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A. Wilson
Western University

L. E. Jansen
London Health Sciences Centre

R. V. Rose
Western University

J. C. Gregor
Western University

T. Ponich
Western University

See next page for additional authors

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HLA-DQA1-HLA-DRB1 polymorphism is a major predictor of azathioprine-induced pancreatitis in patients with inflammatory bowel disease

A. Wilson[1,2,3] | L. E. Jansen[4] | R. V. Rose[5] | J. C. Gregor[2] | T. Ponich[2] | N. Chande[2] | R. Khanna[2] | B. Yan[2] | V. Jairath[2,5] | N. Khanna[2] | M. Sey[2] | M. Beaton[2] | K. McIntosh[2] | W. A. Teft[1] | R. B. Kim[1]

[1]Division of Clinical Pharmacology, Department of Medicine, Western University, London, ON, Canada

[2]Division of Gastroenterology, Department of Medicine, Western University, London, ON, Canada

[3]Department of Physiology and Pharmacology, Western University, London, ON, Canada

[4]London Health Sciences Centre, London, ON, Canada

[5]Department of Epidemiology and Biostatistics, Western University, London, ON, Canada

Correspondence
Dr. R. B. Kim, Division of Clinical Pharmacology, Department of Medicine, Western University, London, ON, Canada.
Email: richard.Kim@lhsc.on.ca

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Summary

Background: Azathioprine (AZA)-induced pancreatitis is an unpredictable and dose-independent adverse event affecting 2%-7% of patients with inflammatory bowel disease (IBD) patients treated with AZA. There are no tools in clinical practice to identify at-risk individuals; however, a genome wide association study (GWAS) identified a strong association between the Class II HLA gene region polymorphism (rs2647087) and thiopurine-induced pancreatitis.

Aim: To independently confirm the findings of the GWAS in an IBD cohort, to evaluate its utility in clinical practice and to offer a novel AZA treatment algorithm for IBD based on pharmacogenomic principles.

Methods: A retrospective cohort study evaluated 373 AZA-exposed IBD patients from a tertiary care academic centre in London, Canada. Due to the limited number of patients taking mercaptopurine (MP), such patients were not included this cohort. All subjects underwent screening for the single nucleotide polymorphism (SNP) rs2647087 mapped to the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype and were sub-divided based on the presence (n = 13) or absence (n = 360) of an AZA-induced pancreatitis diagnosis. The risk of AZA-induced pancreatitis was assessed based on rs2647087 genotype.

Results: The risk of pancreatitis during AZA-therapy was highly predictable and genotype dependent: 0.53% for wild type (A/A), 4.25% (OR = 4.19, 95% CI 1.02-36.45, $P = 0.044$) for heterozygous (A/C), and 14.63% (OR = 15.83, 95% CI 3.80-145.26, $P = 0.0001$) for homozygous variant (C/C) patients.

Conclusions: The class II HLA region (at rs2647087) is an important marker of AZA-induced pancreatitis risk. We propose a simple and clinically implementable algorithm based on rs2647087 and TPMT genotypes for AZA selection and dosing for patients with IBD.

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1 | INTRODUCTION

Immunosuppressive therapy is integral to the management of inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC). Azathioprine (AZA) is one of several treatments used to manage the dysregulated inflammatory response of IBD.[1,2] Due to high costs associated with biologics, payers sometimes require patients to be resistant or intolerant to AZA prior to the approval of such agents. Currently, several international societies and federal agencies discourage AZA use as a monotherapy for IBD; however, its use as combination therapy with anti-TNF agents remains a gold standard in high risk IBD patients.[2-5]

AZA is associated with a few, but significant, toxicities that can limit its use in clinical practice. These include nausea, vomiting, myelosuppression, hepatotoxcity or acute pancreatitis.[6] AZA-induced pancreatitis is an idiosyncratic drug reaction that affects 2%-7% of AZA-treated patients with IBD.[6,7] Risk factors associated with its onset include cigarette smoking and glucocorticoid exposure; however, there are no predictive tests used clinically to identify patients at risk for AZA-induced pancreatitis.[7]

Recently, an association between the human leucocyte antigen (HLA) gene region and AZA-induced pancreatitis has been identified.[8] The genome wide association study (GWAS) recognised an association between AZA-induced pancreatitis and the single nucleotide polymorphism (SNP) rs2647087 that maps to the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype (odds ratio, OR, 2.59). Heterozygous (A/C) and homozygous (C/C) carriers of the variant allele had 2.5- and 5-fold higher risk of developing AZA-induced pancreatitis respectively compared to those who are homozygous for the common allele (A/A).[8]

Given the clinical relevance and potential impact of the findings with regard to the HLA-DQA1*02:01-HLA-DRB1*07:01 haplotype, the aim of our study was to validate the findings in a large and independent cohort of patients with IBD and to further delineate an IBD AZA treatment algorithm based on pharmacogenomic principles.

2 | MATERIALS AND METHODS

2.1 | Subjects

A retrospective cohort study was carried out in 373 individuals with IBD exposed to AZA. Patients taking mercaptopurine (MP) were not included in the final cohort. Subject recruitment took place between July 2012 and March 2017. This cohort was assembled from IBD patients (n = 591) being considered for AZA therapy who were referred to the London Health Sciences Centre Personalized Medicine Service (London, Canada) for thiopurine S-methyltransferase (TPMT) genotyping. Eligible subjects were 18 years of age or older and had a histopathological diagnosis of CD or UC. All subjects were required to have been prescribed AZA by their treating clinician and for their exposure to be documented in their patient chart. Control subjects (no history of AZA-induced pancreatitis) were required to be on AZA for a minimum of 6 months. Subjects diagnosed with AZA-induced pancreatitis were required to meet two of the following three criteria: a minimum elevation in serum lipase three times the upper limit of normal, clinical symptoms of nausea, vomiting and characteristic abdominal pain or radiological evidence of acute pancreatitis.[9] The diagnosis of pancreatitis was made by an independent clinician less than 3 months after exposure to azathioprine in the absence of other causative factors (alcohol, gallstones, other drugs causing pancreatitis, etc.). Severity of acute pancreatitis was classified based on the revised Atlanta criteria.[9] The study protocol was approved by the Western University Health Sciences Research Ethics Board. Data collected on all subjects included IBD diagnosis (CD, UC), age, sex, height, weight, smoking history, disease activity, and AZA response. Additional data specific to AZA-induced pancreatitis cases were collected including dose and duration of AZA exposure preceding the onset of acute pancreatitis, hospitalisation and duration, acute pancreatitis severity, biochemical parameters of pancreatitis, results of abdominal imaging, and concomitant medications. Subjects were followed up for a minimum of 6 months up to 4 years.

2.2 | Genotypic analysis

DNA was extracted from whole blood using a standard DNA extraction protocol (QIAmp DNA Mini Kit, Qiagen, Valencia, California) or using the MagNA Pure Compact instrument (Roche, Laval, Quebec, Canada). TaqMan allelic discrimination assay (assay id: C_16052296_10) (Applied Biosystems, Carlsbad, CA) was used to determine the presence of wild-type and/or variant alleles in the class II HLA gene region at rs2647087 mapped to the *HLA-DQA1*02:01-HLA-DRB1*07-01* haplotype in AZA-exposed IBD subjects (n = 373). Each genotyping experiment included three positive controls and one negative control. Five per cent of samples were genotyped in duplicate. All duplicated genotypes were congruent.

2.3 | Statistical analysis

Statistical analyses were performed using R statistical software with the "epitools" (Epidemiology Tools) package.[10,11] Genotype frequencies were compared using a 2 × 3 contingency table with a correction for small sample size and Fisher's exact test; allele frequencies were compared using a 2 × 2 contingency table and Fisher's exact test. Two-sided *P*-values, odds ratios (OR) and 95% confidence (CI) were calculated. *P*-values <.05 were deemed to be statistically significant.

3 | RESULTS

Figure 1 highlights patient selection based on the STROBE statement.[12] Three hundred and seventy-three subjects with IBD were exposed to AZA and included in this study (245 CD and 128 UC). Thirteen subjects were diagnosed with AZA-induced pancreatitis. Baseline characteristics and demographics are described in Table 1.

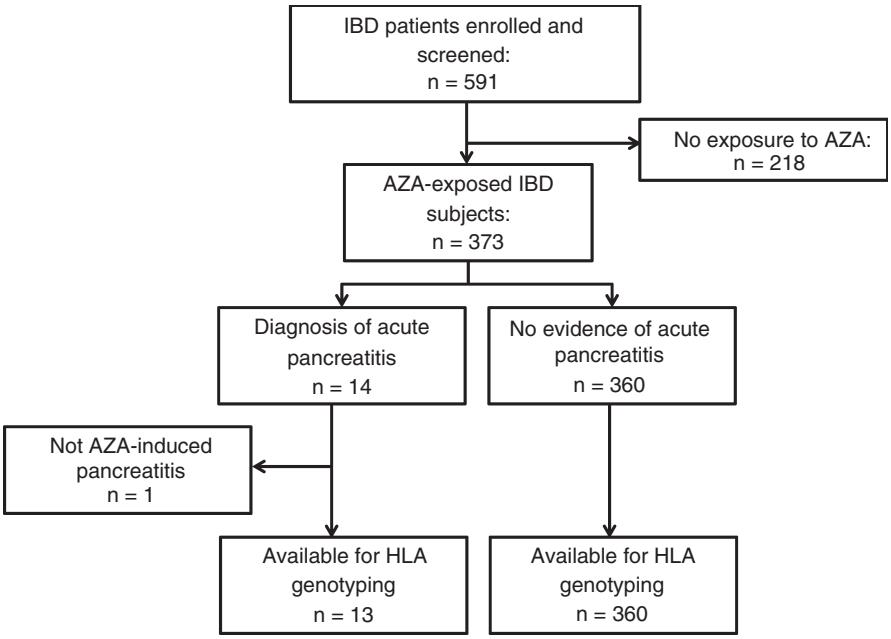

FIGURE 1 Patient selection pathway. Inflammatory bowel disease, IBD; azathioprine, AZA; months, mo

TABLE 1 Subject demographics

	Control	AZA-induced pancreatitis
N	360	13
CD (%)	234 (65)	11 (84.6)
Ileitis (%)	76 (21.1)	3 (23.1)
Ileo-colitis (%)	110 (30.6)	8 (61.5)
Colitis (%)	48 (13.3)	0 (0)
UC (%)	126 (35)	2 (15.4)
Pancolitis (%)	80 (22.2)	1 (7.7)
Left-sided colitis (%)	40 (11.1)	1 (7.7)
Proctitis (%)	6 (1.7)	0 (0)
Female (%)	196 (54.4)	7 (53.8)
Age (mean ± SD; y)	41.25 ± 15.98	46.15 ± 11.87
BMI (mean ± SD; kg/m^2)	26.19 ± 5.99	27.51 ± 7.31
Smoking history (%)	148 (41.1)	5 (38.5)
Disease duration (mean ± SD; mo)	104.79 ± 115.17	95.75 ± 109.18

AZA, Azathioprine; CD, Crohn's disease; UC, ulcerative colitis; BMI, body mass index; SD, standard deviation.

TABLE 2 Acute AZA-induced pancreatitis in an IBD cohort

Subject	AZA dose (mg)	Time to AP (d)	Severity of AP[a]	Smoker	Steroid exposure
1	50	22	Mild	Y	Y
2	125	26	Mild	Y	Y
3	200	9	Mild	N	N
4	300	14	Mild	N	Y
5	200	26	Mild	N	Y
6	100	16	Mild	Y	Y
7	50	14	Mild	N	N
8	50	28	Mild	Y	N
9	50	28	Mild	N	N
10	50	30	Mild	N	N
11	200	25	Mild	N	N
12	250	27	Mild	N	N
13	50	20	Mild	Y	N

AP, Acute pancreatitis; AZA, azathioprine; IBD, inflammatory bowel disease.
[a]Based on the revised Atlanta Criteria (2013).

Table 2 provides further description on the individuals diagnosed with AZA-induced pancreatitis. The mean time to the onset of acute pancreatitis from the time of first exposure to AZA was 21.9 ± 6.7 days. Subjects were exposed to AZA doses ranging from 50 mg to 300 mg, with a mean dose of 128.85 ± 90.05 mg. All cases of acute pancreatitis were mild based on the revised Atlanta criteria.[9] with no associated complications. All subjects required admission to hospital. The mean length of stay was 2 ± 1.63 days.

All subjects underwent genotyping for the class II HLA gene region at rs2647087. The genotype and minor allele frequencies classified by the presence or absence of acute pancreatitis are displayed in Table 3. Across the entire cohort, the HLA rs2647087 genotype was in Hardy-Weinberg equilibrium. There was a strong association between carriers of the variant C allele and AZA-induced pancreatitis (OR = 5.63, 95% CI 2.41-13.15, $P<0.0001$) (Figure 2). The absolute risk of AZA-induced pancreatitis in the total population was 3.49%, with a median time to pancreatitis of 25 days. However, when stratified by rs2647087 genotype, wild-type carriers (A/A) had an absolute risk of 0.53%, while heterozygous (A/C) and homozygous (C/C) variant carriers had absolute risks of AZA-induced pancreatitis of 4.25% (n = 6/135; OR = 4.19, 95% CI 1.02-36.45,

TABLE 3 Genotype and allele frequencies for HLA-DQA1-HLA-DRB1 rs2647087

HLA-DQA1-HLA-DRB1 rs2647087	No pancreatitis (n = 360)	Pancreatitis (n = 13)
Genotype, n (%)		
A/A	189 (52.5)	1 (7.7)
A/C	135 (37.5)	6 (46.15)
C/C	35 (9.7)	6 (46.15)
Allele frequency		
A	0.7125	0.3100
C	0.2847	0.6923

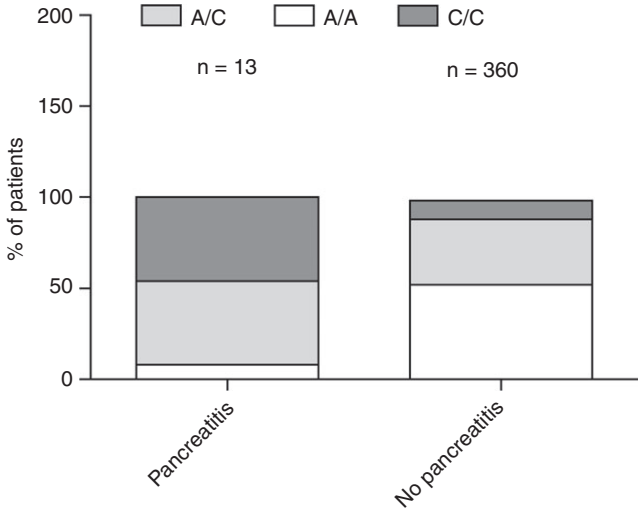

FIGURE 2 Genotype frequency stratified by pancreatitis diagnosis. Genotypes are expressed as a percentage of the total population of the pancreatitis cases (n = 13) and controls (n = 360). Number, n

P = 0.044) and 14.63% (n = 6/35; OR = 15.83, 95% CI 3.80-145.26, *P* = 0.0001) respectively. Of note, A/C and C/C genotypes were not associated with AZA-induced nausea and/or vomiting and/or abdominal pain in the absence of lipase elevation or clinically diagnosed pancreatitis.

4 | DISCUSSION

The use of AZA for the treatment of CD and UC has been well-documented over the last 4 decades. Drug-induced acute pancreatitis is an unfortunate, unpredictable and dose-independent complication that can arise with AZA exposure. The prevalence of AZA-induced acute pancreatitis in our IBD population (3.49%) aligns with the 2%-7% rate reported in the literature.[6,7] Pancreatitis appears to be mild in these individuals, without evidence of organ failure or lasting complication.

Our findings confirm an important role of the class II HLA gene region at rs2647087 as demonstrated in the GWAS by Heap et al.[8] However, we note that those who are wild-type (A/A) for the rs2647087 SNP appear to have a much lower than average risk for pancreatitis (0.54%). The study by Heap et al noted a 2.5- and five-fold higher risk of pancreatitis for those who are rs2647087 A/C or C/C compared to those who are A/A.[8] Our finding suggests the pancreatitis risk is in fact much higher, 4- and 15-fold for rs2647087 A/C and C/C patients respectively. Although patients on MP were not directly assessed in this study, azathioprine is a prodrug of MP. In the study by Heap et al patients on either azathioprine or MP had been enrolled and there was no indication that being on either medication alters the risk for pancreatitis.[8]

Heap et al[8] questioned whether or not this association suggests AZA-associated pancreatitis is a T-cell mediated response given the role of class II HLA molecules in T-lymphocyte activation. However, common genetic polymorphisms identified via GWAS cannot be assumed to causally explain disease phenotypes. These polymorphisms are often only "surrogates of 'unknown' causal factors [inherited in linkage disequilibrium]."[13] It cannot be inferred that a polymorphism in the class II HLA gene region plays a functional role in the onset of AZA-induced pancreatitis. Further mechanistic studies are needed to determine if polymorphisms within the class II HLA gene region play a functional role in the onset of AZA-induced pancreatitis.

Interestingly, this is not the first study to link polymorphisms in HLA molecules to adverse drug reactions. Variant genotypes in class II molecules occur more frequently in β-lactam-associated hepatotoxicity.[14,15] In addition, in a landmark trial by Mallal et al[16] carriers of the HLA-B*5701 allele had a marked increased risk for an abacavir-related hypersensitivity reaction, leading to significant patient morbidity and immediate withdrawal of an otherwise well-tolerated and effective treatment for the human immunodeficiency virus (HIV). It was concluded that prospective screening for this genotype among HIV patients eligible for abacavir was warranted for patient safety and cost-effectiveness. Currently, both professional society guidelines and the US Food and Drug Administration (FDA) recommend that patients be screened for the HLA-B*5701 allele as a companion diagnostic test before being initiated on therapy.[17] Although caution must be taken when interpreting these results given the small number of cases, this study highlights the potential utility of prospectively screening IBD patients being considered for AZA therapy for the rs2647087genotype given its predictive effect and the fact that other, potentially more efficacious, treatments are available for patients deemed to be higher risk for AZA-induced pancreatitis.

Accordingly, we propose a genotype-guided algorithm for the treatment of patients with IBD with AZA, which takes into account TPMT genotype as well as the class II HLA rs2647087 genotype (Figure 3). This would allow rs2647087 homozygous and even heterozygous variant carriers to be excluded from AZA therapy, minimising the risk of adverse drug effects and a more timely and systematic progression to other more effective IBD therapies. Patients who are homozygous for the rs2647087 wild-type allele (A/A) would be candidates for a trial of azathioprine, where the risk of myelosuppression is mitigated through TPMT genotype testing.

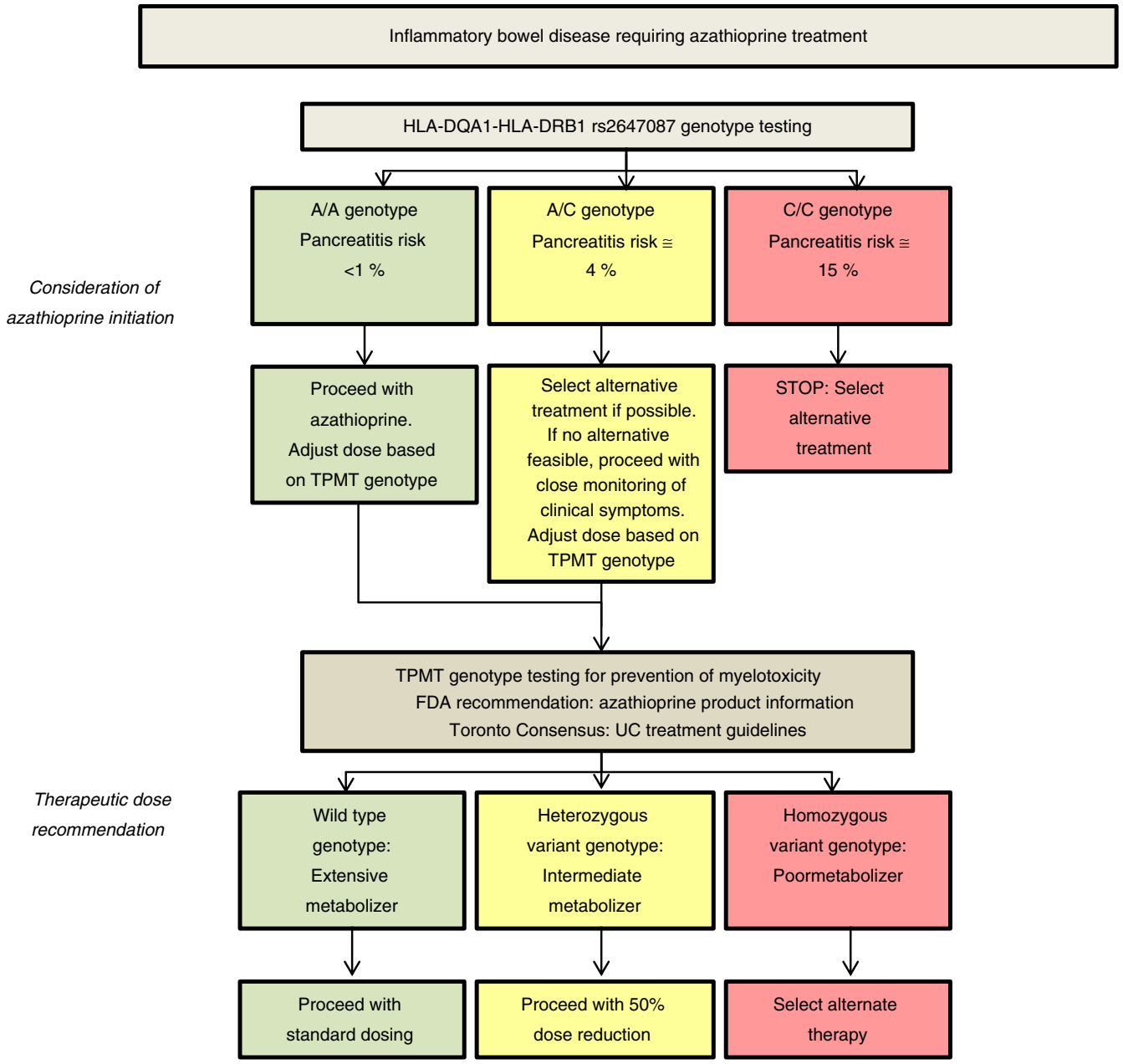

FIGURE 3 Proposed azathioprine treatment algorithm based on HLA rs2647087 and TPMT genotypes. Thiopurine S-methyltransferase, TPMT; Food and Drug Administration, FDA; human leucocyte antigen, HLA

Currently, payers across many jurisdictions require that patients try and fail low cost immunomodulators, such as AZA, prior to the initiation of more effective and costly biological therapies.[18] It should be noted that treatment of AZA-induced pancreatitis results in added health care costs. Hospitalisation costs alone relating to acute pancreatitis can be over $10,000 (US) per individual, depending on the length of stay and investigations pursued.[19,20]

In summary, class II HLA gene region at rs2647087 appears to be an important marker of AZA-induced pancreatitis risk, though caution must be taken due to the small number pancreatitis cases identified in this study. The marked increase in risk for pancreatitis among carriers of the variant allele provides a strong rationale for a companion diagnostic approach for patients who are to be prescribed AZA, similar to what is already the case for medications such as abacavir. We recommend that HLA-DQA1-HLA-DRB (rs2647087) and TPMT genotyping are carried out prior to initiation of AZA where the rs2647087 wild-type (A/A) patients are then prescribed a TPMT genotype-guided AZA dose. Dedicated cost-benefit analyses would be useful to further elucidate the utility of our proposed approach in clinical practice.

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AUTHORSHIP

Guarantor of the article: Dr. Richard B. Kim.

Author contributions: RBK supervised the study. AW, JCG, TP, NC, RK, BY, VJ, NK, MS, MB, and KM were involved in data acquisition. AW, WAT and RBK contributed to the study concept and design. LEJ and AW carried out all data analyses. RBK, AW and WAT were involved in data interpretation. Statistical analyses were performed by RR and AW. AW drafted the manuscript. Critical revisions were carried out by RBK, WAT, JCG, TP, NC, RK, BY, VJ, NK, MS, MB, and KM. All authors had full access to all the data. All authors reviewed and approved the final version of this manuscript. No writing service was used for this manuscript.

ORCID

R. B. Kim http://orcid.org/0000-0001-8148-1632

LINKED CONTENT

This article is linked to Teich et al and Wilson et al papers. To view these articles visit https://doi.org/10.1111/apt.14545 and https://doi.org/10.1111/apt.14562.

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