Use of Pharmacogenetics, Enzymatic Phenotyping, and Metabolite Monitoring to Guide Treatment with Azathioprine in Patients with Systemic Lupus Erythematosus

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ABSTRACT. *Objective.* Individualized therapy based on genetic background and monitoring of metabolites can optimize drug safety and efficacy. Such an approach is available for azathioprine (AZA), the thiopurine antimetabolite. AZA exerts therapeutic effects when metabolized to the active thiopurine nucleotide, 6-thioguanine (6-TGN). In inflammatory bowel disease (IBD), 6-TGN levels in the target range of 235–400 pmol/8×10^8 red blood cells (RBC) are associated with a high likelihood of response. Our objective was to evaluate whether drug escalation based on metabolite levels improves efficacy and maintains safety in patients with systemic lupus erythematosus (SLE).

Methods. We conducted a 6-month open-label dose-escalation clinical study of patients with active SLE treated with azathioprine dosed by body weight and metabolite levels. The primary endpoint was ≥ 50% improvement in any one parameter of disease activity, or 50% decrease in glucocorticoid dose.

Results. Of 50 patients enrolled in the study, 21 achieved clinical responses, 13 of whom had 6-TGN < 235 pmol/8×10^8 RBC. Ten patients had no clinical response at 6 months, yet achieved either therapeutic IBD 6-TGN levels (> 235, n = 4) or received maximum AZA dose ≥ 3.5 mg/kg (n = 6). In 19 patients the drug was discontinued prematurely due to side effects or SLE activity. For those patients in whom either liver function test or white blood cell count abnormalities were encountered, metabolites guided attribution to drug or disease activity.

Conclusion. Clinical responses in SLE can occur at levels of 6-TGN lower than the target range established for IBD. During followup, measurements of AZA metabolites may provide a rational approach to safety. (First Release Dec 1 2008; J Rheumatol 2009;36:89–95; doi:10.3899/jrheum.070968)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS   AZATHIOPRINE   METABOLITE
6-THIOGUANINE   MONITORING   TREATMENT

Azathioprine (AZA, Imuran®) is approved by the US Food and Drug Administration (FDA) for the treatment of rheumatoid arthritis and has been widely used in the treatment of systemic lupus erythematosus (SLE). It is administered during the active stages of the disease as a remittive agent[1], and during periods of quiescence to prevent exacerbations[2]. While AZA is most often considered in the context of a steroid-sparing agent, its use is much more extensive, including virtually every clinical manifestation of SLE. However, in clinical practice the medication has been observed to fall short of expectations, a factor that may relate to underdosing due to concerns of toxicity.

AZA is an inactive prodrug that requires intracellular metabolism in order to produce the active thiopurine nucleotides, 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMPN)[3] (Figure 1). The principal mechanism for the cytotoxic and immunosuppressive effects of this drug is thought to be via incorporation of 6-TGN into nucleic acids and inhibition of purine synthesis.

Recent studies in patients with inflammatory bowel disease (IBD) suggest that concentrations of active drug metabolites, not the absolute dose of AZA, are associated

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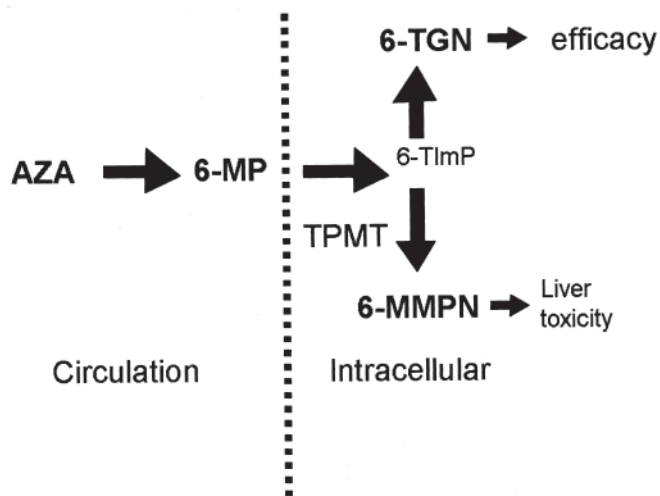
Figure 1. Thiopurine metabolism. Azathioprine (AZA) is rapidly convert-ed to 6-mercaptopurine (6-MP) by an extracellular nonenzymatic process. 6-MP is metabolized intracellularly by thiopurine methyltransferase (TPMT) via 6-thioinosine 5'-monophosphate (6-TImP), yielding 6-methylmercaptopurine ribonucleotides (6-MMPN) and active nucleotide metabolite 6-thioguanine nucleotides (6-TGN).

with clinical responses[4-7]. The efficacy of AZA appears to be optimized at 6-TGN concentrations > 235 pmol/8×10^8 red blood cells (RBC)[4], whereas 6-TGN levels > 400 pmol/8×10^8 RBC are associated with higher risk of bone marrow toxicity. Hepatotoxicity correlates with elevated 6-MMPN levels (> 5700 pmol/8×10^8 RBC)[4]. Moreover, as reported in patients with IBD, inability to achieve therapeutic 6-TGN levels despite dose escalation over time may be due to shunt-ing of 6-mercaptopurine towards the overproduction of 6-MMPN[8]. Therefore, persistence of subtherapeutic 6-TGN and excessive potentially toxic levels of 6-MMPN biochem-ically could also characterize a subgroup of lupus patients resistant to AZA therapy. Finally, low to absent enzyme activity of thiopurine methyltransferase (homozygous $TPMT^L/TPMT^L$), the key enzyme in AZA metabolism to 6-MMPN, is associated with potentially life-threatening bone marrow toxicity[9], and is a contraindication to thiopurine drugs in such individuals[10].

Accordingly, our study was initiated to test the hypothe-sis that in patients receiving AZA for lupus activity, efficacy can be enhanced by serial measurements of 6-TGN levels, aiming for a therapeutic "window" with a target range of 235–400 pmol/8×10^8 RBC. We predicted that dose escala-tion of AZA to achieve 6-TGN levels in the desired target range demonstrated for IBD would improve the likelihood of therapeutic response. In addition, we predicted that metabolite monitoring would provide data regarding com-pliance with drug dosing. To achieve these goals, an open-label dose escalation titrating 6-TGN levels to clinical response in 50 lupus patients with moderate disease activity was undertaken at 2 sites.

MATERIALS AND METHODS

Study design. This was a 6-month open-label dose-escalation clinical study of patients with active SLE treated with AZA based on body weight and metabolite levels. Since AZA is not FDA approved for the treatment of SLE, the study was initially reviewed by the agency and conducted under an Investigational New Drug application. The study was also approved by the institutional review boards.

The primary outcome measure was an improvement of ≥ 50% on the Responder Index for Lupus Erythematosus (RIFLE)[11] or a decrease by 50% in the dose of glucocorticoids (prednisone or equivalent) while main-taining or decreasing the score on the SELENA-SLEDAI (Safety of Estrogens in Lupus Erythematosus: National Assessment–SLE Disease Activity Index) composite[12-14]. Secondary outcomes included: (1) the fre-quency of mild-moderate and severe flares, as defined by SELENA-SLEDAI[12-14]; (2) the mean dose (range) of AZA necessary to achieve a clinical response as defined for the primary outcome in SLE patients with and without heterozygous mutations of TPMT; (3) the level of 6-TGN at which patients met the primary outcome; and (4) the correlation between side effects and metabolite concentrations in patients with SLE.

The RIFLE is a comprehensive instrument that assesses the organ sys-tems affected by SLE, and specifically examines both the extent of disease activity and response to treatment[11]. The RIFLE was selected because it specifically incorporates objective definitions of partial and complete responses to treatment. At screening, the investigators prospectively select-ed for each patient the specific disease manifestation on the RIFLE deemed the most clinically significant and necessitating treatment. The primary out-come for each subject was response of this prospectively identified disease manifestation. A clinically successful outcome for each subject was achievement of a "partial response" or a "complete response" in the pri-mary RIFLE manifestation as specifically predefined by the instrument. Nonresponders were patients that either (1) did not achieve a 50% improve-ment in their primary SLE manifestation at the end of the study period, or (2) experienced a flare after achieving a stable therapeutic dose of AZA as guided by the data from patients with IBD.

Patient selection. Inclusion criteria consisted of all the following: (1) ful-fillment of at least 4 of the American College of Rheumatology criteria for the diagnosis of SLE[15]; (2) active disease in a specific organ as defined by the RIFLE; (3) inability to taper prednisone; (4) diagnosis of SLE for at least 1 year prior to screening; and (5) willingness to comply with the fre-quency of visits and blood draws. Patients were excluded if they were receiving other immunosuppressants. Drugs known to inhibit TPMT activ-ity (salicylic acid and its derivates, sulfasalazine, and furosemide) were allowed, but use of these medications had to be stable for at least 1 month prior to entering the study. Other exclusion criteria included (1) SLE-relat-ed encephalopathy and psychosis; (2) liver transaminases greater than twice the normal value; (3) absolute neutrophil (PMN) count < 1000; (4) absolute lymphocyte count < 500; (5) active infections; and (6) $TPMT^L/TPMT^L$ genotype, associated with low to absent levels of TPMT enzyme activity (and therefore high risk of severe myelotoxicity). Fifty-four patients were screened for participation in the study between March 2003 (when the first patient was enrolled) and October 2004 (completion of the study) in 2 cen-ters, New York University (NYU)/Hospital for Joint Diseases (HJD), New York, and Cedars-Sinai Medical Center, Los Angeles. Patients were recruit-ed from the SLE clinic at HJD, NYU Medical Center, and Cedars Sinai Medical Center. Of the patients seen in these settings about 10% met inclu-sion criteria. Screening failure occurred in 4 of the 54 patients. Fifty patients were enrolled in the study.

Assessment and treatment. At screening, a detailed history and physical examination were performed. Laboratory tests included TPMT genotype and enzyme levels, 6-TGN and 6-MMPN metabolite levels (for background assessment), and complete blood count; and liver function tests (as part of a complete metabolic panel), amylase/lipase, urinalysis (dipstick and microscopic examination), and 24-hour urine collection for measurement of creatinine clearance and protein excretion. Pregnancy tests were done if

indicated. Serologic profiles included measurement of anti-double-stranded DNA antibodies and C3/C4 (at a local commercial laboratory). Patients were seen at an interim visit 1 week after screening and medication was started. Prometheus Laboratories (San Diego, CA, USA) provided AZA (50 mg Imuran tablets); none of the patients were given generic AZA. For patients with TPMT[H]/TPMT[H] genotype, 2 mg/kg AZA, and for patients with TPMT[H]/TPMT[L] genotype, 0.75 mg/kg AZA were prescribed. Two weeks after initiation of therapy, patients were asked to return for repeat blood tests. The dose was escalated by 0.5 mg/kg for homozygotes TPMT[H]/TPMT[H] or 0.25 mg/kg for heterozygotes TPMT[H]/TPMT[L], and up to a maximum dose of 3.5 or 1.75 mg/kg, respectively. Drug dosage was maintained and the patient entered the maintenance phase if the patient achieved one of (1) a partial or complete clinical response at any dose of AZA regardless of 6-TGN level; or (2) target levels of 6-TGN (235–400 pmol/8 × 10^{8} RBC), so even if doing poorly, patients could choose to continue that dose of AZA and not take additional medication; or (3) maximum AZA dose for respective TPMT genotype/kg. Two weeks after each dose escalation, a complete blood count with differential, kidney and liver function tests, amylase/lipase, urinalysis, urine pregnancy test, and 6-TGN and 6-MMPN levels were performed. Once the dose was maintained, patients were followed monthly for 4 months with no adjustment to treatment. The total evaluation time for each patient was between 4 and 8 months. A schematic diagram of the study visits and assessments performed at each visit is presented in Figure 2.

The study stopping rules included any of the following: (1) development of severe flare as defined by the SELENA-SLEDAI composite; (2) any clinical measure for which the physician prescribed an increase in the dose of prednisone by > 10 mg or increased the absolute steroid dose to > 0.5 mg/kg or prescribed additional immunosuppressive therapy; or (3) side effects.

RBC concentrations of 6-TGN and 6-MMPN metabolites were measured by Prometheus Laboratories using commercial assays, which included proprietary modifications of a reversed-phase high-performance liquid chromatography (HPLC) assay[16]. Levels were run blinded to the clinical information. TPMT enzyme activity was measured by Prometheus Laboratories using commercial TPMT assays, which included proprietary modifications of published HPLC methods[16]. TPMT levels > 23.7 indicated normal enzyme activity. All individuals were genotyped for 2 polymorphisms in TPMT transitions[17,18] at position 460 (G460A, Ala to Thr) and at position 719 (A719G, Tyr to Cys) using a polymerase chain reaction allele-specific oligonucleotide hybridization assay[19]. These mutations were used to define 3 distinct alleles: TMPT 3*A (both G460A and A719G), TMPT 3*B (only G460A), and TMPT 3*C (only A719G). By screening for these mutations, it was determined whether the patient was homozygous for the wild-type high-activity alleles (TPMT[H]/TPMT[H]), heterozygous with one wild-type and one low-activity allele (intermediate enzyme activity, TPMT[H]/TPMT[L]), or homozygous for the low-activity alleles (TPMT[L]/TPMT[L]).

Analysis. The proportion of responders with target 6-TGN levels was compared with the proportion of nonresponders with target 6-TGN using Pearson's chi-square test. Data were analyzed for all patients for whom outcome information was available. Differences in the baseline characteristics between the 2 groups were evaluated with the 2-sample t-test for continuous variables and the chi-square test for categorical variables. Correlation between AZA dose and 6-TGN levels was assessed using Pearson's correlation method. All p values were calculated as 2-sided with $p < 0.05$ considered significant.

RESULTS

A total of 54 patients were screened and 50 were enrolled into the study.

The racial and ethnic background as determined by patients was 23 (46%) Caucasians, 11 (22%) Hispanics, 13 (26%) African Americans, and 3 (6%) Asians. The baseline
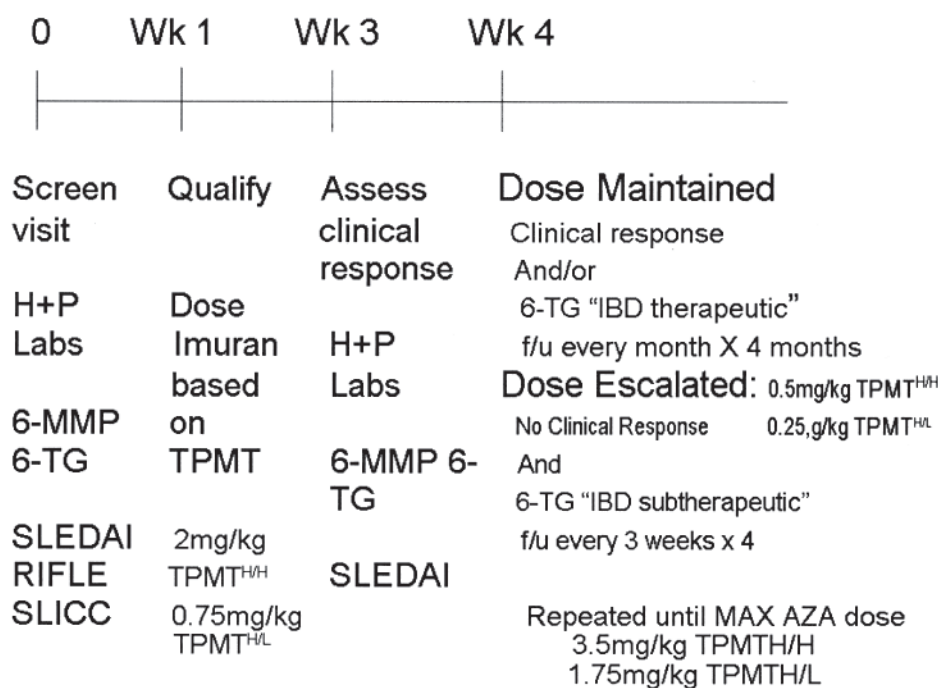
Figure 2. Visits and evaluations were performed as described in the text. Beginning at Week 3, clinical assessments were performed to gauge efficacy. H+P: history and physical examination, Labs: laboratory evaluations, 6-MMPN and 6-TGN are azathioprine metabolites, SLEDAI: SLE Disease Activity Index, RIFLE: Responder Index for Lupus Erythematosus, SLICC: Systemic Lupus International Collaborating Clinics, IBD: inflammatory bowel disease, f/u: followup.

clinical characteristics, clinical manifestations, and the selected primary outcomes (as determined by the investigator) of these 50 patients are presented in Table 1. The distribution of TPMT genotypes was: 47 (94%) TPMT^H/TPMT^H, 3 (6%) TPMT^H/TPMT^L, and none TPMT^L/TPMT^L. The average TPMT concentration in patients with TPMT^H/TPMT^L was 13, and in patients with TPMT^H/TPMT^H this was 30.

Patients' progression through the study is presented in Figure 3. As shown in Figure 3 and Table 2, 31 patients (out of 50) met at least one of the 3 criteria for entry into the dose-maintenance phase. Specifically, 21 achieved a clinical response (responders) and their mean AZA dose was 3 mg/kg; 10 did not achieve clinical response (nonresponders). Four of the 10 nonresponders achieved IBD therapeutic 6-TGN, while 6 were escalated to 3.5 mg/kg yet achieved

Table 1. Characteristics of patients with SLE enrolled in the study.

Characteristic	
Mean age, yrs	41.1 ± 1.1 (22–70)
Sex, F/M, n (%)	51/3 (94.4/5.6)
Race, n (%)	
Caucasian	24 (44.4)
African American	13 (24.1)
Hispanic	13 (24.1)
Asian	4 (7.4)
Clinical manifestations at screening*, n; primary outcome no. (%)	
Arthritis	41; 38 (76)
Rash	12; 6 (12)
Proteinuria	3; 1 (2)
Serositis	1; 1 (2)
Myositis	1; 1 (2)
Thrombocytopenia	1; 1 (2)
Inability to taper prednisone	2; 2 (4)
Average prednisone dose, mg (range)	7.72 (0–60)
Average SLEDAI at screening	7.04
Plaquenil use, n (%)	33 (66)

* Some patients had more than one clinical manifestation at screening.

neither a clinical response nor a therapeutic 6-TGN level. When baseline clinical characteristics were compared between responders and nonresponders (Table 2) the only significant difference was in their initial prednisone dose, 10 mg compared to 2 mg daily, respectively. This difference in steroid use may have contributed to the clinical response in the absence of statistical differences in TGN levels between the groups, especially in patients with arthritis.

At the end of the 4-month maintenance period, of the 21 patients that achieved a therapeutic response, 2 discontinued AZA: one for an allergic rash, the other for low absolute lymphocyte count (ALC). However, the 6-TGN level in the patient with low ALC was 266, suggesting that the decreased ALC was not likely related to AZA. For the 21 patients, the mean 6-TGN level during the maintenance phase was lower than target (159, range 3–562 pmol/8 × 10^8 RBC). However, 8 had 6-TGN IBD therapeutic levels on at least one occasion. Of the 10 nonresponder patients, 4 had therapeutic 6-TGN. For nonresponders, the mean 6-TGN level was 202 (range 3–612) pmol/8 × 10^8 RBC.

There was wide inter- and intra-patient variability in 6-TGN levels. In responders the mean 6-TGN was 196 ± 26 SEM pmol/8 × 10^8 RBC at the start of the maintenance phase and 127 ± 16 SEM pmol/8 × 10^8 RBC at the end of the study. In nonresponders, levels were 160 ± 39 SEM pmol/8 × 10^8 RBC at the beginning of the maintenance phase and 196 ± 60 SEM pmol/8 × 10^8 RBC at the end.

The study was terminated prior to the maintenance phase in 19 patients: 6 had SLE flares (3 severe, 3 mild-moderate); 6 patients had gastrointestinal intolerance (nausea, vomiting, diarrhea, bloating), one of whom also had a low absolute neutrophil count (ANC; 6-TGN was only 21 pmol/8 × 10^8 RBC, suggesting that the low ANC was due to SLE activity, not AZA); 3 patients had liver function test abnormalities (their 6-MMPN levels were 8,141, 14,358, 12,033, suggesting a likely causal relationship with AZA);

Figure 3. Patients' progression through the study, showing flare events and outcomes. AZA: azathioprine, GI: gastrointestinal, LFT: liver function tests, ANC: absolute neutrophil count, ALC: absolute lymphocyte count.

Table 2. Clinical characteristics of responders and nonresponders.

Characteristic	Responders, n = 21	Nonresponders, n = 10	p
Mean age, yrs	39.3 ± 2.1	45.3 ± 4.7	0.06
Female	20	10	NS
Race, n (%)			
Caucasian	6	7	NS
African American	5	2	NS
Hispanic	8	1	NS
Asian	2	0	NS
Arthritis as outcome (%)	16 (76)	9 (90)	NS
History of SLE nephritis (%)	1 (5)	2 (20)	NS
Average prednisone dose, mg	9.88	2.0	0.03*
Average SLEDAI	7.28	7.2	NS
Plaquenil use, n (%)	17 (80)	6 (60)	NS
6-TGN "IBD therapeutic" ≥ 1 occasion (%)	8 (38)	4 (40)	NS
AZA ≥ 3.5 mg/kg (%)	13 (62)	6 (60)	NS
Average 6-TGN, maintenance	159 ± 11	202 ± 27	NS
Average 6-MMPN, maintenance	5166 ± 665	6044 ± 1234	NS

* Statistically different, $p < 0.05$. P values were calculated using unpaired t test for continuous variables and chi-square and Fisher's exact test for categorical variables. NS: nonsignificant.

and one patient each had an allergic rash, zoster, candida esophagitis, and cervical cancer.

Throughout the study there were 8 disease flares, as shown in Figure 3. There were 4 patients in whom the initial manifestation worsened during the trial, requiring prednisone at 1 mg/kg, i.e., they experienced a severe flare (1 nephritis, 1 cytopenia, 1 arthritis, and 1 severe rash). The patient with escalating proteinuria (SLE nephritis) had achieved a therapeutic 6-TGN level. The other 3 patients deteriorated prior to entering dose maintenance. Four other patients with mild-moderate flares required prednisone at < 0.5 mg/kg, and none of them achieved a therapeutic TGN level.

The mean dose of AZA necessary to achieve an adequate clinical response in patients without heterozygous mutations (homozygous for the high-producing alleles) of TPMT (n = 19) was 203 mg/day (range 125–300 mg/day), while in patients with the heterozygous TPMT mutation this dose was 125 mg/day (n = 2).

The correlation between AZA dose and 6-TGN and 6-MMPN levels at each dosing point is shown in Table 3.

Serious adverse events requiring discontinuation of study medication occurred in 46% (n = 23) of the patients (Table 4). In 3 patients there was more than a doubling of the liver transaminases. In each, 6-MMPN levels exceeded the established threshold for hepatotoxicity, > 5700 pmol/8 × 10^{8} RBC[4,8], suggesting a causal relationship.

DISCUSSION

Thiopurine methyltransferase is a key catabolic enzyme in the metabolism of thiopurine drugs. A representation of the AZA/6MP metabolic pathway is shown in Figure 1[5]. TPMT exhibits individual variation as a result of a known genetic polymorphism of its alleles. One in 300 (0.3%) individuals (Caucasians) have low to absent enzyme activity (homozygous TPMT[L]), 11% have intermediate activity (heterozygous TPMT[H]/TPMT[L]), and 89% have normal to high levels of activity (homozygous/wild-type TPMT[H])[9]. The proportion of heterozygotes in our cohort was somewhat lower than expected based on the known distribution of TPMT genoptypes in Caucasians. However, our study involved a limited number of cases in a heterogeneous population, with Caucasians representing 44%.

The average dose of AZA required for therapeutic responses was 3 mg/kg, corresponding to ~200 mg/day. This dose is higher than that currently used in clinical practice (100–150 mg/day). Only 5 of 19 patients achieved a therapeutic response at 100–150 mg/day. The mean level of 6-TGN associated with efficacy for SLE, 159 pmol/8 × 10^{8} RBC, was lower than that validated in IBD[7], which was > 235 pmol/8 × 10^{8} RBC. Based on the dose-response curve (Table 3), it is possible that a portion of patients with SLE never achieve IBD therapeutic 6-TGN concentrations. Despite the lower levels of 6-TGN there was clinical efficacy, which further supports the notion that there are many more variables that ultimately determine responsiveness to thiopurines[20].

Inter-patient 6-TGN variability has been documented[20,21]. The reasons for this variability may include concurrent medications, mode of administration (milk interferes with bioavailability), or noncompliance. Although patients were continuously counseled to report any change in medication, including over the counter nonsteroidal antiinflammatory drugs (which are known inhibitors of TPMT), strict control of patient self-therapy can be challenging in patients with arthritis. Self-therapy may be particularly relevant for

Table 3. Correlation between the azathioprine (AZA) dose and 6–TGN and 6-MMPN levels.

Genotype		AZA Dose, mg/kg	Mean 6–TGN (range), pmol/8 × 10⁸ RBC	Mean 6–MMP (range), pmol/8 × 10⁸ RBC
TPMT^H/TPMT^H	N = 47 P = 47	0	7 (0–52)	270 (192–1001)
	N = 54 P = 47	2	127 (3–680)	3816 (181–10665)
	N = 61 P = 35	2.5	185 (3–612)	4406 (223–17424)
	N = 45 P = 23	3	160 (3–540)	5627 (748–18924)
	N = 56 P = 15	3.5	136 (7–295)	8255 (184–20934)
Pearson correlation coefficient between AZA dose and 6–TGN = 0.39, $p < 0.0001$				
TPMT^H/TPMT^L	N = 3 P = 3	0	5 (3–9)	228 (199–250)
	N = 3 P = 3	0.75	79 (72–93)	235 (217–244)
	N = 3 P = 3	1	154 (123–199)	407 (221–754)
	N = 3 P = 3	1.25	218 (191–224)	489 (220–757)
	N = 11 P = 3	1.5	205 (82–350)	410 (254–788)

N: number of determinations of metabolite levels. P: number of patients. As the dose was escalated patients that achieved clinical response were maintained at the same dose. Subsequently, the number of determinations at higher doses is different from the number of patients. RBC: red blood cells.

Table 4. Adverse events.

Type of Event	N = 24 (in 23 patients)
SLE flares	4 severe flares
	4 mild to moderate flares
GI intolerance (nausea, vomiting, bloating, epigastric pain)	6
LFT abnormalities, possibly related to high 6–MMPN	3, 6–MMPN = 8141, 14358, 12033
Allergic rash	2
ALC < 250	1, 6–TGN = 266
ANC < 500, most likely not due to high 6–TGN	1, 6–TGN = 21*
Zoater	1
Cervical cancer	1
Candida esophagitis	1

* This patient also had GI intolerance. GI: gastrointestinal, LFT: liver function tests, ANC: absolute neutrophil count, ALC: absolute lymphocyte count.

interpretation of this study since patients may have been taking these medications at the time of study enrollment, thus accounting for the higher 6-TGN at the start of maintenance in responders[20,21].

The measurement of metabolites of AZA has been shown to be useful to individualize and optimize IBD therapy for over a decade[22]. A recent metaanalysis confirmed the clinical utility of a target range for 6-TGN levels between 235 and 400 pmol/8 × 10⁸ RBC in IBD[7]. AZA metabolite monitoring is also successfully employed for other clinical disorders, such as autoimmune hepatitis[23], leukemia[24], and solid organ transplantation[25]; however, its use has not been widely applied in the clinical setting of SLE. The target range of desired metabolite levels varies between these clinical indications, and needs to be established by adequately powered prospective studies.

Based on our study, several conclusions regarding the use of genotyping and metabolite measurement in SLE appear justified. Prior to initiation of AZA, genotyping or phenotyping TPMT activity serves to eliminate treating patients (0.3%) with homozygous deficiency, given the unacceptably high risk of potentially life-threatening bone marrow toxicity[10]. This FDA recommendation applies to any indication for use of AZA or 6MP[26]. Moreover, identification of heterozygotes with reduced TPMT activity (11%) facilitates safe, adequate dosing. In individuals with normal TPMT activity (89%), dosing should be based on body weight, 2–4 mg/kg, as the routine dose of 100–150 mg daily provides

inadequate 6-TGN levels. However, our data demonstrate that clinical responses in SLE do not consistently correlate with IBD therapeutic 6-TGN levels, as there were 13 patients that achieved responses despite persistently sub-therapeutic TGN levels. In contrast, 4 of 10 patients achieved IBD therapeutic 6-TGN levels, or maximum AZA (> 3.5 mg/kg, 6 of 10 patients), and did not have clinical responses. During followup, 6-TGN and 6-MMPN metabolite measurements are effective in optimizing AZA therapy in SLE. Finally, routine metabolite measurements are perhaps most important when choosing the appropriate course of therapy for nonresponders and/or patients suspected of incomplete compliance. Identification of low metabolite levels may imply a need to increase dosage, while the absence of detectable metabolites clearly indicates a need to counsel the patient regarding the importance of adherence to therapy in lieu of mistakenly concluding that the drug is ineffective. For patients in whom either liver function test or WBC abnormalities are encountered, metabolites guide attribution to drug or disease activity.

Several limitations of our study are acknowledged. This was an open-label, non-placebo-controlled, nonrandomized study. Hence, it cannot definitely establish either a therapeutic dose or a metabolite range. The investigators selected patients based on their potential benefit from the addition of AZA to their therapeutic regimen.

Our data suggest that serial measurements of azathioprine metabolites are best used for managing compliance and attributing toxicity. Further study is required to identify the ideal target range of 6-TGN in SLE.

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