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SHORT REPORT

Clinical outcomes of patients receiving three versus four doses of methotrexate with concomitant antithymocyte globulin in match unrelated donor allogeneic stem cell transplant: A single-center experience

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Abstract

Methotrexate (MTX) doses on days +1, +3, +6, and +11 after match unrelated donor allogeneic stem cell transplant (MUD HSCT) is a common graft-versus-host disease (GVHD) prophylaxis regimen. However, the overlapping toxicity of MTX with conditioning chemotherapy sometimes warrants the omission of the fourth dose of MTX. Prior single-institution studies showed conflicting results comparing the outcomes of patients who received three versus four doses of MTX, but to our knowledge, the effect of concomitant antithymocyte globulin (ATG) has not been reported. Charts of patients who underwent MUD HSCT between 2009 and 2023 were reviewed. Patients received rabbit ATG (Thymoglobulin), given at 0.5 mg/kg on day −3, 2 mg/kg on day −2, and 2.5 mg/kg on day −1. MTX is given at 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. Severe mucositis was the most common indication for day +11 MTX omission (82%). We identified 292 patients (116 in 3 dose cohort and 176 in 4 dose cohort). Median follow-up was 23 months (range 1–151). Patients in the 4 doses cohort were more frequently male (68% vs. 50%, $p < 0.01$), received a reduced intensity conditioning regimen (38.0% vs. 22%, $p < 0.01$), were older (median 58 vs. 54 years, $p = 0.02$), and received a transplant in the earlier era (median HSCT year 2014 vs. 2018, $p < 0.01$). A statistically significant difference was not evidenced between the cohorts for the following outcomes: acute GVHD (aGVHD) (HR 1.1, 95% CI 0.9–1.5), chronic GVHD (cGVHD) (HR 1.3, 95% CI 0.8–1.6), relapse-free survival (RFS) (HR 1.0, 95% CI 0.6–1.5), non-relapse mortality (NRM) (HR 1.4, 95% CI 0.9–2.2), and overall survival (OS) (HR 1.2, 95% CI 0.9–1.7). Both cohorts had similar median time to neutrophil engraftment at 14 days. When ATG is incorporated, omission of day +11 MTX does not significantly

impact the rate of engraftment or cumulative incidence of aGVHD, cGVHD, RFS, NRM, and OS.

KEYWORDS
antithymocyte globulin, GVHD prophylaxis, match unrelated allogeneic stem cell transplant, methotrexate

1 | INTRODUCTION

To prevent acute and chronic graft-versus-host disease (aGVHD and cGVHD), various combinations of immunosuppressants have been developed. One of the most common graft-versus-host-disease (GVHD) prophylaxis regimens for match unrelated donor allogeneic stem cell transplant (MUD HSCT) includes four doses of methotrexate (MTX) given on days +1, +3, +6, and +11 in combination with a calcineurin inhibitor [1] with or without antithymocyte globulin (ATG) for enhanced T-cell suppression [2, 3]. Due to the common overlapping toxicity of MTX with the conditioning chemotherapy (mucositis, hepatic or renal toxicity, and cytopenia), omission of day +11 MTX is sometimes warranted.

Prior single-institution studies showed conflicting GVHD, engraftment, and survival outcomes when comparing patients who received three versus four doses of MTX [4–7]. These studies did not incorporate ATG into the regimen. Therefore, we aimed to evaluate the outcomes of patients who underwent ATG-based MUD HSCT at our center.

2 | METHODS

Adult (age ≥18 years) patients who underwent a MUD HSCT between 2009 and 2023 for various malignant hematologic conditions were included. HLA typing was determined by high-resolution techniques, and all donor–recipient pairs were HLA matched 10/10 at the A-, B-, C-, DRB1-, and DQB1-loci. We included all types of conditioning therapy, including myeloablative (MAC), reduced intensity conditioning (RIC), and non-MAC regimens. Based on our institutional protocol, rabbit ATG (Thymoglobulin) is given at 0.5 mg/kg on day −3, 2 mg/kg on day −2, and 2.5 mg/kg on day −1. MTX is given at 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. Tacrolimus (Tac) is started at 1 mg oral twice daily starting day −1, and Tac level is measured at least three times per week until a therapeutic level of 5–10 ng/dL is reached. Tapering begins after day +90 in the absence of clinically significant GVHD. Patients who did not complete ATG infusions, those who received <3 doses of MTX, and those who died before day +11 were excluded. Patients who missed the fourth dose of MTX did not receive any additional or alternative immunosuppressants. Patients provided informed consent for the HSCT treatment plan as well as the use of their personal information for research purposes. The study was approved by the University of Iowa Institutional Review Board.

The International Bone Marrow Transplant Registry (IBMTR) severity index and International Consortium and National Institutes of Health (NIH) criteria were used for the diagnosis and grading of aGVHD and cGVHD, respectively [8, 9]. Neutrophil engraftment was defined as the achievement of an absolute neutrophil count ≥500 × 10^6/L for 3 consecutive days. The conditioning regimen was classified as MAC when it included fractionated total body irradiation >8 Gray or intravenous busulfan ≥12.8 mg/kg; otherwise, it was classified as RIC [10]. Mucositis was graded according to the World Health Organization (WHO) criteria [11].

Chi-squared tests were used to compare categorical variables, and Wilcoxon rank-sum tests were used to compare continuous variables among MTX doses. Time was calculated from HSCT to recurrence or death due to any cause for relapse-free survival (RFS) and overall survival (OS), respectively. For aGVHD and cGVHD, time was calculated from HSCT to the onset of acute and cGVHD. Relapse and death due to any cause were considered competing events. For non-relapse mortality (NRM), time was calculated from HSCT to death due to any cause; relapse was considered a competing event. Otherwise, patients were censored at the date of last contact. Cox regression and Fine-Gray competing risk models were used to estimate the effect of patient, disease, and treatment characteristics on outcomes. All statistical testing was two-sided and assessed for significance at the 5% level using SAS v9.4 (SAS Institute, Cary, NC).

3 | RESULTS

A total of 292 patients were included. Median age was 57 (range: 18–74), and acute myeloid leukemia (45%) was the most common indication for HSCT. Median follow-up was 23 months (range: 1–151) and 51 months (range: 3–151) among survivors.

A total of 116 and 176 patients received 3 and 4 doses of MTX, respectively. Patients receiving four doses were more frequently male (68% vs. 50%, $p < 0.01$), were older (median age 58 vs. 54, $p = 0.02$), underwent HSCT in an earlier era (median year 2014 vs. 2018, $p < 0.01$), and more frequently received a RIC regimen (38% vs. 22%, $p < 0.01$, Table 1). The most common reason for day +11 MTX omission was severe mucositis (82%) followed by hepatic toxicity (5%). Median time to neutrophil engraftment was 14 days in both cohorts.

Cumulative incidence of aGVHD at 12 months was 69% and 72% for patients receiving three versus four doses of MTX (Figure 1), whereas grade 3–4 aGVHD was 13% and 18%, respectively. Cumulative incidence of cGVHD at 12 months was 37% and 39% for patients who

TABLE 1 Patients characteristics.

Covariate	Statistics	Level	Methotrexate doses 3 N = 116	4 N = 176	p-Value
Sex	N (Col %)	F	58 (50.0)	56 (31.8)	**<0.01**
	N (Col %)	M	58 (50.0)	120 (68.2)	
Disease	N (Col %)	Acute lymphoblastic leukemia (ALL)	15 (12.9)	26 (14.8)	0.71
	N (Col %)	Acute myelogenous leukemia (AML)	55 (47.4)	76 (43.2)	
	N (Col %)	Chronic myelogenous leukemia (CML)	8 (6.9)	6 (3.4)	
	N (Col %)	Myelodysplastic/myeloproliferative diseases (MDS/MPN)	25 (21.6)	44 (25.0)	
	N (Col %)	Non-Hodgkin lymphoma (NHL)	6 (5.2)	12 (6.8)	
	N (Col %)	Other	7 (6.0)	12 (6.8)	
Prep classification	N (Col %)	Myeloablative	90 (77.6)	110 (62.5)	**<0.01**
	N (Col %)	RIC/Non-myeloablative	26 (22.4)	66 (37.5)	
Cell source	N (Col %)	Marrow	11 (9.5)	9 (5.1)	0.15
	N (Col %)	PBSC	105 (90.5)	167 (94.4)	
Age	Median		54	58	**0.02**
	(Min–max)		(20–73)	(18–74)	
Year of transplant	Median		2018	2014	**<.01**
	(Min–max)		(2009–2023)	(2009–2023)	
CD34 infused	Median		5.0	5.0	0.64
	(Min–max)		(0.9–7.3)	(0.7–8.1)	

Abbreviation: RIC, reduced intensity conditioning.
< 0.05 is considered "statistically significant". Major differences in the two cohorts mentioned in the second paragraph of the result section.

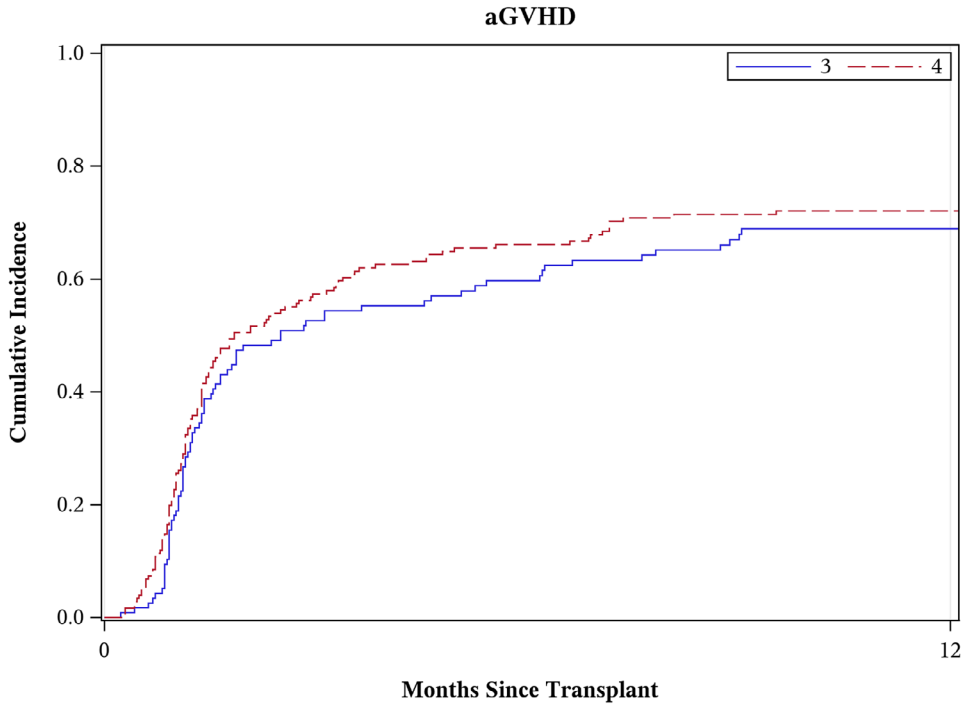

FIGURE 1 Cumulative incidence of acute graft-versus-host disease (aGVHD).

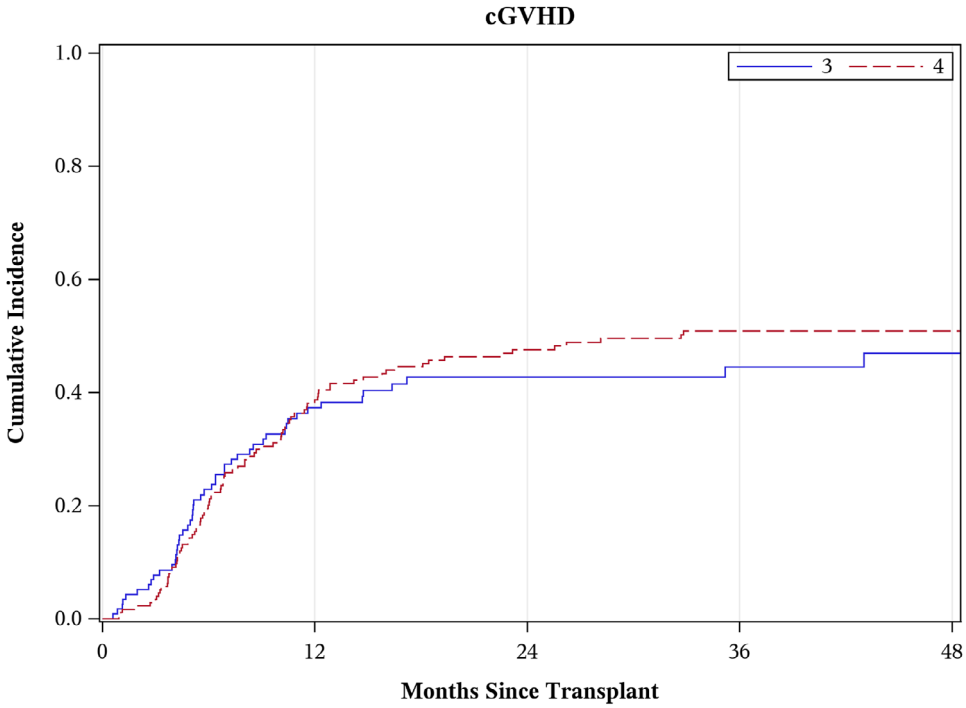

FIGURE 2 Cumulative incidence of chronic graft-versus-host disease (cGVHD).

received three versus four doses of MTX (Figure 2), whereas grade 3–4 cGVHD was 12% and 15%, respectively. Cumulative incidence was similar between patients receiving 4 versus 3 doses of MTX for aGVHD (HR: 1.1, 95% CI: 0.9–1.5), grade 3–4 aGVHD (HR: 1.4, 95% CI: 0.8–2.7), cGVHD (HR: 1.3, 95% CI: 0.8–1.6), and grade 3–4 cGVHD (HR: 1.6, 95% CI: 0.9–3.0).

NRM (Figure S1) at 12 months was 17% and 19% for patients who received three versus four doses of MTX, respectively. A statistically significant increase was not evidenced for patients receiving four versus three doses for NRM (HR: 1.4, 95% CI: 0.9–2.2). RFS (Figure S2) and OS (Figure 3) at 12 months were 75% and 70% for patients treated with three doses and 77% and 68% for patients treated with four doses, respectively. RFS (HR: 1.0, 95% CI: 0.6–1.5) and OS (HR: 1.2, 95% CI: 0.9–1.7) were similar among patients receiving four versus three doses of MTX.

4 | DISCUSSION

Here, we report the outcomes of our single institution experience of patients who received three versus four doses of MTX when ATG is used concomitantly in MUD HSCT.

A few single-institution studies have reported inconsistent outcomes after HSCT when the fourth dose of MTX was omitted. First, Kumar et al. reported in 2002 [4] that omission of day +11 MTX resulted in an increased rate of grade 3–4 aGVHD and decreased OS, whereas a study by Hamilton et al. in 2015 [6] did not find a significant difference in the rate of aGVHD and cGVHD but noted an increased NRM attributed to GVHD in the cohort that omitted day +11 MTX. A study from Japan by Nakamura et al. [5] used "mini-MTX" at 5 mg/m2 on days +1, +3, +6, and +11 and reported decreased OS and increased aGVHD and cGVHD when day +11 MTX was omitted. Finally, a meta-analysis by Kharfan-Dabaja et al. reported [12] no statistical difference in the rates of aGVHD, cGVHD, progression-free survival, NRM, and OS. However, these studies included various doses of MTX and donor platforms, and ATG was not included.

Most recently, a multicenter Italian study reported by Picardi et al. [7] used ATG in the preparative regimen, which actually suggests a protective effect on aGVHD, cGVHD, transplant-related mortality, and OS when the fourth dose of MTX was omitted. Based on these *heterogenous* findings, we set out to evaluate the outcomes from our own institution.

We did not find a statistically significant difference in aGVHD, grade 3–4 aGVHD, cGVHD, grade 3–4 cGHVD, RFS, NRM, and OS outcomes between patients who received 3 versus 4 doses of MTX. Although there were significant differences between patients who received three versus four doses of MTX (Table 1), multivariable modeling further supported the lack of statistical significance observed on univariable analysis for MTX doses. We have no clear explanation of our findings but speculate that the synergistic effect between MTX and ATG may have mitigated the degree of alloreactivity and protected against severe aGVHD and cGVHD without compromising the NRM and OS outcomes [13].

Prior pretransplant cytotoxic therapies, prolonged neutropenia, and malnutrition all contributed to frequent chemotherapy-induced mucositis, and the omission of day +11 MTX can help alleviate further toxicity. We find our data reassuring that the omission of day +11 MTX does not translate to a clinically significant difference in the rate of aGVHD, cGVHD, NRM, RFS, and OS.

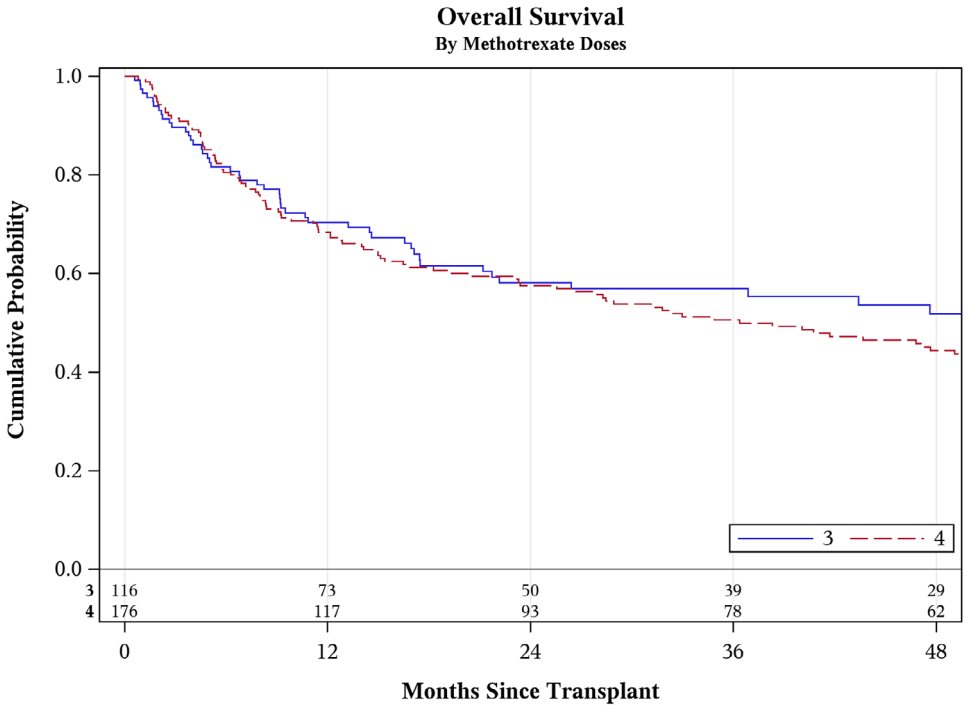

FIGURE 3 Overall survival (OS).

We acknowledge that this is a retrospective, single-institution study with a relatively small sample size. Our patients were transplanted for various hematologic conditions as well as received different intensity conditioning regimens, contributing to the heterogeneity of the outcomes. Moreover, we could not account for inter-patients' variability in methylene tetrahydrofolate reductase gene polymorphism, the role of which could affect MTX metabolism [14]. A larger multicenter prospective study is needed to validate the findings.

5 | CONCLUSION

When ATG is incorporated into the conditioning regimen, omission of the fourth dose of MTX may not significantly impact the incidence of aGVHD, cGVHD, NRM, RFS, and OS after MUD HSCT.

AUTHOR CONTRIBUTIONS

Data collection: Kittika Poonsombudlert, Benda Miller, and Ratdanai Yodsuwan. *Data analysis and interpretation*: Sarah Mott. *Manuscript preparation and review*: Kittika Poonsombudlert, Sarah Mott, Hira Shaikh, Christopher Strouse, Jonathan Lochner, Umar Farooq, and Margarida Magalhaes-Silverman. *Study concept and design*: Kittika Poonsombudlert, and Margarida Magalhaes-Silverman. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest pertinent to this manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the University of Iowa Institutional Review Board.

PATIENT CONSENT STATEMENT

All patients involved provided informed consent authorizing the use of their personal information both for treatment and research purposes.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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