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Title Page

First onset herpesviral infection and lung injury in allogeneic hematopoietic cell transplantation

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Materials and Methods

Human study population and study design

Allogeneic hematopoietic cell transplant (HCT) recipients transplanted at the University of Michigan Medical Center between 2005- 2011 were included in this retrospective analysis. Quantitative polymerase chain reaction (qPCR) for Cytomegalovirus (CMV) was performed weekly on plasma through 100 days post-HCT. CMV reactivation was defined as ≥600 copies/mL. Additional testing, including qPCR, direct fluorescent antibody or enzyme linked immunosorbent assay (ELISA) for human herpesvirus 6 (HHV-6, both HHV-6A and HHV-6B), Epstein Barr Virus (EBV), herpes simplex virus (HSV) and community acquired respiratory viruses (CARV) were analyzed from plasma, bronchoalveolar lavage (BAL) fluid, cerebrospinal fluid, oral mucosa or nasopharyngeal swabs as clinically indicated. Quantitative PCR for HHV-6 was carried out by ViraCor (Lee's Summit, MO). HHV-6 reactivation was defined as ≥100 copies/mL. Quantitative PCR for EBV was carried out at Mayo Clinic Medical Laboratories (Rochester, MN). EBV reactivation was defined as ≥100 copies/mL. One patient persistently had high HHV-6 DNA levels in her plasma (3~4 log10 copies/ml) throughout the treatment and thus likely carried chromosomally integrated HHV-6 (ciHHV-6). However, her status of ciHHV-6 was not verified, and she remained in the patient group of early HHV-6 infection. This subject did not have idiopathic pneumonia syndrome (IPS), but did developed acute and chronic graft-versus-host disease (GVHD) and bronchiolitis obliterans syndrome (BOS).

Viral infections were defined as isolation of viral proteins or nucleic acid from any body fluid or tissue specimen of patients with clinical indication (e.g., plasma, serum, whole blood, cerebral spinal fluid, feces, or tissue), using viral cultures, qPCR, immunohistochemical analysis, or in situ hybridization, with the exception of CMV infections for which we screened plasma samples weekly by qPCR. A first onset viral infection (FOVI) was defined by the presence of the first viral pathogen within 100 days post-HCT. IPS and BOS were diagnosed according to NIH consensus guidelines (1, 2). The study was approved by the Institutional Review Board at University of Michigan (HUM00117533), with all patients signed informed consent permitting data collection.

Murine roseolovirus and infection

Murine roseolovirus (MRV) was previously termed murine thymic lymphotropic virus (MTLV), or murine thymic virus (MTV) and has been described by Dr. Yokoyama's laboratory previously (3, 4). Our virus stocks were produced by *in vivo* passaging as previously described (5, 6). A single virus stock was used for all experiments. Litters of newborn BALB/c mice within 24 hours after birth were infected with MRV by intraperitoneal inoculation with 50µl of a 1:5 dilution of virus stock.

Mouse minor histocompatibility antigen-mismatched BMT

B10.D2($H2^d$) and BALB/c($H2^d$) mice were purchased from Jackson Laboratory (Bar Harbor, ME). BALB/c mice were infected with MRV within 24 hours after birth and allowed to age for 8-10 weeks for the virus to complete lytic infection and establish latency. Bone marrow transplantation was performed as previously reported (7). In brief, BALB/c mice infected at birth with MRV or left uninfected were aged to 8 to 10

weeks before being given 9 Gy total body irradiation (split dose) using a ^{137}Cs irradiator, followed by intravenous injection with 5 x 10^6 marrow cells supplemented with 5 x 10^6 splenocytes prepared from B10.D2 mice. Mice were then assessed at various time points for evidence of MRV reactivation, scoring for clinical signs of GVHD and tissue pathology. GVHD scores were assessed by evaluating weight, fur, skin, activity, and posture; a score from 0 to 2 was assigned to each characteristic, resulting in a maximal GVHD score of 10 (8).

Mouse bronchoalveolar lavage (BAL)

Mice were euthanized with CO_2 asphyxiation and BAL was performed with 1ml of phosphate-buffered saline (PBS) containing 5mM EDTA. BAL fluid was frozen for subsequent analysis of TNFα, total protein and MRV DNA (see below).

TNFα and total protein measurement

The levels of TNFα in BAL fluid were measured with a R&D duoset ELISA kit. Total protein quantification was achieved with the use of Pierce BCA assay kit from Thermo Fisher. Immunofluorescence images were taken with an Olympus 500 confocal microscope.

Quantitative PCR for MRV genes

Total RNA was extracted from mouse tissues using Ambion TRIzol reagent (ThermoFisher, Waltham, MA) following the manufacturer's directions. The relative abundance of transcripts of MRV *DNA polymerase* (*pol*) or *glycoprotein B* (*gB*) was assayed by quantitative RT-PCR using thermocycler ABI Prism 7000 (Applied

Biosystems, Foster City, CA) following a previously described protocol.(9) For quantification of *pol* mRNA, forward primer is 5' AGGAGCTACGGTCTTGGAAC 3', reverse primer is 5' AAAGACGTCCTCTTCAGGCA 3', and probe is Fam 5' TCCAACACCACGGTGGGCGA 3' Tamra; for quantification of *gB* mRNA, forward primer is 5' TTCTGGGCTCGGATCGATAG 3', reverse primer is 5' TTTGCCAGATCAGCAACAGC 3', and probe is Fam 5' ACCGCAGTAACGGCTGACCCT 3' Tamra. Data for the expression of *gB* was similar to that reported for *pol* and thus is not shown. Genomic DNA was prepared from 100 µl mouse BAL fluid using a DNA blood kit (Qiagen, Valencia, CA). MRV virial DNA abundance was determined using an MRV-specific genome primer set previously published (3).

Statistical analysis

Demographic and clinical characteristics of HCT recipients were summarized using median and range for age at HCT and numbers and percentages for categorical characteristics. Kaplan-Meier methods estimated median follow-up time and the corresponding 95% confidence interval.

In studying time-to-event, the relationship between FOVIs and overall mortality was assessed using a Cox proportional hazards regression model with a time-dependent covariate for FOVIs. This time-to-event analyses allows for patients to contribute to models as infection-free prior to their first infection when they shift status. Corresponding survival estimates based on this model adjusted for age, gender, time-dependent aGVHD, underlying diagnosis, HLA disparity, source of hematopoietic cells,

donor relationship and conditioning regiment were displayed graphically. Other time-to-event outcomes that were subject to dependent censoring from competing risks (time to viral infection, relapse mortality, non-relapse mortality, IPS, BOS, aGVHD, cGVHD) were analyzed using stabilized inverse probability censored weighting methodology adjustments to Cox proportional hazards regression (10-13). Infection type was modeled using time-dependent covariates when listed as a predictor (time to relapse mortality, non-relapse mortality, IPS, BOS, aGVHD, cGVHD). Corresponding cumulative incidence curves are graphically displayed based on Cox models that account for dependent censoring from competing risks, where appropriate.

In multivariate modeling of time-to-event, forward selection was initially used to identify independently predictive demographic and clinical risk factors at the 0.05 significance level. To fully account for potential confounders in each of the four models of time-to-first viral infection/reactivation (CMV, HHV-6, EBV or CARV) during the first 100 days post-HCT, risk factors that turned up through forward selection for any one of these four separate outcomes were included across all four multivariable models of these outcomes. Similarly, in multivariable models of time-to-aGVHD and cGVHD, statistically significant risk factors identified via forward selection for either GVHD outcome type were included in both models as potential confounders. In multivariable time-to-event models for IPS and BOS, age, cord blood transplant (CBT) and human leukocyte antigen (HLA) mismatch were identified via forward selection for either IPS or BOS and were included in both models as potential confounders. All analyses were done using R version 3.3.2 (14).

For preclinical mouse studies, Student's *t* tests were used to determine significance between two groups. When three groups or more were compared, one-way analysis of variance was utilized with a Tukey's multiple comparisons test to determine significance.

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Table E1. Univariate analysis for risk factors associated with FOVIs during 100 days post-HCT.

	CMV				HHV-6				EBV				CARV			
	HR	95% CI		*P*	HR	95% CI		*P*	HR	95% CI		*P*	HR	95% CI		*P*
HLA mismatched	1.51	0.99	2.28	0.05	4.46	2.64	7.52	<0.0001	1.99	0.86	4.61	0.11	2.17	0.94	5.03	0.07
Unrelated donor	2.14	1.48	3.10	<0.0001	3.45	1.86	6.41	<0.0001	2.06	0.92	4.63	0.08	1.57	0.72	3.44	0.26
CBT	0.99	0.39	2.55	0.99	14.08	8.15	24.34	<0.0001	--*	--*	--*	--*	1.92	0.56	6.56	0.30
TBI	1.36	0.91	2.02	0.13	2.27	1.33	3.86	0.003	2.10	0.94	4.67	0.07	2.11	0.94	4.72	0.07
Age, 10 years	1.31	1.15	1.50	<0.0001	0.79	0.70	0.90	<0.0001	0.96	0.77	1.19	0.69	0.90	0.72	1.13	0.37
Non-malignant	0.68	0.39	1.18	0.17	1.36	0.70	2.64	0.36	2.30	0.99	5.32	0.05	1.12	0.42	2.99	0.83
Male	0.78	0.55	1.11	0.16	0.91	0.54	1.55	0.74	0.78	0.36	1.69	0.53	0.84	0.38	1.86	0.67

CARV, community acquired respiratory viruses; CBT, umbilical cord blood transplant; CI, confidence interval; CMV, cyclomegalovirus; EBV, Epstein-Barr virus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; HR, hazard ratio; TBI, total body irradiation.

* No first EBV reactivation up to 100 days post-CBT

Table E2. Multivariable analysis for risk factors associated with FOVIs during 100 days post-HCT

	CMV			HHV-6			EBV			CARV		
	aHR	95% CI	*P*	aHR	95% CI	*P*	aHR	95% CI	*P*	aHR	95% CI	*P*
HLA Mismatched	1.12	0.72 - 1.75	0.61	1.79	0.90 - 3.57	0.10	1.64	0.58 - 4.55	0.35	1.85	0.72 - 4.76	0.20
Unrelated Donor	2.08	1.43 - 3.03	<0.001	1.52	0.72 - 3.13	0.27	1.69	0.66 - 4.35	0.27	1.18	0.52 - 2.63	0.69
CBT	1.07	0.41 - 2.80	0.89	7.69	3.72 - 15.87	<0.001	--*	-- *	--*	0.96	0.24 - 3.80	0.95
TBI	1.57	1.05 - 2.36	0.03	1.52	0.83 - 2.79	0.18	2.04	0.86 - 4.83	0.11	1.82	0.73 - 4.58	0.20
Age, 10 yrs	1.36	1.18 - 1.55	<0.001	0.99	0.86 - 1.14	0.88	1.05	0.85 - 1.28	0.66	0.96	0.75 - 1.21	0.71
Non-malignant	0.83	0.48 - 1.45	0.53	1.75	0.83 - 3.70	0.14	2.78	1.23 - 6.25	0.01	1.27	0.46 - 3.45	0.65

All variables in Table E1 were forward selected in an inverse probability weighted Cox regression model for at least one of the infection types.

aHR, adjusted hazard ratio; CARV, community acquired respiratory viruses; CBT, umbilical cord blood transplant; CI, confidence interval; CMV, cyclomegalovirus; EBV, Epstein-Barr virus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; TBI, total body irradiation.

* No first EBV reactivation up to 100 days post-CBT

Table E3. Univariate analysis of FOVIs and other risk factors for time to grade II-IV aGVHD and cGVHD

	Grade II-IV aGVHD				cGVHD			
	HR	95% CI		*P*	HR	95% CI		*P*
First CMV* vs none	1.90	1.30	2.76	0.001	1.16	0.90	1.50	0.26
First HHV-6* vs none	2.17	1.26	3.73	0.005	0.99	0.68	1.44	0.97
First EBV* vs none	1.54	0.71	3.33	0.28	0.74	0.40	1.36	0.33
First CARV* vs none	1.47	0.69	3.13	0.32	0.95	0.57	1.60	0.85
First HSV* vs none	0.83	0.17	3.92	0.81	0.92	0.27	3.09	0.89
Age, 10 years	1.02	0.96	1.08	0.63	1.09	1.03	1.15	0.003
Male	0.92	0.74	1.14	0.44	0.89	0.73	1.09	0.25
Malignant	1.40	1.02	1.91	0.04	1.43	1.05	1.96	0.02
HLA mismatched	2.38	1.89	3.03	<0.001	1.35	1.08	1.69	0.01
CBT	1.92	1.29	2.84	0.001	0.67	0.42	1.09	0.001
Unrelated donor	2.17	1.75	2.70	<0.001	1.41	1.16	1.72	<0.01
TBI	1.38	1.08	1.78	0.01	0.90	0.73	1.11	0.31

aGVHD, acute graft-versus-host disease; CARV, community acquired respiratory viruses; CBT, umbilical cord blood transplant; cGVHD, chronic graft-versus-host disease; CI, confidence interval; CMV, cyclomegalovirus; EBV, Epstein-Barr virus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; HSV, herpes simplex virus; HR, hazard ratio; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

Table E4. Multivariable analysis of total FOVIs and other risk factors for time to grade II-IV aGVHD and cGVHD

Multivariable analysis	Grade II-IV aGVHD C-index=0.663				cGVHD C-index=0.559			
	aHR	95% CI		*P*	aHR	95% CI		*P*
FOVIs*	1.466	1.07	2.009	0.017	0.98	0.78	1.23	0.86
Age, 10 years	1.021	0.954	1.092	0.55	1.05	0.99	1.12	0.13
Malignant	1.265	0.899	1.778	0.177	1.28	0.92	1.78	0.139
HLA mismatched	1.74	1.305	2.32	<0.001	1.31	1.01	1.69	0.04
Unrelated donor	1.741	1.374	2.205	<0.0001	1.39	1.12	1.72	0.002
TBI	1.199	0.926	1.554	0.17	0.90	0.71	1.14	0.38
CBT	0.925	0.571	1.499	0.75	0.56	0.32	0.96	0.04

Specific FOVIs and all variables in Table 1 were forward selected in an inverse probability weighted Cox regression model for either aGVHD or cGVHD.

aGVHD, acute graft-versus-host disease; aHR, adjusted hazard ratio; CBT, umbilical cord blood transplant; cGVHD, chronic graft-versus-host disease; CI, confidence interval; C-index, concordance index; FOVI, first onset viral infection; HCT, stem cell transplantation; HLA, human leukocyte antigen; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

Table E5. Multivariable analysis of FOVI types and other risk factors for time to grade II-IV aGVHD and cGVHD

Multivariable analysis	Grade II-IV aGVHD C-index=0.664				cGVHD C-index=0.561			
	aHR	95% CI		*P*	aHR	95% CI		*P*
First CMV* vs none	1.59	1.06	2.39	0.02	1.05	0.80	1.37	0.73
First HHV-6* vs none	1.55	0.85	2.80	0.15	1.07	0.69	1.67	0.76
Other viral infect* vs none	1.21	0.68	2.14	0.52	0.70	0.41	1.20	0.19
Age, 10 years	1.02	0.95	1.09	0.57	1.05	0.99	1.12	0.13
Malignant	1.25	0.89	1.75	0.20	1.26	0.91	1.75	0.16
HLA mismatched	1.75	1.32	2.33	<0.0001	1.32	1.01	1.69	0.04
Unrelated donor	1.72	1.37	2.17	<0.0001	1.37	1.11	1.69	0.003
TBI	1.22	0.94	1.58	0.14	0.90	0.71	1.14	0.38
CBT	0.90	0.54	1.50	0.69	0.54	0.31	0.94	0.03

Specific FOVIs and all variables in Table 1 were forward selected in an inverse probability weighted Cox regression model for either aGVHD or cGVHD.

aGVHD, acute graft-versus-host disease; aHR, adjusted hazard ratio; CBT, umbilical cord blood transplant; cGVHD, chronic graft-versus-host disease; CI, confidence interval; C-index, concordance index; CMV, cyclomegalovirus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

Table E6. Multivariable analysis of FOVI types and other risk factors for time to grade II-IV aGVHD or cGVHD adjusted by year of HCT.

Multivariable analysis	Grade II-IV aGVHD C-index=0.670				cGVHD C-index=0.576			
	aHR	95% CI		*P*	aHR	95% CI		*P*
First CMV* vs none	1.803	1.213	2.681	0.004	1.138	0.864	1.497	0.358
First HHV-6* vs none	1.828	1.02	3.279	0.043	1.165	0.776	1.75	0.461
Other viral infect* vs none	1.371	0.782	2.402	0.27	0.797	0.464	1.368	0.41
Age, 10 years	1.045	0.975	1.119	0.215	1.064	0.997	1.136	0.061
Malignant	1.215	0.87	1.696	0.254	1.241	0.895	1.72	0.196
HLA mismatched	0.591	0.445	0.785	<0.0001	0.779	0.605	1.004	0.054
Unrelated donor	0.59	0.466	0.747	<0.0001	0.742	0.6	0.917	0.006
TBI	1.339	1.024	1.749	0.033	0.933	0.737	1.182	0.567
CBT	1.026	0.619	1.702	0.92	0.579	0.332	1.01	0.054
Year of HCT	0.893	0.841	0.947	<0.0001	0.923	0.872	0.976	0.005

Specific FOVIs and all variables in Table 1 were forward selected in an inverse probability weighted Cox regression model for either aGVHD or cGVHD.

aGVHD, acute graft-versus-host disease; aHR, adjusted hazard ratio; CBT, umbilical cord blood transplant; cGVHD, chronic graft-versus-host disease; CI, confidence interval; C-index, concordance index; CMV, cyclomegalovirus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

Table E7. Clinical characteristics and demographics of patients developed IPS or BOS

Characteristics	Patients with IPS (N = 41)	Patients with BOS (N = 49)
Age at HCT, median (range), years	45 (1,67)	52 (10,68)
Median follow-up time post-HCT, weeks (95% CI)*	34.0 (20.9-153.9)	189 (136-269)
Sex, n (%)		
Male	28 (68)	30 (61)
Female	13 (32)	19 (39)
Indication, n (%)		
Malignant	34 (83)	42 (86)
Nonmalignant	7 (17)	7 (14)
HLA disparity, (n%)		
Matched	27 (66)	37 (76)
Mismatched	14 (34)	12 (24)
Donor relationship, n (%)		
Related	16 (39)	21 (43)
Unrelated	25 (61)	28 (57)
Number of HCT, n (%)		
First	36 (88)	45 (92)
Second/Third	5 (12)	4 (8)
Conditioning by irradiation, n (%)		
TBI-Based	13 (32)	12 (24)
Chemotherapy-Based	28 (68)	37 (76)
Conditioning by intensity, n (%)		
Myeloablative	30 (73)	33 (67)
Nonmyeloablative	11 (27)	16 (33)
Graft Source, n (%)		
PB	30 (73)	44 (90)
BM	5 (12)	3 (6)
CB	4 (10)	2 (4)

Values are indicated as the number (percentage), except ages at HCT and follow up time as specified.

BM, bone marrow; BOS, bronchiolitis obliterans syndrome; CB, umbilical cord blood; CI, confidence interval; HCT, stem cell transplantation; HLA, human leukocyte antigen; IPS, idiopathic pneumonia syndrome; PB, peripheral blood; TBI, total body irradiation.

* Median follow-up time post-HCT and the corresponding 95% confidence interval was estimated by a Kaplan-Meier method

Table E8. Univariate inverse-weighted Cox regression analysis of risk factors for time from HCT to IPS and BOS, respectively.

	IPS				BOS			
	HR	95% CI		*P*	HR	95% CI		*P*
First CMV* vs none	1.78	0.57	5.56	0.32	2.70	1.42	5.13	<0.01
First HHV-6* vs none	6.96	2.90	16.66	<0.01	2.30	0.89	5.94	0.09
First EBV* vs none	11.10	3.13	39.32	<0.01	1.94	0.45	8.38	0.37
First CARV* vs none	3.54	0.74	17.03	0.12	--	--	--	--
First HSV* vs none	11.13	2.54	48.85	<0.01	3.39	0.49	23.63	0.22
Age, 10 years	0.86	0.74	1.00	0.06	1.05	0.90	1.23	0.57
Male	1.49	0.77	2.89	0.23	1.05	0.57	1.93	0.88
Malignant	0.91	0.40	2.07	0.83	1.35	0.59	3.08	0.48
HLA mismatched	2.00	1.05	3.85	0.03	1.75	0.88	3.45	0.11
CBT	2.61	1.09	6.26	0.03	0.80	0.19	3.30	0.76
Unrelated donor	1.49	0.80	2.78	0.21	1.39	0.76	2.50	0.28
TBI	1.57	0.82	2.98	0.17	0.81	0.41	1.62	0.56

BOS, bronchiolitis obliterans syndrome; CARV, community acquired respiratory viruses; CBT, umbilical cord blood transplant; CI, confidence interval; CMV, cyclomegalovirus; EBV, Epstein-Barr virus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; HSV, herpes simplex virus; HR, hazard ratio; IPS, idiopathic pneumonia syndrome; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

+: No CARV infection up to the first 100 day post-HCT in patients who developed BOS

Table E9. Multivariable inverse weighted analysis of total FOVIs and other risk factors for time from HCT to IPS and BOS, respectively

Multivariable analysis	IPS C-index= 0.721				BOS C-index=0.633			
	aHR	95% CI		*P*	aHR	95% CI		*P*
FOVIs	3.97	1.91	8.25	<0.001	2.13	1.19	3.85	0.012
Age, 10 years	0.88	0.76	1.02	0.08	1.02	0.87	1.20	0.77
HLA mismatched	1.47	0.64	3.34	0.36	1.66	0.82	3.37	0.16
CBT	0.99	0.32	3.03	0.98	0.52	0.12	2.30	0.39

FOVI status is a time-dependent categorical predictor. The additional three variables were forward selected in an inverse probability weighted Cox regression model for either IPS or BOS.

aHR, adjusted hazard ratio; BOS, bronchiolitis obliterans syndrome; CBT, umbilical cord blood transplant; CI, confidence interval; C-index, concordance index; FOVI, first onset viral infection; HCT, stem cell transplantation; HLA, human leukocyte antigen; IPS, idiopathic pneumonia syndrome.

Table E10. Multivariable inverse weighted analysis of risk factors for time from HCT to IPS or BOS adjusted by year of HCT

Multivariable analysis	IPS C-index= 0.761				BOS C-index=0.663			
	aHR	95% CI		*P*	aHR	95% CI		*P*
First CMV* vs none	2.28	0.65	7.99	0.20	2.88	1.47	5.64	0.002
First HHV-6* vs none	7.03	2.19	22.50	0.001	2.20	0.81	5.99	0.12
First EBV* vs none	14.25	3.59	56.56	<0.001	1.60	0.34	7.50	0.55
First HSV* vs none	12.40	2.49	61.80	0.002	4.98	0.78	31.68	0.09
First CARV* vs none	3.83	0.86	17.10	0.08	--†	--†	--†	--†
Age, 10 years	0.90	0.77	1.06	0.21	1.01	0.86	1.18	0.91
HLA mismatched	0.73	0.31	1.68	0.45	0.59	0.30	1.15	0.12
CBT	1.19	0.31	4.62	0.80	0.51	0.12	2.11	0.35
Year of HCT	0.78	0.64	0.95	0.01	1.00	0.86	1.16	1.00

FOVI status is a time-dependent categorical predictor. The additional three variables were forward selected in an inverse probability weighted Cox regression model for either IPS or BOS.

aHR, adjusted hazard ratio; BOS, bronchiolitis obliterans syndrome; CARV, community acquired respiratory viruses; CBT, umbilical cord blood transplant; CI, confidence interval; C-index, concordance index; CMV, cyclomegalovirus; EBV, Epstein-Barr virus; FOVI, first onset viral infection; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; HSV, herpes simplex virus; IPS, idiopathic pneumonia syndrome; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

+: No CARV infection up to the first 100 day post-HCT in patients who developed BOS

Table E11. Multivariable inverse weighted analysis of risk factors for time from HCT to IPS or BOS adjusted by GVHD

Multivariable analysis	IPS C-index= 0.737				BOS C-index=0.691			
	aHR	95% CI		*P*	aHR	95% CI		*P*
First CMV* vs none	1.79	0.53	5.99	0.34	2.91	1.51	5.59	0.001
First HHV-6* vs none	6.04	1.67	21.77	0.006	2.09	0.75	5.83	0.16
First EBV* vs none	9.31	2.66	32.57	0.0004	1.97	0.42	9.36	0.39
First HSV* vs none	10.6	2.55	43.99	0.001	5.24	0.88	31.3	0.07
First CARV* vs none	3.28	0.75	14.38	0.11	--	--	--	--
Age, 10 years	0.9	0.77	1.06	0.20	0.99	0.84	1.17	0.9
HLA mismatched	1.49	0.6	3.69	0.39	1.54	0.77	3.11	0.23
CBT	0.88	0.2	3.77	0.86	0.61	0.15	2.55	0.5
Acute/Chronic GVHD	0.72	0.32	1.64	0.44	3.64	1.27	10.44	0.02

FOVI status is a time-dependent categorical predictor. The additional three variables were forward selected in an inverse probability weighted Cox regression model for either IPS or BOS.

aHR, adjusted hazard ratio; BOS, bronchiolitis obliterans syndrome; CARV, community acquired respiratory viruses; CBT, umbilical cord blood transplant; CI, confidence interval; C-index, concordance index; CMV, cyclomegalovirus; EBV, Epstein-Barr virus; FOVI, first onset viral infection; GVHD, graft-versus-host disease; HCT, stem cell transplantation; HHV-6, human herpesvirus 6; HLA, human leukocyte antigen; HSV, herpes simplex virus; IPS, idiopathic pneumonia syndrome; TBI, total body irradiation.

* Infections modeled with time-dependent covariates through the first 100 days

+: No CARV infection up to the first 100 day post-HCT in patients who developed BOS

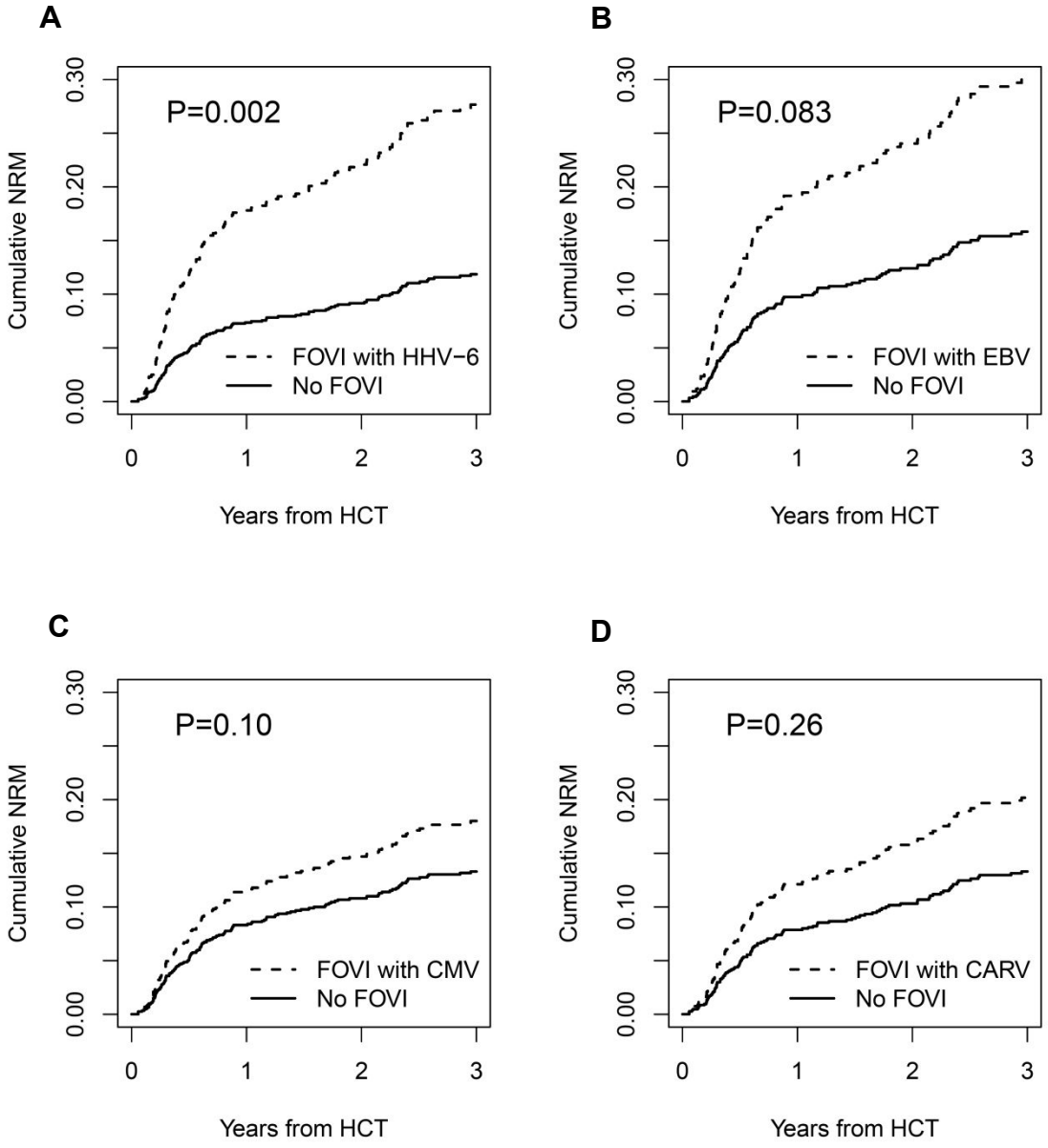
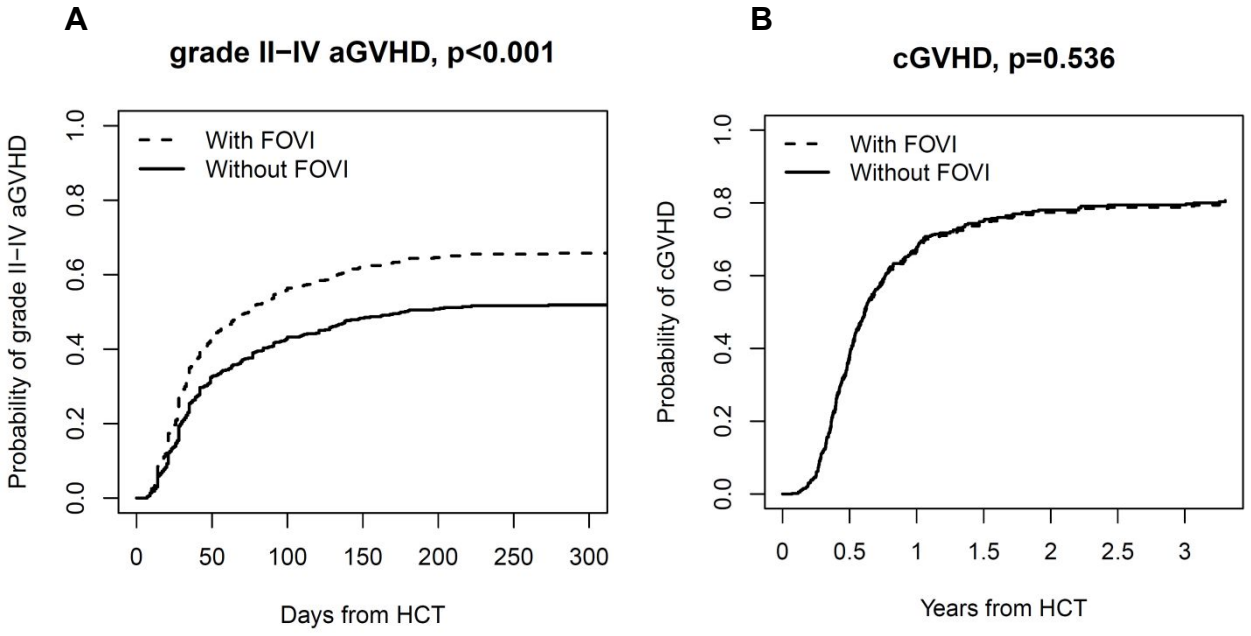


Figure E1. NRM by specific types of FOVI. Inverse weighted cumulative incidence of NRM stratified by FOVI with HHV-6 (aHR, 2.57; 95% CI, 1.43-4.62; *P* = 0.0016) (A), EBV (aHR, 2.08; 95% CI, 0.91-4.74; *P* = 0.083) (B), CMV (aHR, 1.39; 95% CI, 0.94-2.07; *P* = 0.10) (C), or CARV (aHR, 1.58; 95% CI, 0.72-3.47; *P* = 0.10) (D). Infections modeled with time-dependent covariates through the first 100 days. Hazard ratios are adjusted for age, gender, time-dependent aGVHD, underlying diagnosis, HLA disparity, source of hematopoietic cells, donor relationship and conditioning regiment.



Number of patients at risk

Days from HCT	0	100	200	300
With FOVI	0	70	45	39
Without FOVI	738	240	175	160

Number of patients at risk

Yrs from HCT	0	0.5	1.5	2.5
With FOVI	0	93	24	16
Without FOVI	738	238	80	62

Figure E2. Cumulative incidence of grade II-IV aGVHD and cGVHD by FOVIs. In our study cohort, 351 patients (47.6%) developed grade II to IV aGVHD and 414 patients (56.1%) developed chronic GVHD (cGVHD) after transplantation. Viral infections within 100 days post-HCT were modeled as time-dependent covariates. The curves are adjusted for age, underlying diagnosis, HLA disparity, source of hematopoietic cells, donor relationship and conditioning regimen. (A) Inverse weighted cumulative incidence of grade II-IV aGVHD in days after HCT stratified by FOVIs. Number of patients at risk of developing aGVHD at each time point is presented under the plot. (B) Inverse-weighted cumulative incidence of cGVHD in years after HCT by FOVIs. Number of patients at risk of developing cGVHD at each time point is presented under the plot.

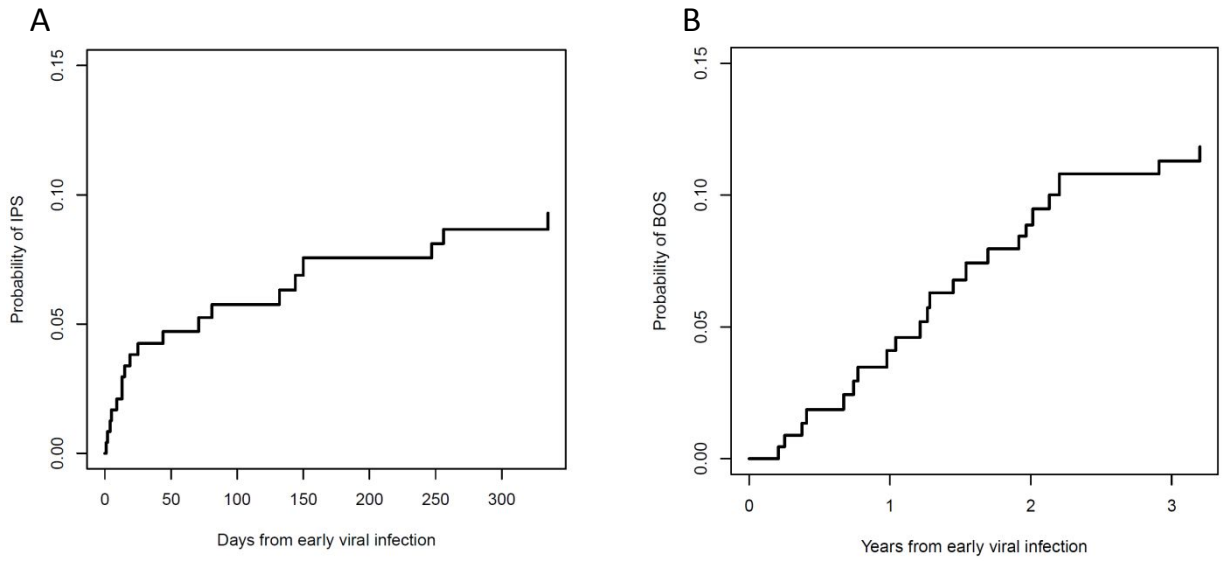


Figure E3. Cumulative incidence of IPS and BOS post early viral infection. A. Inverse-weighted cumulative incidence of IPS in days after early viral infection/reactivation. B. Inverse-weighted cumulative incidence of BOS in years after early viral infection/reactivation.

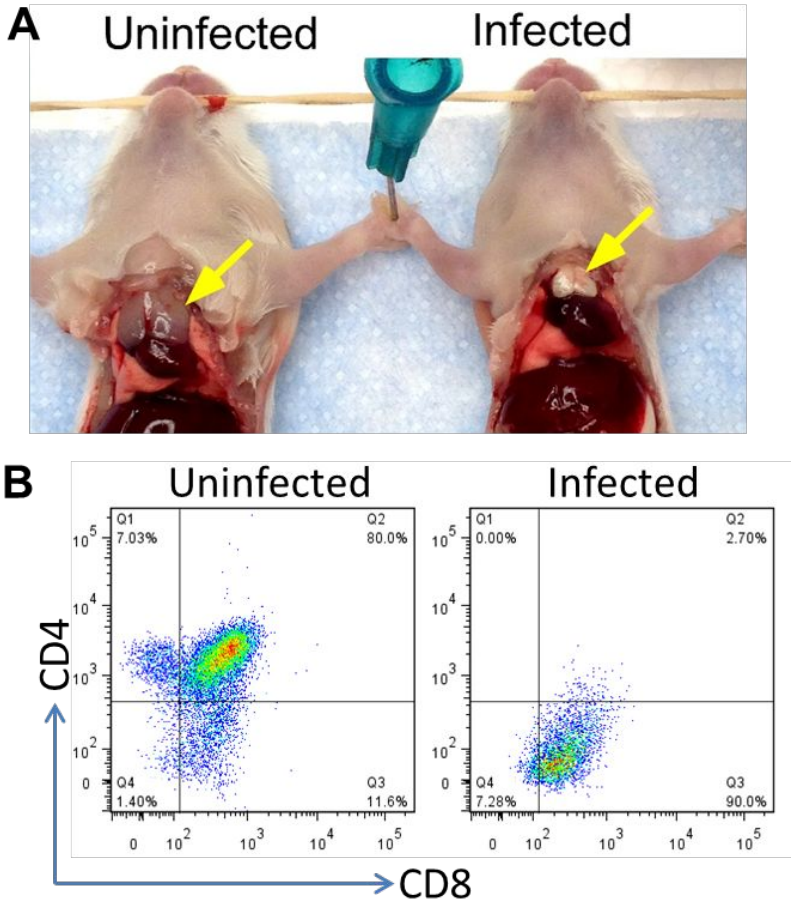


Figure E4. Phenotype of MRV primary infection. A. Macroscopic necrosis of the thymus (yellow arrow) of infected pup vs. uninfected pup. B. Flow cytometry analysis of thymocytes gated on live T cells. Two-color histograms for CD4 and CD8 expression.