Phase I Clinical Trial of the Inosine Monophosphate Dehydrogenase Inhibitor Mycophenolate Mofetil (Cellcept) in Advanced Multiple Myeloma Patients

Naoko Takebe,[1] Xiangfei Cheng,[1] Suhlan Wu,[1] Kenneth Bauer,[1,2] Olga G. Goloubeva,[1] Robert G. Fenton,[1] Meyer Heyman,[1] Aaron P. Rapoport,[1] Ashraf Badros,[1] John Shaughnessy,[4] Douglas Ross,[1,3] Barry Meisenberg,[1] and Guido Tricot[4]

[1]University of Maryland Greenebaum Cancer Center, [2]University of Maryland School of Pharmacy, and [3]Department of Veterans' Affairs Medical Center, Baltimore, Maryland; and [4]University of Arkansas, Myeloma Institute for Research and Therapy, Little Rock, Arkansas

ABSTRACT

Purpose: **Inosine monophosphate dehydrogenase (IMPDH) inhibitors have been used to induce leukemia blast cell differentiation but have not been tested in multiple myeloma for activity. Currently, available IMPDH inhibitor, mycophenolate mofetil (MMF), which is known as an immunosuppressant, was shown to induce apoptosis in myeloma cell lines. On the basis of our preclinical studies, we designed a clinical study to test our hypothesis that MMF has antimyeloma activity.**

Experimental Design: **A Phase I MMF dose escalation study was conducted in relapsed and refractory myeloma patients who had documented disease progression by myeloma markers or bone marrow plasmacytosis to determine the maximum tolerated dose, toxicities, and efficacy of the drug. To assess the activity of IMPDH inhibition in the myeloma cells of patients, we measured intracellular nucleotide triphosphate levels by high-performance liquid chromatography-based analysis and examined the correlation with clinical response.**

Results: **Among the 11 study patients, MMF was generally well tolerated and was administered up to a maximum dose of 5g/day. The most common toxicity was grade 1 fatigue ($n = 4$, 36%). One patient had a partial response (3g/day), four patients had stable disease, and six patients had progression of disease. There was a statistically significant difference in the intracellular dGTP level changes between the stable disease/partial response group *versus* progression of disease.**

Conclusions: **MMF at 1 to 5 g/day daily dose is well tolerated by patients with relapsed and refractory multiple myeloma patients. Positive correlation between clinical response and depletion of intracellular dGTP level was shown. Future drug development to target this enzyme maybe useful in treating myelomas.**

INTRODUCTION

Proliferation and survival of multiple myeloma cells is regulated through cytokine-mediated signal transduction pathways, adhesion molecules, angiogenic factors, and overexpression of antiapoptotic proteins (1). Hypothetical molecular targeting therapies have been proposed by blocking an interleukin-6–dependent pathway to reduce the expression of bcl-xL by inhibition of either Janus-activated kinase or signal transducers and activators of transcription 3 (2). Blocking only one signaling pathway may not be sufficient to inhibit multiple myeloma cell proliferation or induce apoptosis. Thus, the ideal therapy includes targeting multiple pathways associated with the promotion of cell survival and proliferation. Although an increased number of therapeutic approaches have been developed to these molecules and receptors, multiple myeloma is still an incurable disease. Thus, it is essential to conduct studies to look for alternative apoptosis-inducing therapeutic approaches.

Mycophenolate mofetil (MMF; Cellcept), an immunosuppressive drug, is currently used for prevention of organ or hematopoietic stem cell transplant rejection. It has also shown efficacy in rheumatoid arthritis and psoriasis trials (3). Mycophenolic acid is the active metabolite of the MMF, which is a selective inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH catalyzes the rate-limiting reaction of *de novo* GTP biosynthesis at the inosine monophosphate metabolic branch point, and inhibition of this enzyme leads to the reduction of xanthine monophosphate (4). Consequently, inhibition of IMPDH activity depresses GTP and dGTP concentrations and results in inhibition of cell proliferation (Fig. 1). IMPDH inhibition has potent cytostatic effects on both T- and B-lymphocytes because they are critically dependent for their proliferation on *de novo* synthesis of purines (5). By contrast, other cell types can use salvage pathways. IMPDH levels are reportedly higher in B- than T-lymphocytes and in acute leukemia blast cells compared with more differentiated mixed bone marrow cells (6).

IMPDH activity is increased significantly in cancer cells, and it is considered a sensitive target for chemotherapy (7, 8). The biological impact of inhibition of *de novo* GTP synthesis

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Requests for reprints: Naoko Takebe, University of Maryland, Greenebaum Cancer Center, 655 West Baltimore Street, BRB7-029, Baltimore, MD 21201; Phone: (410) 328-6870; Fax: (410) 328-1975; E-mail: ntakebe@som.umaryland.edu.

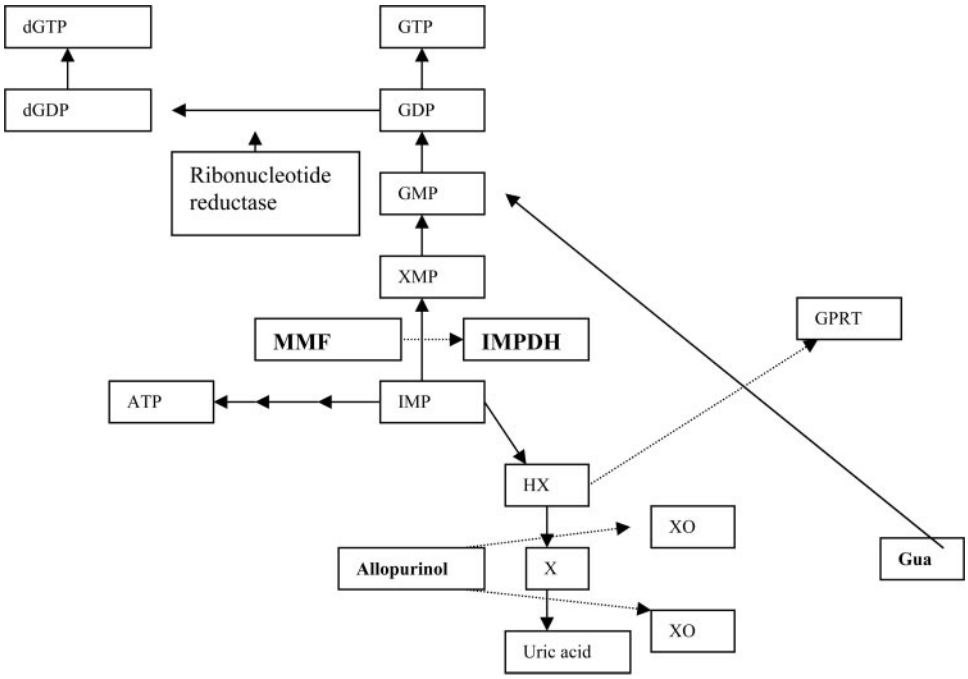

Fig. 1 IMPDH inhibitor MMF and xanthine oxidase inhibitor allopurinol treatment resulting in a decrease in GTP concentration in mononuclear cells. *Dash lines* represent blocking of the pathway by enzyme inhibition. (*IMP*, inosine monophosphate; *GPRT*, guanine phosphoribosyltransferase; *Gua*, guanine; *HX*, hypoxanthine; *X*, xanthine; *XO*, xanthine oxidase)

includes blocking the activity of activation of G proteins (including Ras), tubulin, protein elongation, and cyclic GMP (9). Human tumor cells, including MCF-7, K562 cells, and HL-60 cells, were induced into terminal differentiation by IMPDH inhibitors, which implicated that alterations in the activity of IMPDH and the levels of guanine nucleotides regulated cell growth and differentiation (10). Previously, a number of investigators studied the induction of differentiation in nonlymphocytic leukemia cells by IMPDH inhibitors (11–14). Among them, the most promising agent called tiazofurin (2-b-D-ribofuranosylthiazole-4-carboxamide), which selectively blocks IMPDH activity (14), was studied in a clinical Phase I/II in leukemia patients. The study showed a good correlation between biochemical parameters (decline in IMPDH activity and GTP concentrations in blast cells) and clinical response (14). Unfortunately, this compound was not tested additionally in the clinic because of its toxicity profile.

IMPDH inhibition and its effect on cell proliferation in multiple myeloma cells has not been studied to our knowledge. IMPDH2 gene expression in plasma cells was recently shown with cDNA microarray method. On the basis of the cDNA microarray data, we found that IMPDH2 gene expression in myeloma patient samples was elevated compared with the normal control plasma cells (Fig. 2). Multiple myeloma represents a B-cell malignancy characterized by a monoclonal proliferation of plasma cells, which may be dependent on *de novo* synthesis of purines similar to normal B-lymphocytes. We also showed that MMF induced apoptosis in multiple myeloma cell lines, which could be salvaged by guanosine administration (15). On the basis of our *in vitro* studies, we conducted a Phase I study of

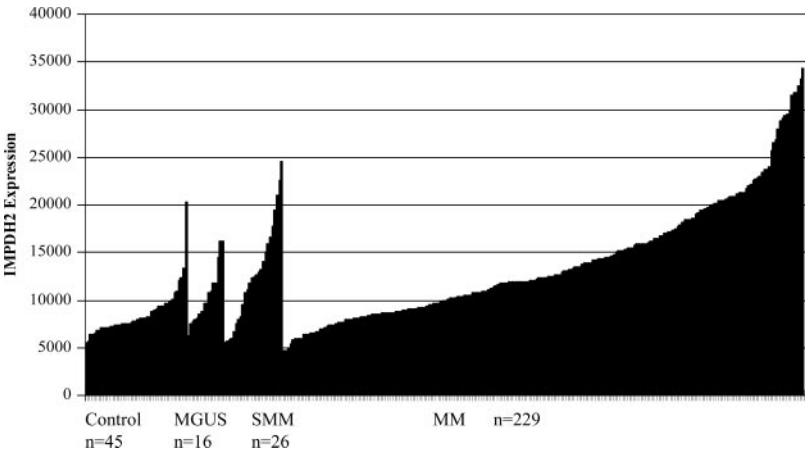

Fig. 2 IMPDH2 gene expression in plasma cells from 45 control subjects, 16 patients with monoclonal gammopathy of undetermined significance, 26 patients with smoldering multiple myeloma, and 229 patients with multiple myeloma. The Affymetrix signal, a quantitative measure of gene expression, is indicated on the *Y* axis. The level of expression of IMPDH2 in each sample is indicated by the height of the *bar*. Samples are ordered from the lowest to highest level of expression of IMPDH2 gene from left to right on the *X* axis. (*MGUS*, monoclonal gammopathy of undetermined significance; *SMM*, smoldering multiple myeloma; *MM*, multiple myeloma)

MMF in relapsed multiple myeloma patients with correlative laboratory studies to measure intracellular nucleotide triphosphate concentrations from patients' samples as a biomarker for IMPDH inhibition.

The present study is based on our hypothesis that MMF inhibits tumor growth of multiple myeloma cells and induces apoptosis by IMPDH inhibition. The objectives of this study were to determine the following: (1) the maximum tolerated dose and the response rate in advanced refractory multiple myeloma patients to MMF; and (2) the relationship between intracellular nucleotide triphosphate levels changes and treatment response with MMF.

PATIENTS AND METHODS

Patient Criteria. Eligible patients were those with multiple myeloma that had relapsed after therapy with standard or investigational regimen or had refractory disease. Enrollment criteria included Karnofsky performance score ≥60, serum asparatate aminotransferase <3.0 × the upper limit of normal, serum alkaline phosphatase <3.0 × the upper limit of normal, direct bilirubin <2.0 mg/dl, no known central nervous system abnormality, and no radiation therapy within 3 weeks of treatment. Patients were required to have measurable disease in bone marrow, or extramedullary sites, and monoclonal proteins in serum and/or urine. Patients with one or more of the following findings were excluded: absolute neutrophil count <1000/μl, platelet count <50,000/μl, and hypocellular bone marrow with <30% estimated cellularity except for pancytopenia secondary to disease progression. If patients experienced a grade 4 toxicity or developed progressive disease after 4 weeks of treatment, MMF therapy was discontinued. Written informed consent was obtained from each participant, and the trial was approved by the University of Maryland School of Medicine Institutional Review Board.

MMF (Cellcept; Roche Laboratories Inc., Nutley, New Jersey), an immunosuppressive agent approved by Food and Drug Administration for organ transplantation and a potent inhibitor of IMPDH, was administered orally twice daily for 4 weeks. The twice-daily dosing regimen was based on Phase I clinical studies in heart and renal transplantation patients showing the half-life of an active metabolite of MMF to be 16 to 18 hours after oral administration. Allopurinol 300 mg orally twice daily was also given to the patient on MMF to block the guanine salvage pathway (16). Allopurinol administration was used to maintain hypoxanthine levels between 40 to 80 μmol/L, which was considered an adequate level to block guanine phosphoribosyltransferase (Fig. 1). The study medication doses were self-administered by the study subjects. Cohorts of three patients were to be treated at each of the planned dose levels of 1, 2, 3, and 5 g/day divided into twice a day. Patients entered at each dose level were followed for 4 weeks before proceeding to enrollment at the next dose level. Toxicities were graded prospectively according to National Cancer Institute Common Toxicity Criteria. Dose escalation was to be halted if 1 of 3 subjects at a dose level developed a grade 3 nonhematologic toxicity deemed related to MMF. If 2 of 3 subjects at a dose level developed a grade 3 nonhematologic toxicity deemed related to MMF, 3 more subjects were to be treated at the next lower dose

Table 1 Distribution of clinical and demographics characteristics (n = 11)

Factor	Frequency
Best response	
PD	6
SD	4
PR	1
Gender	
Male	9
Female	2
Race	
Black	5
White	6
Age at treatment	
Median (range)	63 (55, 74)
Type myeloma	
IgG (kappa or labda)	8
IgA (kappa or labda)	2
IgM (kappa)	1
Stage at diagnosis	
IIIB	4
IIIA	5
IIA	2
Karyotype *	
Normal	6
Multi	3
13q-	2
Number of previous treatments	
1	1
2	3
3	3
4	2
5	2
Number of previous transplant	
0	2
1	6
2	3
Dosage	
1	3
2	3
3	3
5	2
Status	
Dead	6
Alive	5
Renal function	
CrCl <30	2
CrCl >=30	8
Hemodialysis	1
Karnofsky performance status	
Median (range)	90 (70, 90)
β2 = microglobulin	
Median (range)	3.8 (1.3, 27.0)
M = protein (serum)	
>=2 g/dl	4
1–2 g/dl	4
<1 g/dl	3
M = protein (urine)	
1–2 g/dl	1
<1 g/dl	9
Bone marrow plasmacytosis	
>=30%	7
10–30%	2
<10%	2

Abbreviations: PD, progression of disease; SD, stable disease; PR, partial response; CrCl, creatinine clearance.

* Assessed by metaphase analysis.

level. *Pneumocystis carinii* prophylaxis and herpes virus prophylaxis were implemented for subjects with CD4 cell counts <400/μl unless the subject was intolerant or declined. Concomitant use of other investigational agents was not permitted during the study.

All of the patients had a prestudy evaluation within 2 weeks of enrollment. The evaluation included measurements of serum quantitative immunoglobulins, serum protein electrophoresis with immunofixation, and 24 hours urine for total protein, protein electrophoresis, and immunofixation. Also included were, β2-microglobulin, complete blood count, serum electrolytes, liver enzymes, CD4/CD8 levels, and urinalysis. Bone marrow aspiration and biopsy, skeletal survey, chest X-ray, and peripheral blood and bone marrow aspiration samples for laboratory correlative studies were obtained. The same evaluation was repeated after 4 weeks of drug treatment. Patients were evaluated for efficacy, safety, and adverse events every week while receiving the study drug for the initial 4 weeks and every 2 to 4 weeks thereafter. Study endpoints were evaluation of safety and assessment of multiple myeloma disease activity. Assessments of clinical progression of multiple myeloma included new soft tissue plasmacytosis, increase in size of lytic lesions, rising serum calcium level, increasing urine and serum M-proteins by immunofixation, and increasing marrow plasmacytosis.

For patients who achieved a response, response duration was defined as the time from best response (complete or partial) to documented disease progression, death because of any cause or last patient contact. Responses were scored according to the criteria published by Blade *et al.* (17). A partial response was defined as reduction of ≥75% serum myeloma protein production, decrease in monoclonal marrow plasmacytosis to <5%, decrease in Bence-Jones proteinuria by ≥90%, and no new lytic bone lesions or soft tissue plasmacytoma. The status of partial response was confirmed by repeat assessment 6 weeks after the criteria for response was first met. Stable disease was defined as no change in patient status at a minimum interval of 6 weeks. Laboratory correlative studies included measurement of nucleotide triphosphate levels by high-performance liquid chromatography (HPLC) with bone marrow samples of patients from pretreatment and 4 weeks post-treatment.

Statistical Analysis. Two related samples, *i.e.*, pre- and post-treatment values for intracellular nucleotide triphosphate levels, CD4/CD8 levels, and peripheral blood counts parameters were compared with the nonparametric Wilcoxon signed rank test. The Spearman correlation test was applied to estimate the correlation between mycophenolic acid level and changes in dGTP values from patients' samples. Fisher's exact test was used to assess the relationship between best overall response and MMF dose or chromosomal abnormalities. The differences between pre- and post-treatment nucleotide triphosphate levels were compared with nonparametric Kruskal-Wallis test. *P*s reported are two-sided.

Determination of Intracellular Nuclear Triphosphates. We modified and optimized the previously published method (13, 18) for the simultaneous determination of intracellular ATP, GTP, dGTP, and xanthine monophosphate with 8226 and HL60 cell lines. An HPLC method has been developed to separate the four nucleotides of interest. HPLC was done with a Beckman solvent delivery model 110B, Beckman absorbance detector model 406, and Beckman 506A autosampler (Beckman Instruments, Inc., San Ramon, CA). Separation was accomplished with a YMC ODS-AQ column (25 cm × 4.6 mm i.d.; Waters Corp., Milford, MA) with an isocratic mobile phase of 0.2 mol/L ammonium phosphate (23g/L; pH 4.5) at a flow rate of 1 mL/minute. The retention times for xanthine monophosphate, ATP, GTP, and dGTP were 15.09, 11.56, 5.81, and 13.68, respectively. Analytes were detected by UV absorbance at 254 nmol/L, with a quantifiable range of 1.25 to 100 μmol/L for all of the analytes. ATP, GTP, dGTP, and xanthine monophosphate stock standards were at 1 mg/mL concentration in mobile phase (Sigma Chemical Co., St. Louis, MO) and stored at −20°C protected from light. All of the cell suspensions were kept on ice for 1 hour between isolation and nucleotide extraction. Cell counts were obtained with Levy and Levy Hausser counting chamber directly before nucleotide extraction. Cell pellets were stored at −70° 176°C until analysis. Cell pellets (10^6 cells) were resuspended in 60 μL of ice-cold 1 × PBS plus 60 μL of 1 N NaOH. Then, the cells were kept in the 37°C water bath for 30 minutes, followed by neutralization with 60 μL of 1 N HCl, and centrifuged at 10,000 × *g* for 10 minutes at 4°C. Next, the mobile phase was added to the nucleotide mixture to a total volume of 260 μL. From this mixture, 100 μL of aliquot was obtained and injected into HPLC using a microinsert containing injection vial.

Table 2 Summary statistics for the laboratory variables

Marker	Response: SD + PR median (range *) nmol/L/10^6 cells	Response: PDmedian (range) nmol/L/10^6 cells
ATP		
Pre	.466 (.093, 2.579)	.399 (.012, .552)
Post	.458 (.140, .915)	.236 (−.061, 2.514)
XMP		
Pre	1.446 (.149, 17.384)	−.046 (−.252, .552)
Post	.242 (.197, 2.836)	.733 (.198, 14.525)
GTP		
Pre	.07 (−.089, .366)	−.125 (−.335, .082)
Post	−.028 (−.185, .0150)	−.342 (−.455, 1.506)
dGTP		
Pre	2.795 (.886, 5.041)	.146 (.130, .208)
Post	.31 (.107, 2.203)	.577 (.084, 9.983)

Abbreviations: SD, stable disease; PR, partial response; PD, progression of disease; XMP, xanthine monophosphate.

* Range is the interval between minimum and maximum values.

RESULTS

Patients and Toxicity. Twelve patients were enrolled between April 6, 2001 and August 27, 2002. All of the patients showed disease progression at the time of enrollment. One patient received only 1 week of treatment at a dose of 1 g/day, developed no clinical adverse events, but refused to return for follow-up visits, and elected to discontinue the study drug. The patient was excluded from analysis. The entry characteristics of the 11 remaining patients are summarized in Table 1. The data analysis contains one patient at a dose level of 3 g/day who developed progressive disease including hypercalcemia during week 3 to 4 and was taken off the study.

The pre- and post-treatment measurements of intracellular

Table 3 Clinical adverse events and laboratory toxicity

	MMF dose level											
	1 g/day ($n = 3$)			2 g/day ($n = 3$)			3 g/day ($n = 3$)			5 g/day ($n = 2$)		
Toxicity grade	1	2	3	1	2	3	1	2	3	1	2	3
Event												
Fever	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0	0	0	0	0
Night sweats	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue	2	0	0	1	0	0	1	0	0	0	0	0
Nausea	0	0	0	0	0	0	0	0	0	0	0	0
Rash	0	0	0	0	0	0	0	0	0	0	0	0
Herpes zoster	0	1	0	0	0	0	0	0	0	0	0	0
Thrombocytopenia	0	0	0	0	0	0	0	0	0	0	0	1
CD4 count	1	0	0	0	0	0	1	1	0	1	1	0
Hemoglobin	0	0	0	1	0	0	1	0	0	1	0	0
Neutropenia	0	0	0	0	0	0	0	0	0	1	0	0

nucleotide triphosphate levels in blood ($n = 1$) and bone marrow ($n = 8$) are summarized in Table 2. MMF administration was well tolerated. Adverse events are summarized in Table 3. One patient at a dose of 1 g/day developed herpes zoster grade 2. We started prophylactic antiviral therapy for all of the patients after this event. The most common toxicity was fatigue, grade 1, which occurred in four patients. Interestingly, patients who did not develop fatigue during the study actually reported subjective improvement, including increased energy level and increased appetite.

Hematologic toxicities were infrequent (Table 3). Five patients treated between 1 to 5 g/day level showed a decrease in CD4 count (grade 1 and 2) at week 4. MMF is an agent known to be immunosuppressive, and CD4 changes were thus expected. However, no significant differences were found in pre- and post-treatment CD4 and CD8 levels (*P*s 0.15 and 0.29, respectively). One patient at a 5 g/day level had IgM of 5,260 mg/dl and M-protein of 3.1 g/dl, developed grade 3 thrombocytopenia at week 2, and was taken off study because of disease progression and possible drug toxicity. However, thrombocytopenia of patients did not improve after discontinuation of treatment and, thereafter, was thought to be related to disease progression of disease. There were no significant changes identified in white blood cell level, hemoglobin count, and platelet count during MMF administration (*P*s 0.39, 0.67, and 0.95, respectively).

For the four patients who stabilized their disease, the median time to disease progression was 105 days. One patient had no changes in his disease status after a 4-week course of MMF at a 2 g/day and continued to be stable on MMF for another 9 months. One of the patients at the 3 g/day dose showed a decrease in IgG M-protein level by 25% from pretreatment after a 4-week course of MMF. He achieved 50% reduction of M-protein in 8 weeks on continuing MMF. The patient showed additional response to the treatment thereafter; however, after 4 months of treatment, the patient progressed with an extramedullary soft tissue relapse.

MMF metabolite levels such as mycophenolic acid (the active metabolite of MMF) and mycophenolic acid glucuronide (MPAG; a metabolite of mycophenolic acid, not pharmacologically active, and its level increases with impaired kidney function) were measured in six patients including one patient on chronic hemodialysis during the study. The result of mycophenolic acid level, MMF doses, dGTP level, and clinical results are summarized in Table 4. The optimal serum concentration for graft *versus* host disease prophylaxis dose is 1.0 to 3.5 μg/mL for mycophenolic acid and 35 to 100 μg/mL for MPAG (19). One patient at a 1 g/day level who stabilized his disease had a mycophenolic acid level of 4.4 μg/mL and a MPAG of 51 μg/mL. This patient had a pretreatment creatinine clearance of 27 mL/minute. Another patient who was on hemodialysis received MMF 1 g/day daily had a mycophenolic acid level of 2.9 μg/mL and a MPAG of 66 μg/mL. He showed progressive disease. One patient at a 2 g/day level had a low mycophenolic acid level of 0.6 and MPAG of 40 with normal renal function. He developed progressive disease and also did not show a decrease in levels of dGTP or GTP despite compliance with treatment regimen. Two patients at the 3 g/day level had mycophenolic acid of 7.3 and 6.9 and MPAG of 105 and 132, respectively. Both patients had stable disease and showed decreasing levels of dGTP and GTP, and particularly one patient had a partial response within 2 months of initiation of MMF as described above. One patient at a 5 g/day level had mycophenolic acid of 6.2 μg/mL and MPAG of 142 μg/mL. This patient showed decrease in GTP and dGTP levels with stabilizing disease.

There was not sufficient evidence to conclude that an association existed between drug dose and clinical response to treatment (Table 5; Kruskal-Wallis test; Monte Carlo estimate of $P = 0.79$. Patients with multiple metaphase chromosomal abnormalities (five patients) were compared with 6 patients without chromosomal abnormalities; 3 of 5 (60%) and 3 of 6 (50%) patients, respectively, had disease progression, and no difference in response to therapy was found. Patients who progressed had higher bone marrow plasma cell percentage ($P = 0.03$). The β2 microglobulin levels pretreatment were not different between the nonresponders (6 of 11) and patients with stable disease/partial response (5 of 11; $P = 0.20$).

Table 4 Clinical response data as a suppression of dGTP levels in BM or peripheral blood mononuclear cells and serum MPA levels

Patient/MMF dose (g)	dGTP changes (post − pre)	MPA level	Response
1/1	9.853	N/A *	PD
2/1	0.255	4.4	SD
3/1	−0.062	2.9	PD
4/2	1.183	N/A	PD
5/2	−2.838	N/A	SD
6/2	0.369	0.6	PD
7/3	N/A	N/A	PD
8/3	−0.185	7.3	SD
9/3	−0.596	6.9	PR
10/5	N/A	N/A	PD
11/5	−0.737	6.2	SD

Abbreviations: BM, bone marrow; MPA, mycophenolic acid; N/A, not applicable; PD, progression of disease; SD, stable disease; PR, partial response.

∗ Peripheral blood or BM samples were not available from these patients.

Table 5 Number of patients per dose-response combination

Dose	Best response		
	PD	SD	PR
1	2	1	0
2	2	1	0
3	1	1	1
5	1	1	0

Abbreviations: PD, progression of disease; SD, stable disease; PR, partial response.

High-Performance Liquid Chromatography-Based Nucleotide Measurements from Bone Marrow and Peripheral Blood as a Surrogate Marker for Inosine Monophosphate Dehydrogenase Inhibition. Because we hypothesized that MMF-induced apoptosis in multiple myeloma cells is because of the inhibition of IMPDH, resulting in depletion of intracellular GTP and dGTP level, we quantified intracellular nucleotide triphosphate levels by HPLC method as described previously (4, 13, 18, 20). We optimized the assay condition and modified the methods previously published as described in Patients and Methods. Instead of measuring IMPDH levels directly, we developed a more clinically applicable method as a surrogate marker for IMPDH inhibition in multiple clinical samples obtained from either total bone marrow or peripheral blood mononuclear cells. Eight bone marrow samples of patients were analyzed. We used peripheral blood samples in one of our patients whose bone marrow samples were insufficient. The result of HPLC assay from bone marrow and peripheral blood samples were combined, and the pre- and post-treatment measurements in blood and bone marrow ($n = 9$) are summarized in Table 2. The remaining two patients had neither bone marrow nor peripheral blood samples obtained post-treatment and therefore were not included in the analysis. Two related samples such as pre- and post-treatment values for each marker were compared with nonparametric Wilcoxon signed rank test. None of the ATP, xanthine monophosphate, GTP, or dGTP changed because of the treatment, *P*s range from 0.359 to 1.000. There were five patients (45%) who received doses of 3 and 5 g/day. Only three of these five patients were analyzed. There was an excellent correlation between serum mycophenolic acid level and change in dGTP values ($P < 0.0001$). We split nine patients who had data available into two groups, five (55%) responders (stable disease and partial remission) *versus* four (45%) nonresponders (progression of disease). The differences between ATP, xanthine monophosphate, GTP, and dGTP pre- and post-treatment values were compared between these two patient groups. The change in level of dGTP was significantly different for responders *versus* nonresponders ($P = 0.03$; Fig. 3, *A* and *B*). No statistically significant difference in any other marker was found (*P* range from 0.14 to 0.33). We also tested whether patients who developed toxicity had a different magnitude of change in levels of ATP, xanthine monophosphate, GTP, and dGTP when compared with those who did not have any toxicity. No differences were observed; also, no clear dose/effect correlation was observed. The Kruskal-Wallis test was applied to assess whether patients who received low dose MMF (1 and 2 g/day, 6 patients) experienced lesser changes in level of the four different nucleotides when compared with three patients who received 3 and 5 g/day of the MMF. We did not find sufficient evidence of a difference.

DISCUSSION

This article is the first report of MMF (Cellcept), a potent IMPDH inhibitor, being tested in a Phase I study in patients with relapsed multiple myeloma. MMF was developed as a prodrug of mycophenolic acid to improve its bioavailability (21). MMF is approved for clinical use in the prevention of acute allograft rejection after organ transplants and has shown efficacy in autoimmune diseases (22). Previous studies have shown that mycophenolic acid inhibits DNA synthesis in T- and B-lymphocytes by blocking *de novo* guanosine synthesis and that mycophenolic acid induces monocyte differentiation. The mechanism of T-cell apoptosis induction by inhibition of IMPDH is through the depletion of GTP and dGTP in activated human peripheral blood lymphocytes (23). One study additionally delineated the mechanism of apoptosis in human CEM leukemia cells induced by this IMPDH inhibitor and found that it was related to the inhibition of primer RNA synthesis that was directly induced by GTP depletion alone (24).

Presently, a few IMPDH inhibitors are used as clinical agents such as ribavirin (antiviral) and MMF (antirejection; ref. 25). Agents such as tiazofurin (antitumor), selenazofurin (anti-

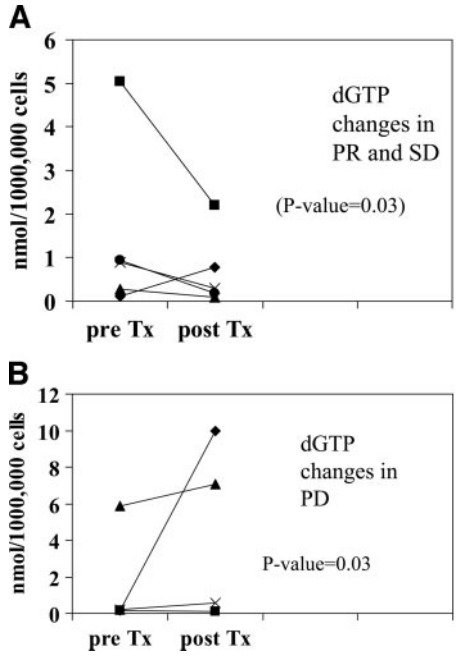

Fig. 3 *A* and *B*. Five patients with stable disease or partial response (*A*) and 4 patients with progression of disease (*B*) after 4 weeks of MMF trial were grouped to analyze intracellular dGTP changes from pre- and post-treatment samples obtained from bone marrow as described in Patients and Methods. In *A*, patient (*pt*)2 at 1g/day —◆—, pt5 at 2 g/day —■—, pt8 at 3 g/day —▲—, pt9 at 3 g/day —×—, and pt11 at 5 g/day —●— . In *B*, pt1 at 1 g/day —◆—, pt3 at 1 g/day —■—, pt4 at 2 g/day —▲—, and pt6 at 2 g/day —×—. The dGTP changes were statistically significant ($P = 0.03$) in both groups. (*SD*, stable disease; *PR*, partial response)

tumor), and benzamine riboside (antitumor) were tested previously (25). Acute leukemia is probably one of the best studied tumors for IMPDH inhibitors, which induce differentiation of blast cells through a nucleotide pool depletion mechanism (12, 13, 16). Recent cDNA microarray analysis data (Fig. 2) provides a strong rationale for testing IMPDH targeting drugs such as MMF in myeloma patients as well.

We conducted a Phase I dose escalation study in advanced relapsed myeloma patients to evaluate the toxicity and clinical response to MMF. HPLC-based cellular nucleotide triphosphate measurements were done on bone marrow cells to correlate clinical responses with nucleotide level changes caused by the IMPDH inhibitor MMF. In the dose range tested, we found that MMF was well tolerated even at the maximum planned dose of 5 g/day. Among 11 refractory myeloma patients, one patient had a partial response (9%), and four patients had stable disease (36%). The remaining patients had disease progression during the first 4 weeks of treatment. Among the five patients with either stable disease or partial response, four patients reported significant subjective improvements.

The major reason for lack of association between drug dose and clinical response is probably the few patients included in this study. Other explanations are related to a difference in drug clearance and metabolism within an individual patient, which lead to a variable range of mycophenolic acid level. As shown in Table 4, patient 2 who achieved stable disease took only 1 g/day dose, but her mycophenolic acid level was elevated to 4.4 μg/mL. This patient had a decreased creatinine clearance of 27 mL/minute. Additionally, the drug dose was not adjusted for body surface area or body weight, which is commonly used for anticancer agent dosing. For instance, patient 6 who developed disease progression while on MMF 2 g/day with his mycophenolic acid level at 0.6 μg/mL had a body surface area of 2.3 *versus* patient 2 at 1.7. Because of these problems, we started to look at the level of mycophenolic acid and the clinical response of patients. There is a plausible association between an elevated mycophenolic acid level and best response to treatment (Fisher's Exact test, $P = 0.07$). Statistical significance is marginal, probably because of a small sample size (six patients).

However, we found a significant positive correlation between mycophenolic acid levels and decrease in dGTP levels ($P < 0.0001$). For future studies with MMF as an antimyeloma agent, it is crucial to adjust MMF dose depending on measured mycophenolic acid level in an individual patient to keep mycophenolic acid level above 3.5 μg/mL. Statistically significant correlation between dGTP changes and clinical response suggests that bone marrow or peripheral blood-derived mononuclear cell dGTP levels may become a useful marker for IMPDH-targeted myeloma treatment response. A study with larger number of patients will be required to confirm this result. We did not separate the myeloma cells of patients from bone marrow mononuclear cells. Therefore, dGTP changes from pre- and post-treatment may not necessarily reflect intracellular level of dGTP in myeloma cells.

Although, a strong rationale exists for using IMPDH inhibitors in patients with multiple myeloma, there may be several explanations for the absence of significant clinical effect in this trial. First, poor clinical response could possibly be because of the patient population enrolled into this trial. Enrolled patients all had advanced disease and had received multiple treatments before the study, including autologous transplants.

Second, depletion of intracellular guanylates, especially by inhibition of *de novo* synthesis via the IMPDH pathway, is known to be a potent signal for inhibition of proliferation and induction of apoptosis. However, if IMPDH expression is downregulated by the p53 tumor suppressor gene (25), or if IMPDH inhibition may be salvaged by a guanine salvage pathway (Fig. 1; ref. 16), we may not see any significant clinical response as the result of IMPDH inhibition. We do not know the p53 status in our patients. However, p53 mutations in myeloma are very infrequent. Although, all of the patients received allopurinol 300 mg by mouth twice a day during the study period to inhibit the guanine salvage pathway (Fig. 1), the inhibition may not have been complete. Anticipating these concerns, we included the laboratory correlative study to measure intracellular nucleotide triphosphate levels from treated patients.

A third and perhaps the most plausible explanation is that possibly multiple myeloma cell growth is most likely controlled by multiple pathways, and it may be extremely difficult for inhibition of a single pathway to result in clinical efficacy. We found that MMF induces apoptosis partly because of the mitochondrial pathway, and therefore myeloma cells with high levels of antiapoptotic proteins may be resistant to MMF-induced apoptosis.

If more potent IMPDH inhibitors are available in the future, it may be possible to overcome antiapoptotic mechanism. Interestingly, a new compound, VX-944, which is a novel IMPDH inhibitor and 3- to 40-fold more potent than MMF, has been tested in lymphoid and multiple myeloma cell lines and primary acute myelogenous leukemia samples for apoptosis induction *in vitro* (26, 27).

In myeloma therapy, antimetabolites are not commonly used. This study reports the first potential IMPDH-targeted drug in this area. As described previously, MMF induces apoptosis partly through the mitochondrial pathway, suggesting that combining it with bcl–2 antisense may overcome MMF-resistant cells to undergo apoptosis. Dexamethasone could be combined with MMF because this agent is relatively well tolerated and an effective drug for myeloma. It also exerts its antiapoptotic activity through the mitochondrial pathway (28). Similarly, arsenic trioxide (As_2O_3) is a mitochondrial poison (29). Combination with thalidomide should be tested because this drug has a totally different mechanism of action from MMF, and toxicities are not overlapping. If MMF proves to be effective in myeloma, it should also be combined with high-dose melphalan, the most active agent in myeloma. Thus, our data adds evidence that inhibition of IMPDH pathway may be a promising novel target therapy to overcome drug resistance in multiple myeloma.

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