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Recipient Pretransplant Inosine Monophosphate Dehydrogenase Activity in Nonmyeloablative Hematopoietic Cell Transplantation

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Mycophenolic acid, the active metabolite of mycophenolate mofetil (MMF), inhibits inosine monophosphate dehydrogenase (IMPDH) activity. IMPDH is the rate-limiting enzyme involved in de novo synthesis of guanosine nucleotides and catalyzes the oxidation of inosine 5′-monophosphate to xanthosine 5′-monophosphate (XMP). We developed a highly sensitive liquid chromatography–mass spectrometry method to quantitate XMP concentrations in peripheral blood mononuclear cells (PMNCs) isolated from the recipient pretransplant and used this method to determine IMPDH activity in 86 nonmyeloablative allogeneic hematopoietic cell transplantation (HCT) patients. The incubation procedure and analytical method yielded acceptable within-sample and within-individual variability. Considerable between-individual variability was observed (12.2-fold). Low recipient pretransplant IMPDH activity was associated with increased day +28 donor T cell chimerism, more acute graft-versus-host disease (GVHD), lower neutrophil nadirs, and more cytomegalovirus reactivation but not with chronic GVHD, relapse, nonrelapse mortality, or overall mortality. We conclude that quantitation of the recipient's pretransplant IMPDH activity in PMNC lysate could provide a useful biomarker to evaluate a recipient's sensitivity to MMF. Further trials should be conducted to confirm our findings and to optimize postgrafting immunosuppression in nonmyeloablative HCT recipients.

INTRODUCTION

Postgrafting immunosuppression for allogeneic hematopoietic cell transplantation (HCT) recipients often consists of the combination of a calcineurin inhibitor and mycophenolate mofetil (MMF) [1,2]. The development of lower dose, nonmyeloablative conditioning increased the availability of this potentially curative procedure to patients who could not tolerate the toxicity of high-dose conditioning regimens due to age or comorbidity [3]. Nonmyeloablative HCT relies on achieving a delicate balance between recipient and donor cells, with the goal of ensuring sufficient immunosuppression of the recipient to maximize graft-versus-tumor effect but minimize toxicity.

After nonmyeloablative HCT, the recipient experiences at least a short-term mixed chimerism state in which the recipient and donor hematological cells coexist in the blood of the recipient. The level and rate of change in donor T cell chimerism have been correlated with several clinical outcomes such as graft rejection, graft-versus-host disease (GVHD), disease relapse/progression (ie, graft-versus-tumor effect), and progression-free survival [4,5]. The observed associations between donor T cell chimerism and subsequent clinical responses could, in part, reflect differences in each recipient's sensitivity to MMF. Early studies in nonmyeloablative HCT recipients administered MMF every 12 hours, regardless of donor type. Although recipients of related donor grafts had acceptable engraftment rates with MMF every 12 hours, patients receiving unrelated donor grafts had persistent problems with graft rejection. Engraftment

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Table 1
Participant Characteristics

	Donor Type		
	Related	Unrelated	All Participants
Total number	22	64	86
Sex, female/male (% female)	9/13 (41)	23/41 (36)	32/54 (37)
HCT-CI			
0	1 (5)	8 (13)	9 (10)
1-2	3 (14)	14 (22)	17 (20)
3-4	10 (45)	23 (36)	33 (38)
≥5	8 (36)	19 (30)	27 (31)
Median recipient age, yr (range)	55 (20-69)	62 (27-75)	62 (20-75)
CMV-seropositive recipients	10 (45)	35 (56)	45 (53)
Kahl disease risk [27]			
Low	4 (18)	22 (34)	26 (30)
Standard	14 (64)	26 (41)	40 (47)
High	4 (18)	16 (25)	20 (23)
Female donor to male recipient	8 (36)	17 (27)	25 (29)
Median donor age, yr (range)	55 (23-73)	31 (20-58)	35 (20-73)
HLA-mismatched graft	1 (5)	2 (3)	3 (4)
Conditioning regimen			
2 Gy TBI + FLU ± auto	9 (41)	25 (39)	34 (40)
2 Gy TBI + FLU + rituximab* ± auto	11 (50)	20 (31)	31 (36)
3 Gy TBI + FLU ± rituximab*	2 (9)	12 (19)	14 (16)
4-4.5 Gy TBI + FLU	0	7 (11)	7 (8)
Postgrafting immunosuppression			
MMF every 8 h	1 (5)	64 (100)	65 (76)
MMF every 12 h	21 (95)	0	21 (25)
Cyclosporine + MMF ± sirolimus†	12 (55)	49 (77)	62 (72)
Tacrolimus + MMF ± sirolimus‡	10 (45)	15 (23)	24 (28)

HCT-CI indicates HCT comorbidity index; FLU, fludarabine monophosphate; auto, autologous transplant.
Values are number of cases, with percents in parentheses, unless otherwise indicated.
* Rituximab given on days −3, +10, +24, and +38 relative to transplant.
† Ten participants received cyclosporine + sirolimus, 1 with a matched donor and 9 with unrelated donors.
‡ Five participants received tacrolimus + sirolimus, all with unrelated donors.

rates in these patients improved when the daily MMF dose was increased by shortening the administration interval to every 8 hours [1,6]. Because reliable engraftment was achieved, efforts have been ongoing to separate the graft-versus-tumor effect from GVHD. Examples of such efforts include examining the association of day +28 T cell chimerism or neutrophil nadirs within the first 3 weeks post-HCT with relapse rates (ie, graft-versus-tumor effect) and GVHD [4,5,7].

The active metabolite of MMF, mycophenolic acid (MPA), is a selective, reversible, and noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH is the rate-limiting enzyme involved in de novo synthesis of guanosine nucleotides; IMPDH catalyzes the oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) by a nicotinamide adenine dinucleotide $(NAD)^+$-dependent pathway [8]. In renal transplantation patients, high recipient IMPDH activity is associated with rejection [9]. To date, no studies have evaluated the association of clinical outcomes in HCT participants with recipient pretransplant IMPDH activity, which is determined before allograft infusion and MMF administration. Characterizing the relationship between recipient pretransplant IMPDH activity and clinical outcomes is critical to understanding the potential benefit of alternative postgrafting immunosuppression or MMF dosing strategies to improve outcomes in HCT recipients.

Even with nonmyeloablative HCT, however, the conditioning regimen administered before the donor graft infusion suppresses the bone marrow and thus decreases the number of peripheral blood mononuclear cells (PMNCs) available to determine IMPDH activity. Various nonradioactive methods using chromatographic separations have been used to quantify XMP, the catalytic product of the enzyme, to indirectly evaluate IMPDH activity. Only recently were mass spectrometry–based detection methods, which provide more specificity and sensitivity, reported for XMP quantitation [8]. Here we report a liquid chromatography–mass spectrometry (LC-MS) method to measure recipient pretransplant IMPDH activity in PMNCs (ie, ex vivo) based on the quantification of XMP formation normalized by cell count. We evaluated and validated this method in PMNC lysates from healthy volunteers and nonmyeloablative HCT recipients. We also evaluated factors associated with recipient pretransplant IMPDH activity and the association of recipient pretransplant IMPDH activity with clinical outcomes in nonmyeloablative HCT recipients.

METHODS

Participant Characteristics

From November 2008 to February 2012, 105 patients participated in a prospective ancillary biomarker study in nonmyeloablative HCT recipients who received either a related or unrelated donor graft. Study participation did not influence the HCT procedure, including the conditioning regimen or postgrafting immunosuppression. Participants receiving fludarabine monophosphate (fludarabine) and total body irradiation (TBI) conditioning with postgrafting immuosuppression of a calcineurin inhibitor (cyclosporine or tacrolimus) with MMF were eligible to participate. This protocol was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center (Protocol 1980, Clinicaltrials.gov identifier NCT00764829). Written informed consent was obtained from all participants before study procedures. Participant characteristics are summarized in Table 1.

The conditioning regimen (summarized in Supplemental Figure 1) comprised fludarabine (30 mg/m^2/day i.v.) from day −4 to day −2 (cumulative dose 90 mg/m^2) followed by a single fraction of 2 to 4.5 Gy TBI on day 0 [1]. In general, the postgrafting calcineurin inhibitor was either cyclosporine or tacrolimus given through day +177, although some participants also received sirolimus as part of postgrafting immunosuppression. MMF, at a dose of 15 mg/kg, was given at 2 different dose frequencies, either 3 times a day (every 8 hours) to unrelated graft recipients or twice a day (every 12 hours) to related graft recipients. Adjusted ideal body weight [10] was used to determine MMF dosing, and all doses were rounded to the nearest