample at diagnosis†	1.00	1.0–1.0	.46			

All variables in Table 1 were used for the univariate analysis. Co-pathogen and ribavirin use at diagnosis of URTI/possible LRTD were included in the analysis. Only variables with  $P < .05$  in any analysis are shown in this table. The following parameters were also shown regardless of  $P$  values; steroid dose after diagnosis, ribavirin use.

PBSC indicates peripheral blood stem cell; BM, bone marrow; CB, cord blood; NP, nasopharyngeal.

\* These variables were analyzed as time-dependent.

† Viral load was analyzed as a continuous variable.

before PIV infection were significantly associated with a high progression rate to probable/proven LRTD (Table 2). Additional multivariable models were formed with different combinations of significant risk factors in the univariable analysis including transplant year but did not yield additional factors that remained significant in the models (data not shown). The probability of progression by each PIV type was shown in Figure 3C. Co-pathogens in the blood were mainly bacteria (*Staphylococcus aureus* in 5 cases, *Pseudomonas aeruginosa* in 4 cases), with candidemia and Epstein-Barr virus viremia observed in 2 cases each. The probability of progression to

probable/proven LRTD by a combination of pairs of the most important factors (steroid use and monocyte count) is shown in Figure 3D, resulting in progression rates of approximately 30%.

We then analyzed progression rates relative to the number of risk factors present at the detection of URTI. No patient progressed to LRTD in the absence of all 4 risk factors, whereas there was an escalating risk with increasing number of risk factors (Figure 4). We also performed a similar analysis among patients who presented with URTI alone, as generally done in the published literature. Probabilities of progression from URTI to all LRTD including possible LRTD (Supplemental Figure 1A) or to probable/proven LRTD only (Supplemental Figure 1B) by the number of risk factors showed similar patterns. Overall, the risk of progression among patients with URTI alone resulted in somewhat higher rates of than those observed among patients with URTI/possible.

Among 136 patients with quantitated viral loads in nasal wash samples at diagnosis, an association between viral load and progression was not observed (Table 2).

#### Effect of Ribavirin Use at URTI Diagnosis

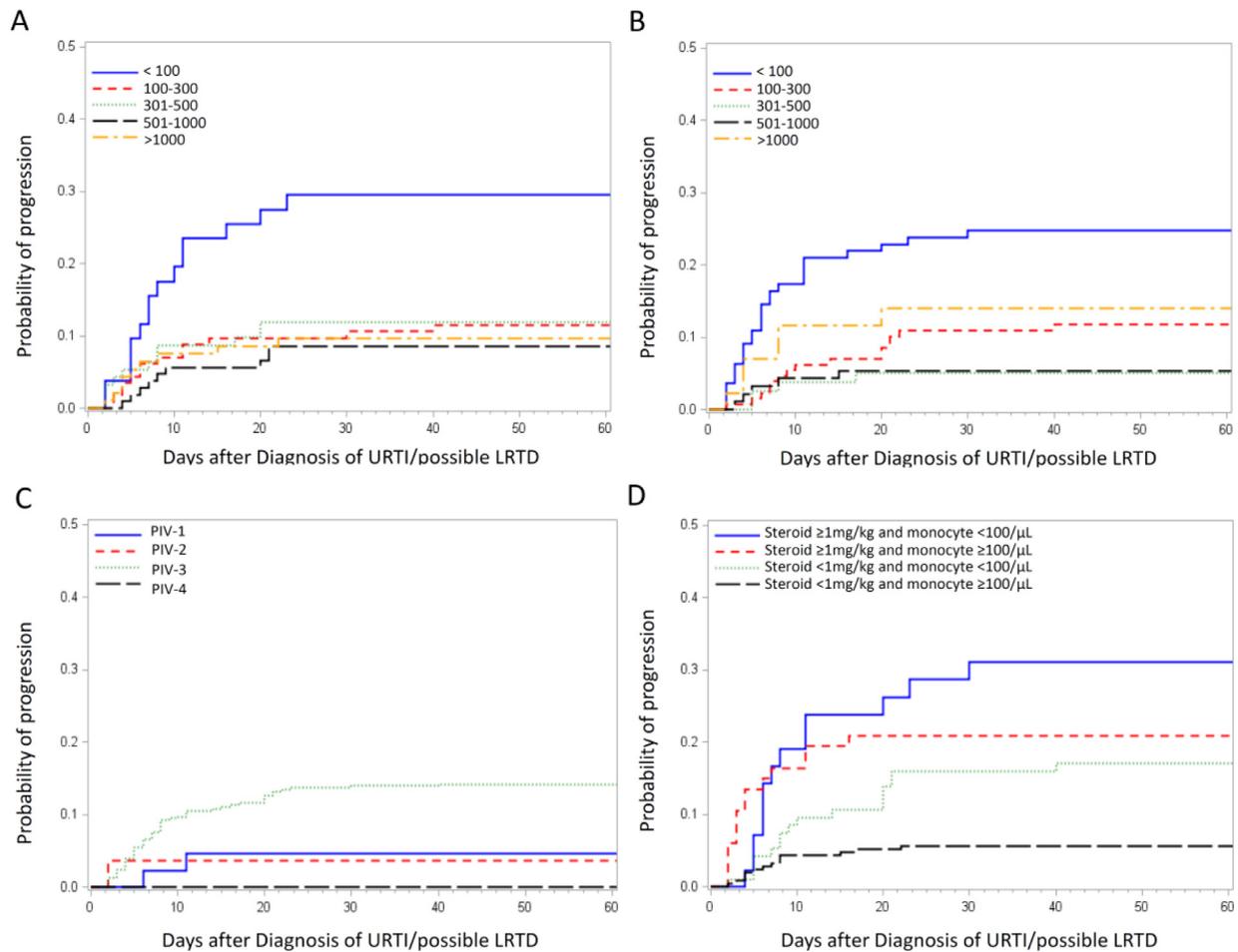
Eighteen patients received ribavirin therapy (inhalation, 13; systemic, 3; both, 1; unknown, 1) after the diagnosis of URTI or possible LRTD, and 3 of these patients progressed to probable/proven LRTD. In a univariable Cox regression model of risk factors for progression to probable/proven LRTD, ribavirin use at URTI or possible LRTD was not significantly associated with progression (Table 2). In bivariate analyses adjusting for each important risk factor, ribavirin use also had no preventive effect on progression to probable/proven LRTD (adjustment variable, PIV type: aHR, 2.17; 95% CI, .67 to 6.96;  $P = .19$ ; adjustment variable, co-pathogen: aHR, 2.32; 95% CI, .72 to 7.46;  $P = .16$ ; adjustment variable, monocyte count: aHR, 1.39; 95% CI, .43 to 4.53;  $P = .59$ ; adjustment variable, steroid use: aHR, 2.29; 95% CI, .71 to 7.35;  $P = .16$ ).

#### Risk Factors for LRTD without Prior URTI among Probable/Proven LRTD Cases

Among 121 probable/proven LRTD cases, 64 (53%) had LRTD without first presenting as URTI. The median time to PIV LRTD after HCT was 76 days (range, 3 to 2343) and 81 days (range, 5 to 2862) in LRTD cases with and without prior URTI, respectively ( $P = .51$ ). Demographics of each patient group are shown in Supplemental Table 1. Of the 64 patients initially presenting with probable/proven LRTD, 13 (20%) had a nasopharyngeal test performed using direct fluorescent antibody or culture in 7 (54%) and PCR in 6 (46%), and all results were negative. In multivariable logistic regression models, factors associated with the development of LRTD without prior URTI versus LRTD that progressed from URTI were low percentage of total lung capacity (<80%) before PIV infection, absence of documented URTI symptoms, oxygen requirement at diagnosis, and co-pathogen detection in the lower respiratory tract (Table 3). The main co-pathogens were *Aspergillus* spp. (n = 9), cytomegalovirus (n = 6), and *P. aeruginosa* (n = 5). Among 48 cases with calculated viral loads in BAL samples, the viral loads were not significantly different between LRTD cases with and without prior URTI. The mortality rate after LRTD without prior URTI was similar to that after LRTD with prior URTI (Figure 5;  $P = .98$ ).

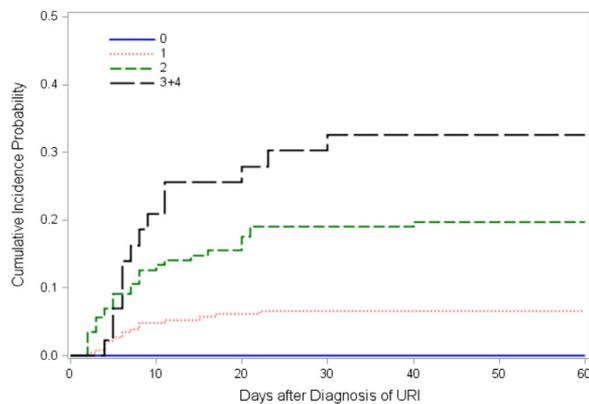
#### DISCUSSION

This study demonstrated that the progression rate of PIV-associated URTI to probable/proven PIV LRTD was approximately 12% and that the total number of risk factors (presence of PIV-3, co-pathogen in the blood, low monocyte counts, and



**Figure 3.** Probability of progression from URTI/possible LRTD to probable/proven LRTD. (A) Cumulative incidence of progression by lymphocyte levels. (B) Cumulative incidence of progression by monocyte levels. (C) Cumulative incidence of progression by PIV type. (D) Cumulative incidence of progression to LRTD by steroid dose before diagnosis and PIV type.

steroid use  $\geq 1$  mg/kg at the time of diagnosis) that are present at the time of URTI diagnosis predicts the probability of progression. Probable/proven LRTD without prior URTI was observed in about half of LRTD cases and occurred more frequently in patients with restrictive lung function who had a co-infection in their lungs. These findings provide new



**Figure 4.** Probability of progression from URTI/possible LRTD to probable/proven LRTD by the number of risk factors. Cumulative incidence of progression by the number of risk factors (PIV type, co-pathogen, monocyte count, and steroid dose;  $n = 64$  in 0,  $n = 227$  in 1,  $n = 142$  in 2, and  $n = 43$  in  $\geq 3$  risk factors).

information for those caring for HCT recipients, particularly those with respiratory symptoms during the post-HCT period.

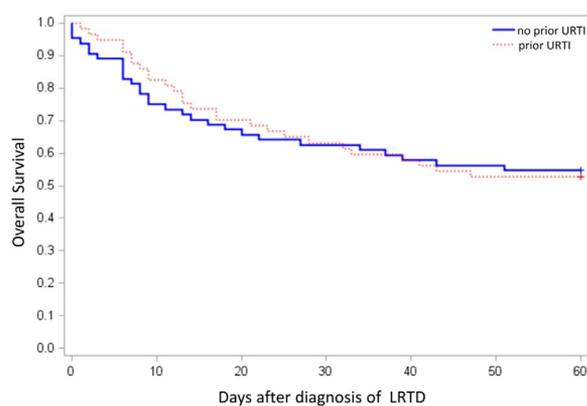
Several studies on the incidence or outcomes of PIV infections in patients with hematologic malignancies or transplant recipients have reported highly variable progression rates from URTI to LRTD ranging from 0 to 74% [3,9,11,12,17], with an average rate of 36%. This wide range could be due to varying cohort sizes (median subjects, 13; range, 4 to 213) or because the definition of LRTD was inconsistent among the studies. We reported previously that the survival after possible LRTD (PIV detection only in upper respiratory tract) is similar to that after URTI and much better than that after probable/proven LRTD (PIV detection in lower respiratory tract) [14]. In this current cohort study of 540 subjects the progression rate was approximately 50% higher when proven/probable and possible LRTD cases were combined (19% versus 12% for proven/probable only) (Figure 2A,B). Considering the differential outcomes after LRTD [14], we recommend reporting probable and proven LRTD as among LRTD cases in future studies, which would permit the consistent interpretation of study endpoints and results.

Using Cox regression models we identified 4 risk factors for progression from URTI/possible LRTD to probable/proven LRTD. Low monocyte count and PIV-3 type were newly identified risk factors. Previous studies including studies of other respiratory viruses showed the importance of lymphocyte

**Table 3**  
Risk Factors for LRTD without Prior URTI (n = 121)

	Univariable Analysis			Multivariable Analysis		
	OR	95% CI	P	OR	95% CI	P
Age at PIV infection, yr						
≤20	.25	.1-1.3	.09			
21-60	.44	.1-1.5	.19			
>60	1.00					
Cell source						
PBSC	1.00			1.00		
BM or CB	.39	.2-.8	.012	.47	.1-1.7	.25
%FEV <sub>1</sub> /FVC before PIV infection						
≥70	1.00					
<70	2.25	.9-5.6	.08			
%TLC before PIV infection						
≥80	1.00			1.00		
<80	3.41	1.1-10.3	.030	5.56	1.1-25.0	.036
Days between transplantation and PIV infection						
≤30	.92	.4-2.4	.87			
31-100	.42	.2-1.0	.047			
>100	1.00					
Inpatient at diagnosis						
No	1.00			1.00		
Yes	3.44	1.6-7.3	.001	1.61	.4-6.0	.48
Diagnosis on weekday						
No	1.00					
Yes	.51	.2-1.5	.21			
Documented URTI symptom at diagnosis						
No	1.00			1.00		
Yes	.19	.1-4	<.001	.17	.0-.7	.017
LRTD symptom at diagnosis						
No	1.00					
Yes	3.37	1.1-10.3	.033			
PIV type						
PIV-1, -2, -4	1.00					
PIV-3	.26	.1-1.0	.049			
Co-pathogen						
No/blood alone	1.00			1.00		
Upper respiratory tract	.38	.0-3.5	.40	.41	.0-40.1	.24
Lower respiratory tract	14.60	4.1-52.0	<.001	43.70	6.0-321	.006
Oxygen use at diagnosis						
No	1.00			1.00		
Yes	10.30	4.4-24.5	<.001	9.51	2.2-41.2	.003
Steroid dose before diagnosis						
No	1.00					
<1 mg/kg	1.80	.7-4.7	.23			
1-2 mg/kg	.38	.2-0.9	.034			
>2 mg/kg	1.96	.3-11.3	.45			

All variables in Supplemental Table 1 were used for the univariate analysis. Only variables with  $P < .1$  in any analysis except diagnosis on weekday are shown in this table. The most informative multivariable model is shown here and additional models with different combinations of risk factors did not yield other factors that remained after adjustment.



**Figure 5.** Probability of overall survival comparing LRTD with and without prior URTI. Kaplan-Meier estimate of overall survival among 121 probable/proven LRTD cases ( $P = 1.00$ ).

count [9,16,18,19]. In our study, however, low monocyte count appeared to be more important in progression to LRTD than lymphopenia, which is similar to the results of risk factors for mortality after PIV LRTD [14]. Previous reports have shown that monocytes are important to protect viral infection [20, 21]. However, the role of monocytopenia in the progression from PIV URTI to LRTD should be considered for further investigation. Importantly, the 4 factors we identified can stratify the risk for progression in HCT recipients with a positive nasopharyngeal sample. Indeed, the absence of any of the 4 risk factors was associated with protection from progression while increasing numbers of risk factors correlated with increased risk. Nevertheless, monocytopenia and corticosteroid dose, a surrogate marker of graft-versus-host disease, appear to be the most relevant of these 4 factors. Of interest, viral load in nasopharyngeal specimen as a possible predictor for progression was analyzed in a subset of patients and was not predictive of progression to LRTD. This is consistent with a

lack of a viral load as a risk for progression after HCT with human metapneumovirus infection [16].

Administration of an antiviral drug may prevent progression to LRTD for other viral infections such as respiratory syncytial virus (RSV) or influenza, based on data from non-randomized studies [19,22,23]. Although studies have suggested a preventive effect of ribavirin [24,25], other studies and a meta-analysis failed to show the efficacy of ribavirin both in disease progression and mortality from PIV LRTD [3, 5,14,17]. In our study, adjusted analyses did not suggest a preventive effect of preemptive ribavirin. Steroid use has been consistently shown to be important for progression in this and other studies [11,17]. Based on our study, we recommend reducing the steroid dose whenever feasible to <1 mg/kg when PIV is detected until a new antiviral drug such as DAS181 or virus-specific T cell therapy are shown to be effective and available for use [26–30].

Although we could not evaluate differences between each PIV subtype because of the insufficient number of cases, PIV-3 had a significantly higher progression rate than the other types in the multivariable analysis. Because the incidence of PIV-1, -2, and -4 infections are low, less is known about the pathogenesis of these PIV types, especially in PIV-4. Further studies are needed to examine the role of strain differences in pathogenicity.

In immunocompromised patients LRTDs are occasionally documented without manifestation of URTI. In our study 53% of patients with probable/proven LRTD had no prior presentation of URTI, whereas previous reports showed frequencies between 13% and 58% [9,11–13]. The differences in the observed frequency rates may be due to how diagnostic workups proceed for PIV using BAL or biopsy samples. We generally test for respiratory viruses including PIV by PCR when we perform bronchoscopic examination regardless of the presence or absence of URTI symptoms. We confirmed that the absence of URTI symptoms was associated with LRTD without URTI, which is plausible and would argue against the possibility that we missed earlier testing in patients who should have been tested. One possible reason for the lack of URTI symptoms may be the inability to mount an inflammatory response after viral acquisition. However, the maximum steroid dose in the 2 weeks before diagnosis did not appear to be an important factor, whereas oxygen requirement at diagnosis and a pulmonary co-pathogen were other significant factors. We hypothesize that inpatients have a higher likelihood of diagnostic imaging, possibly in the context of pre-existing restrictive lung function and/or hypoxia, which may have led to more bronchoscopic procedures. Meanwhile, early bronchoscopic examination for LRTD with a broad diagnostic panel that includes respiratory viruses regardless of upper respiratory symptoms may maximize diagnostic yield for pulmonary disease.

This study has the advantage of including the largest number of HCT recipients with PIV infections, which permitted the performance of multivariable Cox regression analyses of risk factors for progression to LRTD that can clearly stratify the risk groups. Moreover, we applied the stringent definition of LRTD based on a previous outcome study [14] and examined for the first time risk factors that might explain the phenomenon of LRTD without URTI. Limitations of this study include the inclusion of cases over a relatively long period of time and insufficient cases to analyze the effect of preemptive ribavirin, viral load on progression, or the differences by each subtype despite of data collection for 20 years. To decrease the effect of a long time period on the analyzed results, we included transplant year and the factors that have changed during 20 years such as

diagnostic methods or cell source in multivariable model. Moreover, because of the retrospective nature of our study, we might have missed information of URTI symptoms from medical charts because of inconsistent documentation practices or failure of patients to report mild URTI symptoms, especially late after HCT. We generally performed examination for respiratory viruses using a nasopharyngeal sample when patients had URTI symptoms and bronchoscopy when patients had lower respiratory tract symptoms and radiographic abnormalities. Nevertheless, the final decision was left to the physicians who managed the patients. Therefore, an aggressive workup was declined in some cases.

In conclusion, the progression rate of PIV to probable/proven LRTD in our large retrospective series was approximately 12%, and it exceeded 30% in patients who had at least 3 risk factors, with low monocyte counts and steroid use  $\geq$  1 mg/kg before URTI being the most important predictors. Our finding that monocytes rather than lymphocytes appear to play an important role in progressive disease suggests that further studies to evaluate host defense pathways for PIV are needed. Ribavirin did not appear to have a protective effect on progression. These findings define populations that appear to be protected from progression and those at high risk. This should facilitate the rational development of preventive and therapeutic strategies in immunocompromised hosts. Considering that approximately half of HCT recipients with LRTD showed absence of URTI symptoms, intensive virologic testing is recommended in patients with pneumonia or evidence of lower respiratory tract disease.

#### ACKNOWLEDGMENTS

The authors thank Zachary Stednick for database services and Terry Stevens-Ayers for laboratory assistance.

*Financial disclosure:* This work was partially supported by grants from the National Institutes of Health (K24HL093294 and CA15704). S.S. is a recipient of a fellowship from the Joel Meyers Endowment Scholarship. Specimens were provided by the Vaccine and Infectious Disease Division Biospecimen Repository at Fred Hutchinson Cancer Research Center.

*Conflict of interest statement:* S.S. and M.B. received research support from Ansun Biopharma, Inc. M.B. also serves as a consultant to Ansun Biopharma, Inc.

*Authorship statement:* S.S. co-designed the study, collected the clinical data, assisted in data interpretation, and wrote the manuscript. H.X. and W.L. did the statistical analyses and edited the manuscript. J.K. performed the PCR assay and edited the manuscript. F.S., S.G., and L.K. collected the clinical data and edited the manuscript. A.C. and J.E. co-designed the study and edited the manuscript. K.J. managed the PCR assay and edited the manuscript. M.B. co-designed the study, assisted in data interpretation, and wrote the manuscript.

#### SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.08.021.

#### REFERENCES

1. Renaud C, Campbell AP. Changing epidemiology of respiratory viral infections in hematopoietic cell transplant recipients and solid organ transplant recipients. *Curr Opin Infect Dis.* 2011;24:333–343.
2. Schiffer JT, Kirby K, Sandmaier B, Storb R, Corey L, Boeckh M. Timing and severity of community acquired respiratory virus infections after myeloablative versus non-myeloablative hematopoietic stem cell transplantation. *Haematologica.* 2009;94:1101–1108.
3. Shah DP, Shah PK, Azzi JM, Chemaly RF. Parainfluenza virus infections in hematopoietic cell transplant recipients and hematologic malignancy patients: a systematic review. *Cancer Lett.* 2016;370:358–364.

4. Henrickson KJ. Parainfluenza viruses. *Clin Microbiol Rev.* 2003;16:242–264.
5. Fry AM, Curns AT, Harbour K, Hutwagner L, Holman RC, Anderson LJ. Seasonal trends of human parainfluenza viral infections: United States, 1990–2004. *Clin Infect Dis.* 2006;43:1016–1022.
6. Hodson A, Kasliwal M, Streetly M, MacMahon E, Raj K. A parainfluenza-3 outbreak in a SCT unit: sepsis with multi-organ failure and multiple co-pathogens are associated with increased mortality. *Bone Marrow Transplant.* 2011;46:1545–1550.
7. Maziarz RT, Sridharan P, Slater S, et al. Control of an outbreak of human parainfluenza virus 3 in hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant.* 2010;16:192–198.
8. Cortez KJ, Erdman DD, Peret TC, et al. Outbreak of human parainfluenza virus 3 infections in a hematopoietic stem cell transplant population. *J Infect Dis.* 2001;184:1093–1097.
9. Chemaly RF, Hanmod SS, Rathod DB, et al. The characteristics and outcomes of parainfluenza virus infections in 200 patients with leukemia or recipients of hematopoietic stem cell transplantation. *Blood.* 2012;119:2738–2745. quiz 969.
10. Ustun C, Slaby J, Shanley RM, et al. Human parainfluenza virus infection after hematopoietic stem cell transplantation: risk factors, management, mortality, and changes over time. *Biol Blood Marrow Transplant.* 2012;18:1580–1588.
11. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. Parainfluenza virus infections after hematopoietic stem cell transplantation: risk factors, response to antiviral therapy, and effect on transplant outcome. *Blood.* 2001;98:573–578.
12. Lewis VA, Champlin R, Englund J, et al. Respiratory disease due to parainfluenza virus in adult bone marrow transplant recipients. *Clin Infect Dis.* 1996;23:1033–1037.
13. Wendt CH, Weisdorf DJ, Jordan MC, Balfour Jr HH, Hertz MI. Parainfluenza virus respiratory infection after bone marrow transplantation. *N Engl J Med.* 1992;326:921–926.
14. Seo S, Xie H, Campbell AP, et al. Parainfluenza virus lower respiratory tract disease after hematopoietic cell transplant: viral detection in the lung predicts outcome. *Clin Infect Dis.* 2014;58:1357–1368.
15. Kuypers J, Wright N, Ferrenberg J, et al. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. *J Clin Microbiol.* 2006;44:2382–2388.
16. Seo S, Gooley TA, Kuypers JM, et al. Human metapneumovirus infections following hematopoietic cell transplantation: factors associated with disease progression. *Clin Infect Dis.* 2016;63:178–185.
17. Srinivasan A, Wang C, Yang J, Shenep JL, Leung WH, Hayden RT. Symptomatic parainfluenza virus infections in children undergoing hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2011;17:1520–1527.
18. Kim YJ, Guthrie KA, Waghmare A, et al. Respiratory syncytial virus in hematopoietic cell transplant recipients: factors determining progression to lower respiratory tract disease. *J Infect Dis.* 2014;209:1195–1204.
19. Shah DP, Ghantaji SS, Shah JN, et al. Impact of aerosolized ribavirin on mortality in 280 allogeneic haematopoietic stem cell transplant recipients with respiratory syncytial virus infections. *J Antimicrob Chemother.* 2013;68:1872–1880.
20. Barbalat R, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol.* 2009;10:1200–1207.
21. Cros J, Cagnard N, Woollard K, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity.* 2010;33:375–386.
22. Shah DP, Ghantaji SS, Ariza-Heredia EJ, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. *Blood.* 2014;123:3263–3268.
23. Kmeid J, Vanichanan J, Shah DP, et al. Outcomes of influenza infections in hematopoietic cell transplant recipients: application of an immunodeficiency scoring index. *Biol Blood Marrow Transplant.* 2016;22:542–548.
24. Chakrabarti S, Collingham KE, Holder K, Oyaide S, Pillay D, Milligan DW. Parainfluenza virus type 3 infections in hematopoietic stem cell transplant recipients: response to ribavirin therapy. *Clin Infect Dis.* 2000;31:1516–1518.
25. Elizaga J, Olavarria E, Apperley J, Goldman J, Ward K. Parainfluenza virus 3 infection after stem cell transplant: relevance to outcome of rapid diagnosis and ribavirin treatment. *Clin Infect Dis.* 2001;32:413–418.
26. Guzman-Suarez BB, Buckley MW, Gilmore ET, et al. Clinical potential of DAS181 for treatment of parainfluenza-3 infections in transplant recipients. *Transplant Infect Dis.* 2012;14:427–433.
27. Drozd DR, Limaye AP, Moss RB, et al. DAS181 treatment of severe parainfluenza type 3 pneumonia in a lung transplant recipient. *Transplant Infect Dis.* 2013;15:E28–E32.
28. Waghmare A, Wagner T, Andrews R, et al. Successful treatment of parainfluenza virus respiratory tract infection with DAS181 in 4 immunocompromised children. *J Pediatr Infect Dis Soc.* 2015;4:114–118.
29. Salvatore M, Satlin MJ, Jacobs SE, et al. DAS181 for treatment of parainfluenza virus infections in hematopoietic stem cell transplant recipients at a single center. *Biol Blood Marrow Transplant.* 2016;22:965–970.
30. Aguayo-Hiraldo PI, Arasaratnam RJ, Tzannou I, et al. Characterizing the cellular immune response to parainfluenza virus 3. *J Infect Dis.* 2017;216:153–161.