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Sirolimus, tacrolimus and low-dose methotrexate based GVHD prophylaxis after non-ablative or reduced intensity conditioning in related and unrelated donor allo-HCT

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Abstract

Encouraging results have been reported with sirolimus, tacrolimus, and low-dose methotrexate after non-myeloablative allogeneic hematopoietic cell transplantation. We conducted a retrospective analysis of 71 patients with lymphoid malignancies treated with this prophylaxis regimen after non-myeloablative or reduced intensity allogeneic hematopoietic cell transplantation. Grafts were HLA-matched related in 29 (41%), matched unrelated in 36 (51%), and 9/10 HLA-matched unrelated in 6 (8%) patients. The regimen was well tolerated and over 90% of patients completed the planned treatment. The cumulative incidences of 1-year grade B–D and C–D acute graft-versus-host disease were 0.28 (95% CI, 0.18–0.39) and 0.07 (95% CI, 0.03–0.15), respectively; and 1 and 2-year chronic graft-versus-host disease (National Institutes of Health criteria) in 70 evaluable patients 0.15 (95% CI, 0.08–0.24) and 0.33 (95% CI, 0.22–0.44), respectively. The median day of onset of acute graft-versus-host disease was 123 days (range, 17–268 days). Peri-transplant rituximab or anti-thymocyte globulin did not affect graft-versus-host disease. The cumulative incidence of 1-year non-relapse mortality and relapse were 4% and 20%, respectively. With a median follow-up of 3.5 (range: 0.18–5.1) years, overall survival and progression-free survival at 2 years were 82% and 66%, respectively. This graft-versus-host disease regimen results in a low incidence and severity of acute and chronic graft-versus-host disease after reduced intensity and non-myeloablative allogeneic hematopoietic cell transplantation for lymphoid malignancies. The study also highlights the incidence of late onset acute graft-versus-host disease in non-myeloablative/reduced intensity conditioning, and the

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contribution of the new graft-versus-host disease staging system that more accurately reflects clinical outcomes.

Keywords

Sirolimus; Reduced-intensity Non-Myeloablative conditioning; Graft-versus-host disease

INTRODUCTION

Graft-versus-host disease (GVHD) remains the main cause of morbidity and non-relapse mortality (NRM) after allogeneic hematopoietic cell transplantation (allo-HCT), and limits the broader application of this treatment modality. Methotrexate (MTX) combined with a calcineurin inhibitor (CNI) has been the most widely accepted standard of GVHD prophylaxis for the past 25 years [1–4]. Despite the use of these drugs, the incidence of grade II–IV acute GVHD (aGVHD) ranges from 35% to 50% in recipients of matched related donor (MRD) grafts, and 40% to 70% after matched unrelated donor (MUD) transplant [3,4]. Sirolimus (rapamycin), an mTOR inhibitor, is structurally similar to tacrolimus, but binds to FK-506 binding proteins at a distinct site. Sirolimus and the CNIs work synergistically. Sirolimus may also have advantages over other immunosuppressive drugs. In experimental models, sirolimus promotes the expansion of regulatory T cells, suggesting it could preserve graft-versus-tumor effects and protect against chronic GVHD (cGVHD) [5–8]. It has shown antineoplastic activity in hematological malignancies [9–13], and is associated with a lower incidence of cytomegalovirus (CMV) reactivation [14,15]. Tacrolimus combined with sirolimus has shown higher efficacy and lower toxicity when compared to the combination with cyclosporine [16]. In RIC allo-HCT, the combination of sirolimus/tacrolimus with or without MTX has shown promise in reducing aGVHD with a low toxicity profile [17–19]. Based on these promising results in single-arm Phase II trials [17,20], we adopted the combination of sirolimus/tacrolimus and low-dose MTX for GVHD prophylaxis at our center in 2008. We now report our results in 71 patients who were treated with this regimen and demonstrate low incidences and severity of acute and chronic GVHD, and encouraging outcomes with this three drug prophylaxis combination after RIC/NMA Allo-HCT.

METHODS

Patient Characteristics

Seventy-one consecutive patients who received a NMA or RIC allo-HCT from related or unrelated donors for treatment of lymphoid malignancies were given the combination of sirolimus/tacrolimus with low-dose MTX between April 2008 and June 2011. The prospective phase II studies that included patients were registered at www.clinicaltrials.gov as NCT00425802 and NCT00574496. The primary transplant outcomes of 31 of the patients treated on a phase II study for patients with CD20+ B cell lymphoid malignancies (NCT00574496) were recently reported [21]. Written informed consent for treatment was obtained from all patients. Approval for this retrospective review was obtained from the Institutional Review and Privacy Board.

Eligibility criteria for transplant included a diagnosis of chemosensitive hematologic malignancy; availability of an HLA-identical or single-allele mismatched donor (A, B, C, DR); absence of active infection; and lack of coexisting cardiac, pulmonary, hepatic, or renal dysfunction that would preclude administration of the cytoreductive regimen. HLA matching was established by DNA sequence-specific oligonucleotide typing for HLA-A, -B, -C, DR-B1 and DQ-B1 loci.

Transplant Procedure and Supportive Care

All patients received a lower-intensity conditioning regimen categorized as RIC (n=12) or NMA (n=59), using established criteria [22]. GVHD prophylaxis consisted of sirolimus and tacrolimus that were started on day -3, followed by methotrexate 5mg/m² on days +1, 3, 6. Doses were adjusted to maintain target serum trough levels of 3–12 and 5–10 ng/mL for sirolimus and tacrolimus, respectively. Tapering of sirolimus and tacrolimus on an alternating schedule typically began at day 60 in the absence of GVHD or relapse, with the goal of complete discontinuation by 6 months or earlier in patients not in CR at transplant. Thirty-one of 37 (84%) recipients of MUD and all recipients of MMUD (n=6) grafts were given 2 and 3 doses, respectively, of 30 mg/kg/dose equine anti-thymocyte globulin (ATG). While commonly used in recipients of MUD, ATG is no longer mandated at our center, particularly given recent CIBMTR data suggesting an increased risk of relapse associated with ATG in recipients of RIC transplants [23]. Forty-eight patients with CD20+ malignancies (68%) received rituximab on days -8 and +21, 28, 35, and 42. Patients received supportive care and prophylaxis against opportunistic infections in accordance with standard guidelines. Monitoring of CMV viremia was performed regularly through day +100 and pre-emptive therapy was instituted in patients with documented CMV viremia.

Chimerism analysis of bone marrow (n=66) and peripheral blood (n=5) was performed by RQ-PCR method based on single-nucleotide polymorphism [24]. Myeloid and platelet engraftment were defined using standard criteria. GVHD was diagnosed clinically, confirmed pathologically whenever possible, and classified according to standard criteria. Patients who engrafted were evaluable for aGVHD [25], and patients surviving at least 100 days were evaluable for cGVHD according to historical and National Institutes of Health (NIH) consensus criteria [26,27]. Patients who relapsed or received donor lymphocyte infusions (DLI) were censored for GVHD at that time. Cause of death was determined using a standard algorithm [28]. Diagnosis of thrombotic microangiopathy (TMA) was established according to defined criteria.

Data Collection and Statistical Analysis

Demographic, disease and treatment characteristics were summarized using descriptive statistics. Analyses were performed as of June 30, 2013. The incidence of acute and chronic GVHD was estimated using cumulative incidence functions, treating relapse and death unrelated to GVHD as competing events. Patients who suffered early disease progression or relapse and subsequently developed acute GVHD in the setting of rapid tapering of the immunosuppression or DLI were categorized as having relapse. Gray's test was used to compare the incidence of GVHD across patient and treatment characteristics. These characteristics included: patient age at transplantation (below or above 50), donor type

(related versus unrelated), donor-patient gender match (female to male versus other combinations), patient CMV serostatus, CD34+ cell dose (above or below median), use of ATG, use of rituximab and conditioning regimen (RIC versus NMA). The incidence of relapse and TRM were estimated using cumulative incidence functions. OS and PFS probabilities were estimated using Kaplan-Meier methodology [29]. The following pre-transplant variables were assessed for their effects on OS, PFS and the cumulative incidence of progression: disease status (CR vs. PR vs. PD/SD), ATG (yes vs. no), LDH (<246 vs. >246), KPS (<80 vs. ≥ 80), chemosensitive response defined by PET scan (sensitive vs. refractory), and prior autologous SCT (yes/no). Survival distributions were compared across pre-transplant variables using the logrank test, while Gray's test was used to compare the incidence of disease progression across pre-transplant variables.

RESULTS

Patient Characteristics

Patient characteristics are shown in Table 1. The median age at transplant was 51 years (range: 23 to 69 years). All patients were treated for lymphoid malignancies, with 72% having non-Hodgkin's lymphoma. At transplantation, 30 patients (42%) were in CR and the other 41 (58%) had active disease. Twenty-seven percent of the patients had received a prior Auto-SCT. Conditioning regimen was NMA in 59 patients (83%) and RIC in 12 patients (17%).

Donor and Stem Cell Source

All patients except one received peripheral blood stem cells. Twenty-nine patients (41%) received a transplantation from an HLA-identical sibling donor, while the remaining 42 received unrelated donor transplantation (10/10 matched in 36 patients, and 9/10 matched in 6 patients). The median infused CD34+ cell dose was 7.4×10^6 CD34+/kg (range $1.5 - 13 \times 10^6$). In 86% of patients the CD34+ dose was capped at 8×10^6 /kg [30,31].

Engraftment and Toxicity

All patients were evaluable for engraftment and 70 achieved initial engraftment. One patient who received a HLA-matched graft from an unrelated donor with ATG had graft failure with autologous recovery and remains alive in remission at last follow-up. The median times to neutrophil and platelet engraftment were 11 days (range, 7–23 days) and 17 days (range, 9–23 days), respectively. At day 30, 99% of patients achieved $\geq 50\%$ donor chimerism and 89% of patients achieved $\geq 90\%$ donor chimerism.

The sirolimus/tacrolimus with low-dose MTX regimen was well tolerated. Five patients (7%) required a change in the immunosuppression treatment because of early toxicity (median day 9, range 2–19): one patient developed reversible sirolimus-related TMA with an average sirolimus level of 6 ng/mL and four patients had tacrolimus-related renal toxicity. Two of the patients with renal toxicity did not receive the full course of methotrexate. All 5 patients developed GVHD with a median day of onset of 200 days (range, 44–232 days), despite having received another immunosuppressant agent (mycophenolate mofetil in 4 patients and mycophenolate mofetil plus steroids in 1). Three

of them developed aGVHD, 1 cGVHD and 1 patient acute and chronic GVHD. There were no cases of sinusoidal obstructive syndrome (SOS).

GVHD

The 100-day cumulative incidences of grades B–D and C–D aGVHD were 0.14 (95%CI, 0.0–0.23) and 0.03 (95%CI, 0.005–0.09), respectively. The cumulative incidence of 1 year grade B–D aGVHD was 0.28 (95%CI, 0.18–0.39; Figure 1A) and grade C–D aGVHD was 0.07 (95%CI, 0.03–0.15; Figure 1B). The median day of onset of aGVHD was 123 days (range, 17–268 days). Three patients (4%) developed grade D aGVHD, and 2 of them died of refractory gut GVHD. The organs involved included skin only in 8 patients, skin and gastrointestinal tract in 8, gastrointestinal tract only in 5, liver only in 1, and gastrointestinal tract and liver in 1 patient.

The cumulative incidence of cGVHD by NIH criteria in 70 evaluable patients at 1 and 2 years was 0.15 (95%CI, 0.08–0.24) and 0.32 (95%CI, 0.22–0.44; Figure 2), respectively, and that of moderate-severe cGVHD at 2 years was 0.22 (95%CI, 0.13–0.32). The two-year cumulative incidence of overall and extensive cGVHD by pre-NIH criteria was 0.56 (95%CI, 0.44–0.67) and 0.45 (95%CI, 0.33–0.56; Figure 2), respectively. Four patients developed severe cGVHD at 2 years, including two who died of bronchiolitis obliterans syndrome (BOS).

Factors Associated with GVHD

Table 2 shows the incidence of 1-year grade B–D aGVHD and 2-year cGVHD by patient and treatment characteristics. Patients older than 50 appeared to have a significantly higher incidence of aGVHD at 1-year, but less cGVHD at 2-years. In this regard, more patients older than 50 received ATG (67%) compared to the younger cohort (27%; $p=0.004$). The use of peri-transplant rituximab did not affect the 1-year incidence of Grade B–D aGVHD (0.31 vs. 0.22 in rituximab cohort, $p=0.38$) or the 2-year incidence of cGVHD (0.34 vs. 0.30 in rituximab cohort, $p=0.91$). There was also no difference in the incidence of Grade B–D aGVHD between ATG and no ATG cohorts (0.27 vs. 0.29, $p=0.89$). There was a trend towards a modestly higher 2-year incidence of cGVHD in patients who did not receive ATG (0.38 vs. 0.27 in ATG cohort, $p=0.24$, Figure 3), despite the fact that these were predominantly recipients of MRD. Additional factors that did not affect acute or chronic GVHD included HLA-match (MRD vs. other), recipient CMV serologic status, donor gender (female donor into male recipient vs. other), and CD34+ stem cell dose.

Reactivation of Cytomegalovirus

The incidence of CMV reactivation among CMV seropositive patients was 18%: 43% (3/7) when the donor was CMV seronegative and 9% (2/21) when the donor was CMV seropositive ($p=0.07$). The median time to CMV reactivation was 62 days (range 36–99 days). There were no cases of CMV disease. None of the 43 CMV seronegative patients reactivated CMV, including 14 with a seropositive donor.

Relapse, Non-relapse Mortality and Survival

With a median follow-up for surviving patients of 3.5 years (range, 0.18–5.1 years), the 2-year overall survival (OS) and progression-free survival (PFS) rates were 0.82 (95%CI, 0.73–0.91) and 0.66 (95%CI, 0.56–0.78), respectively. The non-relapse mortality (NRM) at day 100, 1 and 2 years was 0.01 (95%CI, 0.001–0.07), 0.04 (95%CI, 0.01–0.11) and 0.08 (95%CI, 0.03–0.16, Figure 4), respectively. Eighteen patients died. The primary cause of treatment failure was relapse of disease (n=11), followed by GVHD (n=6). Patients whose cause of death was GVHD (4 aGVHD and 2 cGVHD) died at a median time of 531 days (range, 88–1318); 3 of refractory gastrointestinal aGVHD, 2 of BOS and one of sepsis. The following pre-transplant factors did not have a significant on 2-year OS or PFS: LDH, disease status, chemosensitivity defined by PET scan or having had a prior autologous transplant (data not shown). KPS was not included in the analysis as too few patients had a KPS < 80. The cumulative incidence of relapse or progression at 2 years was 0.28 (95%CI, 0.19–0.37). The 1-year relapse or progression rate in the ATG (MUD/MMUD) vs. no ATG (MRD) cohort was 0.11 and 0.29 (p=0.09), respectively. There was no statistically significant effect on the cumulative incidence of progression of the other pre-transplant factors evaluated (data not shown).

DISCUSSION

Consistent with prior studies, we demonstrate that the combination of sirolimus/tacrolimus with low-dose MTX results in low rates and severity of acute and chronic GVHD, with low toxicity and favorable OS and PFS after RIC/NMA allo-HCT. Furthermore, we show that this combination can be used with a number of conditioning regimens. The cumulative incidence of 1-year grade B–D and C–D aGVHD were 0.28 and 0.07, respectively, with only 3 patients developing grade D aGVHD. As noted above, 3 patients who received DLI were censored at time of DLI. The median day of onset of aGVHD was > 100 days and many patients developed aGVHD in the setting of immunosuppression tapering. This explains a higher reported rate of aGVHD than in the initial report of this regimen, where the cumulative incidence of grade II–IV and III–IV aGVHD (at day 100) were 16% and 7%, respectively [17]. These day 100 rates, however, are almost identical to the day 100 rates of aGVHD in the present series. The corollary of a higher rate of aGVHD, with the inclusion of late-onset aGVHD in our series, is a lower rate of cGVHD than previously reported [19,32]. The cumulative incidence of cGVHD using NIH consensus criteria was low (0.33), with only 6% of patients developing severe cGVHD. Furthermore, when we used the classic definition for cGVHD, our rate of extensive cGVHD of 0.45 again was consistent with the prior report [17]. Although these results highlight potential issues associated with the implementation of newer classification systems when comparing to older published data, further studies will be required to determine the prognostic value of distinguishing late onset aGVHD and cGVHD.

After the initial report, the group at the Dana Farber showed that methotrexate could be safely eliminated in the setting of MRD with no adverse impact on GVHD and outcomes [18]. Pidala *et al.* [7] recently compared sirolimus/tacrolimus to tacrolimus-methotrexate in a prospective randomized study, and reported a statistically significant decrease in overall

day 100 aGVHD (43% vs. 89%, $p<0.0001$) and moderate-severe cGVHD (24% vs. 64%, $p=0.008$) in patients receiving sirolimus. In another report, recipients of unrelated donor grafts treated with sirolimus and tacrolimus after RIC on a prospective multicenter study had a lower 1-year cumulative incidence of cGVHD when compared to historical controls treated with cyclosporine and mycophenolate (55 vs. 21%, $p=0.003$) [8]. This translated into better OS (70% vs. 45%, $p=0.018$).

The low incidence of grade C–D aGVHD we observed also translated into a low NRM (4% at 1 year and 8% at 2 years), an expected result given the contribution of severe aGVHD to NRM. Furthermore, the regimen was well tolerated. Only one patient developed TMA that resolved after IS change, and no cases of SOS were observed. These results are consistent with those of others in patients receiving non-ablative conditioning. The 2-year OS of 0.82 and PFS of 0.66 are consistent with previous reports after RIC/NMA transplantation [8,19,32]. Our survival data are very encouraging considering that 58% of patients had active disease at transplant and 27% had aggressive NHL. These results also compare favorably to a recent series we reported of patients undergoing RIC conditioning with alemtuzumab, where the rate of grade 2–4 aGVHD at day 100 was 10.5%, but was associated with a significant risk of infections as well as a PFS 30% at 3 years [33]. However, only 34% of patients in this older series had chemosensitive disease, unlike the current study where the majority of patients had chemosensitive disease.

In contrast to results in NMA/RIC transplants, co-administration of tacrolimus and sirolimus in myeloablative transplants has been associated with an increased risk of endothelial injury syndromes, including SOS and TMA, particularly in patients receiving busulfan and cyclophosphamide [7,34–38]. In particular Cutler et al [38] recently reported that results of a randomized phase III study in which patients were randomized to either tacrolimus/sirolimus or tacrolimus/MTX after a TBI containing ablative transplant. While tacrolimus/sirolimus was associated with less mucositis and severe aGVHD, there was no improvement in survival and an increase in cGVHD as well as SOS and TMA.

In order to identify potential additional factors that may have contributed to lower rates of GVHD, we studied the effects of ATG and rituximab. The role of ATG in preventing aGVHD is controversial, particularly in RIC/NMA transplants. A recently published meta-analysis including 733 patients from 7 studies showed that ATG was effective in preventing grade III/IV aGVHD, but did not improve OS because of an increase in infectious complications and NRM [39]. This is in contrast to a report of the Center for International Blood and Marrow Transplant Research (CIBMTR), which found that ATG had no impact on grade II–IV aGVHD in patients given RIC (38% vs. 40%), but that it did adversely impact relapse (51% vs. 38%, $p<0.001$) [23]. In our study, we did not find an effect of ATG on the incidence of Grade B–D aGVHD at day 100. However, there was a trend towards a lower incidence of cGVHD at 2 years in the ATG cohort with no increase in relapse rate or infections, despite the fact that these were recipients of unrelated donors. Although there is increasing evidence of the role of B cells in the pathogenesis of acute and particularly chronic GVHD [40], and some studies have reported a low incidence of cGVHD with prophylactic rituximab after transplantation [41], the use of peri-transplant rituximab did not affect the incidence acute or chronic GVHD in our series. A similar outcome was observed

in a recent randomized study where rituximab did not affect acute or chronic GVHD after myeloablative conditioning [42].

Sirolimus prophylaxis has been shown to protect against CMV reactivation after solid organ transplantation and HCT [14,15]. Only 7% of our patients reactivated CMV (18% of seropositive recipients) and no patients developed CMV disease, despite the fact that 40% of patients were CMV seropositive and 51% received ATG. This finding may be related to the possible protective effect of sirolimus and to the small number of patients who experienced GVHD.

Despite the fact that sirolimus may reduce the incidence of disease progression in patients with lymphoma that undergo an allo-HCT [43], disease progression was the leading cause of treatment failure in our study. Additional prospective studies are needed to investigate the contribution of sirolimus to lymphoma relapse after allo-HCT.

In summary, our experience with the combination of sirolimus/tacrolimus and low-dose MTX further supports the safety and efficacy of this GVHD prophylaxis combination. An open question is whether patients would benefit from a more prolonged prophylaxis with sirolimus, tacrolimus, or the combination, with the intention of reducing late onset aGVHD and promoting immune tolerance. In addition, the inclusion of low-dose methotrexate in this regimen also warrants further study, particularly for unrelated donors.

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Figure 1.
Cumulative incidence of grade B–D (A) and C–D (B) acute GVHD.

Figure 2.
Cumulative incidence of overall chronic GVHD by pre and post NIH Consensus Criteria.

Figure 3.
Cumulative incidence of chronic GVHD by NIH consensus criteria in ATG/No ATG cohorts.

Figure 4.
Cumulative incidence of relapse and non-relapse mortality.

Table 1**Patient Characteristics**

Patients Characteristic	N (%)
Sample size	71
Patient median age, years (range)	51.1 (23.6–69.3)
Disease at transplantation	
Non-Hodgkin lymphoma	51 (72)
Aggressive B-cell NHL *	19 (27)
Follicular	16 (23)
T-cell Lymphoma	6 (8)
Mantle cell lymphoma	6 (8)
Marginal zone Lymphoma	3 (4)
Plasmablastic lymphoma	1 (1)
Hodgkin's Lymphoma	10 (14)
Chronic lymphoid leukemia/SLL	10 (14)
Remission status at time of transplant	
CR	30 (42)
PR	29 (41)
SD	11 (16)
PD	1 (1)
PET Scan	
Sensitive	51 (72)
Refractory	10 (14)
Missing	10 (14)
LDH	
246	54 (76)
> 246	17 (24)
KPS	
< 80	4 (6)
80	67 (94)
Sex, no. (%)	
Male	43 (61)
Female	28 (39)
Donor/recipient sex	
M/M	25 (35)
M/F	16 (22)
F/M	18 (25)
F/F	12 (17)
Donor Type	
HLA-identical related	29 (41)
HLA-matched unrelated	36 (51)
HLA-mismatched unrelated	6 (8)

Patients Characteristic	N (%)
1 HLA-DQ mismatch	5
1 HLA-A mismatch	1
Conditioning regimen	
Non-myeloablative	59 (83)
Reduced intensity	12 (17)
Conditioning regimen ^{**}	
Cy/Flu/TBI	58 (82)
Mel/Flu	9 (13)
Cy/Flu/Thio/TBI	2 (3)
Flu/TBI	1 (1)
BEAM	1 (1)
Conditioning regimen	
Rituximab only	20 (28)
ATG only	8 (11)
Rituximab+ATG	28 (40)
No Rituximab/No ATG	15 (21)
Source of stem cells	
Bone marrow	1 (1)
Mobilized blood	70 (99)
Prior Transplantation	19 (27)
Donor/recipient CMV status	
+/+	21 (30)
+/-	14 (20)
-/+	7 (10)
-/-	29 (41)

* Including diffuse large B-cell lymphoma, transformed low-grade lymphoma, transformed CLL/SLL and lymphoblastic lymphoma

** Cy/Flu/TBI: cyclophosphamide (50mg/kg), fludarabine (25mg/m² daily for 5 days) and TBI (200 cGy); Mel/Flu: melphalan (70mg/m² daily for 2 days) and fludarabine (30mg/m² daily for 5 days); Cy/Flu/Thio/TBI: cyclophosphamide (50mg/kg), fludarabine (30mg/m² daily for 5 days), thiotepa (5mg/kg daily for 2 days) and TBI (400 cGy); Flu/TBI: fludarabine (25mg/m² daily for 5 days) and TBI (200 cGy); and BEAM: carmustine (300mg/m²), cytarabine (200mg/m²/12 hours for 8 doses), etoposide (200mg/m² daily for 4 days) and melphalan (140 mg/m²).

Table 2

Incidence of 1-year acute (grade B–D) and 2-year chronic GVHD by patient and treatment characteristics.

Variable	N	Acute GvHD, grade B–D		Chronic GvHD	
		1-yr Estimate	P-value	2-yr Estimate	P-value
Overall	71	0.28 (0.18–0.39)		0.33 (0.22–0.44)	
Age			0.07		0.04
< 50 years	26	0.15 (0.05–0.32)		0.47 (0.26–0.65)	
> 50 years	45	0.36 (0.22–0.50)		0.24 (0.13–0.38)	
Conditioning			0.75		0.59
NMA	59	0.27 (0.17–0.40)		0.34 (0.22–0.46)	
RIC	12	0.33 (0.09–0.61)		0.25 (0.05–0.53)	
Pt CMV Status			0.23		0.61
Sero +	28	0.36 (0.18–0.53)		0.29 (0.13–0.48)	
Sero –	43	0.23 (0.12–0.37)		0.35 (0.21–0.49)	
Donor			0.25		0.57
MRD	29	0.21 (0.08–0.37)		0.34 (0.18–0.52)	
MUD/MMUD	42	0.33 (0.20–0.48)		0.31 (0.18–0.45)	
Donor: Patient Sex			0.08		0.18
Female: Male	18	0.11 (0.02–0.33)		0.17 (0.04–0.37)	
Other	53	0.34 (0.22–0.47)		0.38 (0.25–0.51)	
Rituximab Use			0.38		0.91
Yes	48	0.31 (0.19–0.45)		0.34 (0.20–0.47)	
No	23	0.22 (0.08–0.40)		0.30 (0.13–0.50)	
ATG Use			0.89		0.24
Yes	37	0.27 (0.14–0.42)		0.27 (0.14–0.42)	
No	34	0.29 (0.15–0.45)		0.38 (0.22–0.54)	
CD34 Dose			0.64		0.16
Below Median *	36	0.25 (0.12–0.40)		0.25 (0.12–0.40)	
Above Median *	35	0.31 (0.17–0.47)		0.40 (0.24–0.57)	

* Median dose 7.39×10^6 CD34+/kg