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Characterization of Viral Infections after Antithymocyte Globulin–Based Conditioning in Adults Undergoing Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Antithymocyte globulin (ATG) has been shown to reduce the incidence of graft-versus-host-disease (GVHD) after matched related donor (MRD) and matched unrelated donor (MUD) hematopoietic stem cell transplantation (HCT); however, because of increased risks of infection and relapse, this use has not translated into a significant improvement in post-transplant survival. The goal of this single-center, retrospective cohort analysis was to quantify the incidence of viral reactivation and viral end-organ disease (EOD) within the first 100 days after MUD HCT with ATG-based conditioning compared with MRD HCT without ATG. Fifty-nine adult patients underwent ATG-based MUD HCT compared with 64 patients receiving MRD HCT without ATG. Cytomegalovirus reactivation was the most frequent event in both groups (65% MUD versus 61% MRD), followed by BK virus reactivation (26% versus 24%) and Epstein-Barr virus reactivation (20% versus 9%). A higher percentage of MUD patients experienced viral EOD by day +100 when compared with MRD patients (34% versus 16%, $P = .022$). This was most notable for EOD involving BK virus (15% versus 6%, $P = .14$) and Epstein-Barr virus (7% versus 0%, $P = .050$). Correspondingly, more patients in the MUD group experienced virus-related complications, including hospitalization (24% versus 3%, $P < .001$), intensive care unit admission (10% versus 6%, $P = .19$), and mortality (8% versus 4%, $P = .44$). There were no significant differences in either relapse-free survival (RFS; 62% versus 78%, $P = .07$) or overall survival (OS; 72% versus 86%, $P = .07$) at 6 months post-HCT. However, when using the final time point of 21 months in the MUD/ATG group and 23 months in the MRD/no ATG group, MUD patients who received ATG had inferior survival (OS: 27% versus 77%, $P = .009$; RFS: 40% versus 59%, $P = .042$). Our results add to and further quantify the infectious risks associated with the use of ATG in MUD transplants and promote the implementation of more intensive preemptive viral monitoring practices in patients receiving ATG-based MUD transplants.

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INTRODUCTION

Despite improvements in HLA matching and supportive care measures, graft-versus-host-disease (GVHD) remains a significant source of morbidity and mortality for patients undergoing allogeneic hematopoietic stem cell transplantation (HCT). Use of peripheral blood grafts in addition to the emergence of alternative donor transplantation have contributed to the persistent burden of this complication in the modern era. Antithymocyte globulin (ATG), an in vivo method of T cell depletion, has been shown to reduce the incidence of acute and chronic GVHD when given to patients undergoing HLA-matched related donor (MRD) and matched unrelated donor (MUD) HCT in multiple randomized controlled trials [1-7]. Consequently, consensus guidelines published in 2013 by the European Society for Blood and Marrow Transplantation and the European Leukemia Net recommends the use of ATG-based GVHD prophylaxis for all patients undergoing unrelated donor HCT [8].

Despite reductions in GVHD and associated improvements in quality of life, use of ATG has not been shown to improve post-HCT survival. This may be explained by increased rates of disease relapse and a higher frequency of infections after the use of ATG; however, these associations have been inconsistently

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demonstrated in prospective trials [1-7]. Use of varying ATG formulations also precludes direct study comparison, especially given evidence of differing mechanisms of action, effects on T regulatory cells, and depth of lymphopenia between horse-derived (eg, ATGAM, Pfizer, New York, NY) and rabbit-derived (eg, Thymoglobulin, Sanofi, Cambridge, MA; ATG-Fresenius, Neovii Biotech, Rapperswil, Switzerland) products.

In spite of heterogeneous designs, studies have repeatedly demonstrated an association between use of ATG and viral infections, most notably involving cytomegalovirus (CMV) and Epstein-Barr virus (EBV) [3,9-12]. Limited data exist to quantify the incidence of other clinically relevant viruses associated with significant morbidity, including adenovirus (ADV), BK virus (BKV), human herpes virus-6 (HHV-6), herpes simplex virus (HSV), and varicella-zoster virus (VZV). Additionally, the occurrence of tissue-invasive viral end-organ disease (EOD) resulting in hospitalization, organ damage, and/or death is infrequently reported. These outcomes are relevant, as evidenced by a recent retrospective study suggesting that a patient's cumulative exposure burden to double-stranded DNA viruses after HCT is independently associated with both early and late mortality [13]. Furthermore, no consensus recommendations guide diagnosis, antiviral drug selection, and treatment duration for all clinically relevant viruses. This has translated into widely variable monitoring, prophylaxis, and treatment practices among transplant providers.

This retrospective study was conducted to quantify the incidence of viral reactivation and EOD within the first 100 days after MUD HCT in those who received ATG. These patients were compared with a cohort of patients who underwent MRD HCT using similar conditioning regimens without ATG to determine the influence of ATG on other outcomes, including acute GVHD, time to neutrophil engraftment and platelet recovery, infection-related mortality, relapse-free survival (RFS), and overall survival (OS).

METHODS

This was a retrospective review of patients ages ≥ 18 years who underwent HLA MRD or MUD allogeneic HCT at The University of Texas MD Anderson Cancer Center from March 2016 through June 2017. After obtaining approval from the Investigational Review Board, patients were retrospectively identified using the electronic medical record. Patients who underwent MUD HCT received prophylactic rabbit ATG (Thymoglobulin) in addition to tacrolimus and either mini-methotrexate (5 mg/m^2 on days +1, +3, +6, and +11) or mycophenolate mofetil (1000 mg p.o. every 8 hours starting on day −3). ATG was given at a total dose of 4 mg/kg, administered as .5, 1.5, and 2 mg/kg i.v. on days −3, −2, and −1, respectively, before stem cell infusion on day 0. Those who underwent MRD HCT received tacrolimus plus either methotrexate or mycophenolate mofetil. Both MUD and MRD HCT patients received valacyclovir 500 to 1000 mg p.o. daily starting on day −1 through at least 1 year after allogeneic HCT per institutional protocol, along with standard antibacterial and antifungal agents. All patients, regardless of donor/recipient serostatus, were preemptively screened for CMV reactivation by serum nucleic acid amplification testing 1 to 2 times weekly until day +100. No patients received prophylactic antivirals for CMV (eg, ganciclovir, valganciclovir, letermovir). Patients were treated for CMV preemptively per institutional protocol. MUD recipients who received ATG were treated for CMV at a lower serum PCR threshold (500 copies/mL) compared with MRD recipients who did not receive ATG (1000 copies/mL). Testing for other viruses was only performed in those patients presenting with symptoms suggestive of a viral etiology. Treatment for these other viruses was based on physician discretion, often with guidance from infectious disease specialists. Exclusion criteria included HLA-mismatched or haploidentical transplant, cord blood graft source, previous allogeneic HCT, or use of post-transplant cyclophosphamide (PTCy) as GVHD prophylaxis.

Primary outcomes of interest included cumulative incidence rates for viral reactivation and EOD occurring within the first 100 days after MUD and MRD HCT. Viruses of interest included CMV, EBV, BKV, HSV, VZV, ADV, and HHV-6. Reactivation was defined as any detection of viral DNA from bodily fluids using PCR assays or the detection of virus via positive staining of tissue biopsy samples. EOD was defined as viral reactivation associated with concurrent organ dysfunction (Table 1). Patients with concurrent nonviral etiologies of EOD (eg, veno-occlusive disease, GVHD, thrombotic microangiopathy) were not classified as having viral EOD for the purposes of this analysis. Several noninfectious outcomes were also assessed, including time to neutrophil engraftment, incidence of acute GVHD ≥ grade II by day +100 using the modified Glucksberg criteria [14], RFS, and OS.

Demographic characteristics and clinical outcomes were summarized and compared between patients who received ATG and those who did not. Group differences for continuous measures were evaluated using Wilcoxon's rank sum test, whereas categorical measures were assessed by Fisher's exact test or its generalization. RFS and OS were estimated using the Kaplan-Meier method, and patients who were still alive and did not experience disease progression were censored for RFS, whereas those who were still alive were censored for OS. Cumulative incidences of time to viral reactivation were constructed, and patients who did not experience a viral reactivation were censored. Group differences in survival and time to viral reactivation were determined using the log-rank test.

RESULTS

One hundred twenty-three patients met inclusion criteria and were included in the analysis; of these, 59 (48%) underwent MUD HCT with use of ATG and 64 (52%) underwent MRD HCT without ATG. Demographic characteristics (Table 2) were similar in terms of patient age, pre-HCT disease remission status, conditioning regimen intensity, high-dose steroid use, and backbone GVHD prophylaxis regimen. Regarding underlying malignancy type the 2 groups were also similar, except for a

Table 1
Definitions of viral EOD

Virus	Viral Detection Associated with Concurrent Organ Dysfunction	
	Method(s) of Virus Detection	Concurrent Organ Dysfunction
ADV	• Positive IHC staining of tissue biopsy	• Pneumonitis: requirement for mechanical ventilation
	• Positive PCR analysis of BAL specimen	• Hepatitis: serum bilirubin > 2 mg/dL and/or transaminases > 3 times upper limit of normal
BKV	• Positive PCR analysis of urine	• Cystitis: dysuria, hematuria, increased frequency
	• Positive IHC staining of renal biopsy	• Nephritis: serum creatinine > 2 mg/dL or >1.5 times baseline value
CMV	• Positive IHC staining of tissue biopsy	• Colitis: diarrhea
	• Positive PCR analysis of BAL specimen	• Pneumonitis: requirement for mechanical ventilation
		• Retinitis: new visual disturbances
EBV	• Positive PCR analysis of serum	• Post-transplant lymphoproliferative disorder: new or worsened lymphadenopathy confirmed by positron emission tomography
HHV-6	• Positive PCR analysis of CSF	• Encephalitis: altered mental status, seizures
HSV	• Positive PCR analysis of CSF or oral swab specimen	• Encephalitis: altered mental status, seizures
		• Oral ulceration
VZV	• Positive PCR analysis of CSF	• Encephalitis: altered mental status, seizures

IHC indicates immunohistochemical; BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

Table 2
Patient and Clinical Characteristics (N = 123).

Characteristic	MUD/ATG (n = 59)	MRD/No ATG (n = 64)	*P*
Median age, yr (range)	54 (19-77)	54 (21-72)	.59
Male gender	34 (58)	35 (55)	.86
Underlying malignancy			
AML	24 (41)	24 (38)	.11
ALL	7 (12)	9 (14)	
Myelodysplastic syndrome	4 (7)	8 (13)	
Chronic lymphocytic leukemia	7 (12)	3 (5)	
Myeloproliferative disorder	6 (10)	5 (8)	
Multiple myeloma	5 (8)	3 (5)	
Lymphomas	2 (3)	11 (17)	
Other	4 (7)	1 (2)	
Pretransplant disease status			
Complete remission	45 (76)	49 (77)	1.00
Non–complete remission	14 (24)	15 (23)	
Donor/recipient CMV serostatus			
D–/R–	3 (5)	7 (11)	.036
D+/R–	3 (5)	3 (5)	
D–/R+	33 (56)	20 (31)	
D+R+	20 (34)	34 (53)	
Donor/recipient gender			
M/M	28 (47)	16 (25)	<.001
M/F	19 (32)	12 (19)	
F/M	5 (8)	18 (28)	
F/F	7 (12)	18 (28)	
Conditioning regimen intensity			
Myeloablative	36 (61)	38 (59)	.59
Reduced intensity	19 (32)	24 (38)	
Nonmyeloablative	4 (7)	2 (3)	
Median cell dose (range)	7.6 (2.9-21.3)	5.4 (2.4-13.1)	<.001
Median pre-ATG ALC (range)	80 (0-670)	N/A	
GHVD prophylaxis regimen			
Tacrolimus/ methotrexate	56 (95)	56 (88)	.21
Tacrolimus/ mycophenolate	3 (5)	8 (13)	
Use of high-dose steroids (≥1 mg/kg prednisone) for acute GVHD Values are n (%) unless otherwise defined.	18 (53)	17 (63)	.45

higher number of patients with lymphoma noted in the MRD group (17% versus 3%). Additionally, there were significant between-group differences in donor–recipient CMV serostatus and donor–recipient gender. Patients in the MUD/ATG group also received a higher cell dose than those in the MRD/no ATG group (median, 7.6 versus 5.4 × 10^6 cells/kg; $P < .001$). Median follow-up time for the entire cohort was 12.8 months (range, 1.0 to 23.1), for the MUD/ATG group was 9.8 months (range, 1.0 to 21.0), and for the MRD/no ATG group 14.4 months (range, 2.3 to 23.1).

Analysis of viral infection-related outcomes (Table 3) revealed that a higher percentage of MUD patients who received ATG experienced viral EOD by day +100 when compared with MRD patients who did not receive ATG (34% versus 16%, $P = .022$). This elevated risk was most notable for EOD involving BKV (15% versus 6%, $P = .14$) and EBV (7% versus 0%, $P = .050$) (Table 4). Correspondingly, more patients in the MUD group experienced complications related to viral infections, including hospitalization (24% versus 3%, $P < .001$), intensive care unit admission (10% versus 6%, $P = .19$), and mortality (8% versus 4%, $P = .44$). When examined by specific virus (Table 4, Figure 1), CMV reactivation was the most frequent event (65% versus 61%), followed by BKV reactivation (26% versus 24%) and EBV reactivation (20% versus 9%). Events involving ADV, HHV-6, and HSV were infrequent in both groups, each occurring in less than 10%. No patients in either group experienced VZV reactivation or EOD.

Analysis of noninfectious outcomes (Table 5) did not reveal any statistically significant differences in the incidence of grade ≥ II acute GVHD (40% versus 38%, $P = .61$) or median time to engraftment of either neutrophils or platelets. Analysis of survival outcomes at 6 months post-HCT revealed no significant differences in either RFS (62% versus 78%, $P = .07$) or OS (72% versus 86%, $P = .07$), However, when using the final time point of 21 months in the MUD/ATG group and 23 months in the MRD/no ATG group, MUD patients who received ATG had inferior survival (OS: 27% versus 77%, $P = .009$ [Figure 2]; RFS: 40% versus 59%, $P = .042$).

Because of higher rates of reactivation and EOD, a higher percentage of MUD patients who received ATG were also treated with nonprophylactic antiviral therapy (Table 6) within the first 100 days of transplant compared with those in the MRD group (86% versus 34%, $P < .001$). This most frequently entailed use of foscarnet (39% versus 35%, $P = .12$) and ganciclovir/valganciclovir (34% versus 8%, $P < .001$), with only sporadic use of acyclovir/valacyclovir (5% versus 2%, $P = .35$) and cidofovir (9% versus 0%, $P = .023$).

DISCUSSION

Consistent with previous reports, use of ATG in the current analysis was associated with a higher risk of viral infections and associated complications. Most notably, a statistically

Table 3
Infectious Outcomes

Outcome	MUD/ATG (n = 59)	MRD/No ATG (n = 64)	*P*
Incidence of any viral reactivation by day +100	48 (81)	44 (72)	.28
Median number of viral infections by day +100 (range)	1 (0-4)	1 (0-4)	.14
Incidence of any viral EOD by day +100	20 (34)	10 (16)	.022
Viral illness–related hospitalization by day +100	14 (24)	2 (3)	<.001
Viral illness–related intensive care unit admission by day +100	6 (10)	4 (6)	.52
Six-month viral infection–related mortality Values are n (%) unless otherwise defined.	5 (8)	2 (4)	.44

Table 4
Outcomes by Virus Through Day +100

	Cumulative Incidence of Reactivation (%)			Incidence of EOD (%)		
Virus	MUD/ATG (n = 59)	MRD/No ATG (n = 64)	*P*	MUD/ATG (n = 59)	MRD/No ATG (n = 64)	*P*
CMV	65	61	.19	8	8	1.00
EBV	20	9	.08	7	0	.050
BKV	26	24	.86	15	6	.14
ADV	5	0	.08	5	0	.11
HHV-6	6	7	.71	0	2	1.00
HSV	9	4	.25	3	2	.61

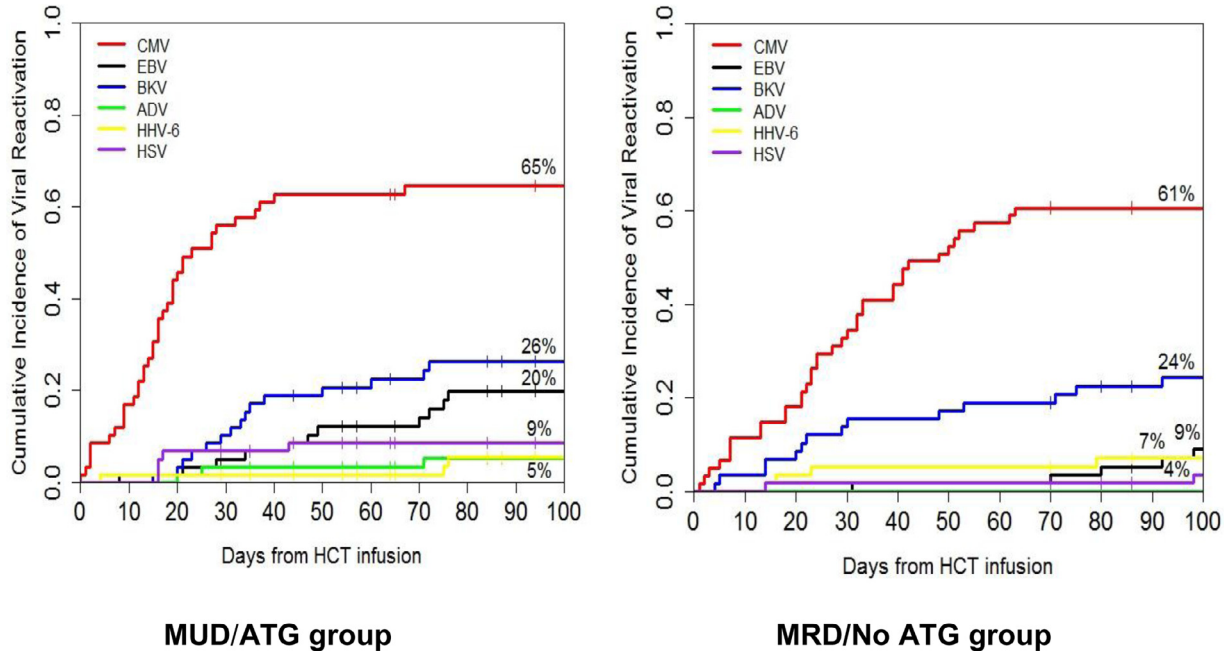

Figure 1. Cumulative incidence of reactivation by virus.

Table 5
Noninfectious Outcomes

Outcome	MUD/ATG (n = 59)	MRD/No ATG (n = 64)	*P*
Median time to neutrophil engraftment, days (range)	11 (0-25)	11 (0-44)	.35
Median time to platelet engraftment, days (range)	12 (0-322)	12 (0-163)	.54
Cumulative incidence of acute GVHD ≥ grade II, %	40	38	.61
6-month RFS, %	62	78	.07
RFS rate (end of assessment period), %	40	59	.042
6-month OS, %	72	86	.07
OS rate (end of assessment period), %	27	77	.009

significant 2.1-fold higher risk of viral EOD was noted in those who received ATG versus those who did not. Although EBV was the only statistically significant virus to reactivate and cause EOD, patients in the MUD/ATG group did have more nonprophylactic antiviral use, which may be attributed to our institutional protocol that indicates treatment for lower viral thresholds in the MUD/ATG recipients versus MRD/No ATG recipients. Additionally, a higher percentage of patients in the MUD/ATG group required hospitalization secondary to viral infections within the first 100 days after HCT. Although there was no significant difference in the 6-month OS between groups, the OS at the final time points did reveal a statistically significant difference (27% versus. 77%, *P* = .009), possibly related to the long-term immunosuppressive effects of ATG. Virus-specific results confirmed previously reported findings of increased CMV and EBV reactivation with ATG use; however, the current analysis additionally found increased reactivation rates with BKV, ADV, and HSV [3,9-12].

Similar to our study, Kaminski et al. [15] retrospectively evaluated viral infection outcomes in 250 patients who

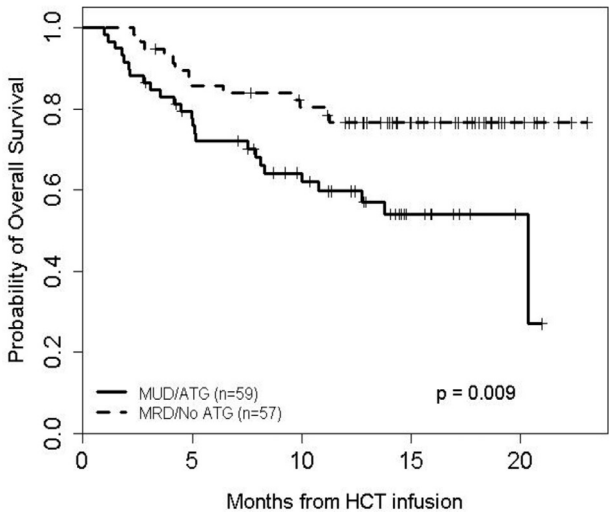

Figure 2. Overall survival.

underwent MUD HCT with ATG or MRD HCT without ATG. Use of ATG in that analysis was also associated with increased incidence of infections involving CMV (112 versus 61 cases), HHV-6 (47 versus 13 cases), and HSV (32 versus 8 cases) by day +180. When further subdivided according to conditioning regimen intensity, ATG patients who received reduced-intensity conditioning versus myeloablative conditioning had a substantially higher risk of viral infections (CMV, 2.5- versus 1.5-fold; HHV-6, 10.5- versus 2.4-fold; HSV, 6.5- versus 3.2-fold). In our analysis, there was no association between conditioning regimen intensity (myeloablative, reduced intensity, and nonmyeloablative) and viral infection reactivation in the MUD/ATG group and MRD/no ATG group.

Although studied using ex vivo $CD34^+$-selected peripheral blood grafts, Huang et al. [16] more recently published their findings in a prospective observational study of 156 adults with myelodysplastic syndrome or acute leukemia who received HCT. All patients were preemptively monitored for CMV, ADV, HHV-6, and EBV viremias through day +180 via serum PCR analysis. Cumulative viremia incidence rates were 44%, 61%, 7%, and 16% for CMV, HHV-6, ADV, and EBV, respectively. Our analysis revealed a much higher rate of CMV reactivation in the MUD/ATG group (65%), which may be reflective of the higher percentage of CMV-seropositive recipients in our study (90% versus 62%). It is well described that CMV serostatus is 1 of the most important risk factors in predicting CMV reactivation, with recipient positivity being the highest risk group [17]. This could have driven other viral-related morbidity in our analysis. Additionally, in contrast to this study, the substantially lower rate of HHV-6 reactivation found in our analysis may be due to the lack of preemptive monitoring for this pathogen at our institution. Viral EOD at 1 year was reported in 18% of patients after ex vivo TCD HCT, which is substantially lower than reported in our analysis at 100 days post-HCT with ATG (34%). Although these results may suggest improved outcomes with the use of ex vivo versus in vivo TCD, a direct comparison cannot be made because of variations in EOD definitions and study populations; however, it is possible that preemptive monitoring of all clinically relevant double-stranded DNA viruses could aid in the detection of reactivation episodes before progression to symptomatic EOD. At our center, testing for viruses other than CMV in the allogeneic MRD and MUD setting is performed only on clinical suspicion of infection (eg, hematuria, new-onset cytopenias). Additional routine monitoring for other double-stranded DNA viruses in those who receive ATG may reduce the incidence of EOD and should be evaluated in future studies.

Despite decades of ATG use in the setting of HCT, an optimal dosing regimen to limit overexposure has not been established; however, several studies assessing alternative ATG dosing to lower infection incidence have been published. Bryant et al. [18] retrospectively examined the use of low-dose ATG (2.5 mg/kg total, given as .5 mg/kg on day −2 and 2 mg/kg on day −1) in 110 patients who received a MUD HCT. When these patients were directly compared with 77 patients who underwent MRD HCT without ATG, the low-dose ATG regimen was associated with a statistically significant improvement in GVHD-free RFS at 2 years (23% versus 3%, $P = .003$) yet with no difference in the incidence of overall viral infections (52% versus 45%, $P = .32$) or CMV infection (25% versus 25%, $P = 1.00$). Similar studies examining lower doses of another ATG product (ATG-Fresenius) have reported similar relationships for CMV and EBV infection [19,20]. Prospective, randomized ATG dose comparison studies are needed to determine if the use of lower doses can reduce infectious complications without compromising efficacy.

The concept of ATG dose personalization according to pre-ATG absolute lymphocyte count (ALC) has also been addressed by recent publications. Kennedy et al. [21] performed a retrospective study of 135 patients who received prophylactic ATG at 1 of 3 doses (10 mg/kg, 7.5 mg/kg, or 5 mg/kg) before MUD HCT. A nonsignificant trend for increased 2-year CMV reactivation rates was noted with higher ATG doses (54% versus 47% versus 41%). Notably, a multivariate analysis revealed that median ALC on the first day of ATG administration and the total amount of ATG interacted to predict OS (hazard ratio, .09; $P = .03$). For those with low ALC (<56 cells/μL), a higher total dose of ATG was independently associated with a greater risk of death. Admiraal et al. [22] recently developed an ALC-based ATG dosing equation derived from a population-based pharmacokinetic model as an alternative to traditional weight-based dosing. All patients included in our study received an empiric weight-based ATG dose of 4 mg/kg; our exploratory analysis did not reveal any associations between pre-ATG ALC

Table 6
Nonprophylactic Antiviral Use

Drug	Cumulative Usage n (%)		Median Duration of Therapy days (range)	
	MUD/ATG (n = 59)	MRD/No ATG (n = 64)	MUD/ATG (n = 59)	MRD/No ATG (n = 64)
Acyclovir/valacyclovir	3 (5)	1 (2)	15 (7-22)	8 (N/A)
Ganciclovir/valganciclovir	20 (34)	5 (8)	29 (5-77)	19 (3-44)
Foscarnet	23 (39)	16 (25)	16 (1-80)	22 (6-56)
Cidofovir	5 (9)	0	7 (1-13)	N/A
Overall	51 (86)	22 (34)	14 (0-80)	0 (0-77)

(ie, ALC on the morning of day −3) and any viral infection outcomes. Prospective studies of ALC-based ATG dosing are needed to evaluate the efficacy and safety of this approach.

Two relatively recent advancements in the field could change the discussion surrounding ATG and its viral-related risks to MUD transplant recipients. The emerging use of prophylactic letermovir in CMV-seropositive transplant recipients has been shown to significantly reduce the incidence of clinically significant CMV and all-cause mortality when administered through day +100 after allogeneic transplant [23]. In this study by Marty et al. [23] 35% of the CMV-seropositive recipients received ATG. None of the patients in our study received letermovir. Although primary prophylaxis with letermovir could change the landscape of CMV reactivation and its viral-related morbidity, particularly in MUD transplant patients receiving ATG, other viruses will remain problematic. Second, the use of PTCy, originally studied in the haploidentical HCT population, is beginning to emerge as a new GVHD prophylaxis strategy as an alternative to ATG-based prophylaxis in both MRD and MUD transplants [24-28]. Kanakry et al. [27] studied PTCy as the sole GVHD prophylaxis in 92 adult patients receiving either MRD or MUD HCT. They reported no deaths from CMV disease, a rate of CMV reactivation of 35%, and no post-transplant lymphoproliferative disorder. In a similar study, Shah et al. [28] studied the use of PTCy with tacrolimus and mycophenolate in 22 MUD patients. CMV and BK reactivation by day +100 were reported in 64% and 23% of patients, respectively. Although this is similar to the rates of CMV and BK reactivation in our analysis, the advantage of PTCy on the immune system may not be realized until after day +100. PTCy, in contrast to ATG, can provide protection from late infectious events because of its inability to affect naïve and nonactivated memory T cells [29]. More studies are needed to determine the infection risk with PTCy in MRD and MUD HCT, along with other transplant outcomes.

The results of the current analysis should be considered with the acknowledgement of some potential limitations. This was a retrospective study with a limited sample obtained from a single center; thus, the results may not be directly applicable to other centers with varying GVHD prophylaxis and viral infection monitoring practices. The lack of routine preemptive monitoring for viruses beyond CMV may have resulted in an underestimation of asymptomatic reactivation episodes for other viruses. Correspondingly, testing for these other viruses purely based on the presence of symptoms suggestive of viral infection may have missed periods of asymptomatic reactivation before the development of EOD. Finally, the limited follow-up time of the current study may have reduced the ability to measure outcomes occurring beyond day +100, including late-onset viral infections, chronic GVHD, RFS, and OS.

In conclusion, use of ATG for GVHD prophylaxis before MUD HCT was associated with significantly higher risks of viral EOD and inferior OS. Patients who received MUD HCT with ATG were also more likely to experience hospitalization and intensive care unit admission as a result of viral infections. Our results suggest that all patients who undergo MUD HCT with ATG should be preemptively monitored for both CMV and EBV reactivation. The prophylactic use of letermovir in CMV-seropositive recipients receiving ATG has promise to change further analyses of ATG use in this population. Future analyses should aim to directly compare post-HCT viral infection outcomes past day +100 in those who received ATG with those who received other forms of GVHD prophylaxis, such as PTCy or ex vivo T cell–depleted grafts.

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