RESEARCH REPORT

PROTOCOL NUMBER: N/A
VERSION NUMBER: Final 1.0 **DATE: 09 January 2014**

TITLE

A descriptive study to determine the prevalence of TPMT polymorphism in Healthy Male Subjects in Central South Africa.

ADMINISTRATIVE STRUCTURE

Primary Researcher	Name: Dr M.M Ferreira Qualifications: M.B.,Ch.B.; M.Fam.Med. Title: Medical Director	
Collaborator(s)	Name(s) Qualification(s) Title	Prof B Rosenkranz MD (Germany) PhD (Germany) FFPM Head, Division of Clinical Pharmacology
Head of Department	Name: Prof B Rosenkranz Qualification: MD (Germany) PhD (Germany) FFPM Title: Head, Division of Clinical Pharmacology	
Site of Research	PAREXEL Bloemfontein, Early Phase Clinical Unit, South Africa	
Statisticians	Name: Justin Harvey Head Statistician, Centre for Statistical Consultation, Stellenbosch University Ph.D (Mathematical Statistics) Name: Ruaan van Zyl Senior Biostatistician, Early Phase Biostatistics and Programming Division, PAREXEL Bloemfontein, Early Phase M.Sc (Statistics)	

ABBREVIATIONS

6-TGN	6-thioguanine
Dr	Doctor
et.al.	Et alii (and others)
GCP	Good Clinical Practice
HREC	Health Research Ethics Committee
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
NA	Not available
N/A	Not applicable
Prof	Professor
SOP	Standard Operating Procedure
TPMT	Thiopurine methyltransferase
USA	United States of America
VNTR	Variable number of tandem repeats

TABLE OF CONTENTS

1. REPORT SYNOPSIS

TITLE:	**A descriptive study to determine the prevalence of TPMT polymorphism in Healthy Male Subjects in Central South Africa**
PRIMARY RESEARCHER:	Dr M.M. Ferreira
SITE of RESEARCH:	PAREXEL Bloemfontein, Early Phase Clinical Unit, South Africa
SPONSOR:	N/A
PROTOCOL NUMBER:	N/A
FINAL PROTOCOL DATE:	09 January 2014
INTRODUCTION	Thiopurine medications e.g. Azathioprine and 6-Mercaptopurine are metabolized by the thiopurine methyltransferase (TPMT) enzyme. This enzyme activity is highly variable due to polymorphism of the TPMT gene. TPMT genotyping can be performed to tailor the treatment with thiopurine medications specifically to the patient's TPMT activity.
HYPOTHESIS	It was expected that the prevalence of TPMT polymorphism in Central South Africa will correlate with that available in the literature in terms of most prevalent polymorphisms, as well as differences in Caucasians, Africans and Mixed Race healthy male population.
RATIONALE and OBJECTIVES:	Determine the prevalence of TPMT polymorphism in healthy male subjects in Central South Africa.
STUDY DESIGN:	This is a descriptive retrospective study to determine the prevalence of TPMT polymorphism in healthy male subjects in Central South Africa
SELECTION CRITERIA:	• Healthy male subjects who took part in 4 clinical trials performed between 23 February 2009 and 29 February 2012 at the PAREXEL Bloemfontein Early Phase unit were included in the study. • Five hundred and forty (540) samples with available TPMT blood tests were included in the analysis; since some of the studies only tested for certain allele mutations, there were missing data for some of the analysis.
STUDY DURATION:	Approval was received from the Stellenbosch University HREC on 02 December 2013. Study data collection and data analysis were performed between 02 December 2013 and 07 January 2014. TPMT were analysed during 4 clinical trials performed between 23 February 2009 and 29 February 2012 at the PAREXEL Bloemfontein Early Phase unit.
ENDPOINTS/ MEASUREMENTS:	Primary • to determine the prevalence of TPMT polymorphisms in healthy male subjects in Central South Africa • to determine the different alleles that were most prevalent in different racial groups in healthy male subjects in Central South Africa

	• to compare the prevalence of mutant alleles in healthy male subjects in Central South Africa with data from literature.
INTERVENTION:	Not applicable
SAMPLE SIZE:	No formal sample size calculation was performed for this study, but 540 samples were available for analysis.
STATISTICAL ANALYSIS:	The statistical evaluation of the study data were performed by the Center of Statistical Consultation at the University of Stellenbosch and the Division of Biostatistics and Programming, PAREXEL Early Phase Bloemfontein. Data were analyzed using SAS 9.2 and 9.3. The 95% confidence interval for the binary proportions was used to estimate the study population prevalence. If small prevalence rates were observed, exact methods were used to calculate these confidence limits. In comparing the prevalence across different ethnic groups, a Chi-squared test was used. When small expected frequencies were observed a Fishers exact test for 2x2 tables and an exact test for rxc tables were used to determine the association. To compare the prevalence rates to literature data, the assumption was made that the literature data are described as a constant; the analysis were a single sample T-test for proportions and a Chi-squared test. A 5% significance level was assumed throughout.
RESULTS	Of the total study population, 89.63% tested normal (homozygous wild type) for TPMT enzyme activity. The remaining study population had polymorphisms for the TPMT enzyme activity. Of these approximate 10%, 6.11% were intermediate metabolizers and 4.26% were poor metabolizers. For exon *2 0.89% Black and 2.43% Caucasian subjects tested heterozygous. None of the Mixed Race subjects tested heterozygous for exon *2. For exon *3A 4.81% Caucasians and 4.76% Mixed Race subjects tested heterozygous. Caucasians tested homozygous mutant in only 1.07% of the total study population, but 9.52% of the Mixed Race subjects tested homozygous mutant. None of the Black subjects tested either heterozygous or homozygous mutant for exon *3A. For exon *3B, 5.65% Black, 7.51% Caucasian and 20.00% Mixed Race subjects tested heterozygous. Homozygous mutant results were only seen in 0.87% Black subjects, but not at all in Caucasian and Mixed Race subjects. In Caucasian subjects 2.82% tested positive for *3B, but a breakdown of the different mutations were not done by the applicable laboratory, so it can be either heterozygous or

	homozygous mutant. With exon *3C, 1.59% Black, 1.53% Caucasian and 7.41% Mixed Race subjects tested heterozygous. No Black subjects tested homozygous mutant, but 3.45% Caucasians and 3.7% Mixed Race subjects tested homozygous mutant. There were no Mixed Race subjects testing positive for exon *3C, but 1.99% Black and 2.3% Caucasian subjects tested positive. Again, no breakdown of the different mutations was done by the applicable laboratory for this exon. None of the subjects tested abnormal for exon *4.
DISCUSSION	In comparison to the literature, there is a difference for the intermediate and poor metabolizer prevalence. The difference is 4.26% for poor metabolizers compared to 0.3% according to the literature. There were 6.11% intermediate metabolizers in this study, compared to approximately 10% according to the literature. This difference may be clinically important as poor metabolizers can present with severe, even life threatening adverse events when treated with thiopurine medications. The most prevalent mutation in all the races in the study population was *3B, which is different from other populations in the world. It might be possible that Black patients don not have to be tested for exon *3A when started on thiopurine medications. Also, no patient might need testing for exon *4, as none of the 540 subjects tested abnormal for this exon. Another interesting finding was that 20% of Mixed Race subjects had a heterozygous polymorphism for exon *3B and therefore a 5th of this population group may be intermediate or poor metabolizers. This information can be used to tailor each patient's treatment with thiopurine medications. More research is needed to determine if routine testing before starting these medications is cost-effective or if these tests should only be considered when a patient experiences adverse effects.
CONCLUSION	Although it were expected that the number of poor and intermediate metabolizers would be similar to data that are available in the literature, it was confirmed that the percentage of poor metabolizers were more than reported in the literature. The percentage of normal metabolizers was similar to literature data. The most prevalent mutation in healthy male subjects in Central South Africa were *3B, which is different from literature of other populations in the world.

2. ETHICAL AND LEGAL OBLIGATIONS

2.1. Good Clinical Practice

The clinical studies were conducted according to the protocol and to Standard Operating Procedures (SOPs) that meet the guidelines laid down by the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) in clinical trials and Guidelines for Good Practice in the Conduct of Clinical Trials in South Africa.

2.2. Health Research Ethics Committee Approval (HREC)

Written approval of the final version of the study protocol and applicable documents were obtained from the HREC of the University of Stellenbosch (HREC number: S13/10/185) on 03 December 2013 where after the first collection of data commenced.

2.3. Regulatory Authority Approval (if applicable)

Not applicable.

2.4. Subject Confidentiality

All data were handled in a coded format and all subject personal information remained confidential and was documented anonymously.

All the sponsors, as well as the specific study investigation medicinal products (IMP) of the clinical trials performed remained confidential during this research study.

3. LITERATURE REVIEW/ RATIONALE

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of thiopurine medications e.g. azathioprine and 6-mercaptopurine. The metabolism of 6-mercaptopurine is competitive between TPMT and two other enzymes (xanthine and hypoxanthine guanine phosphoribosyltranferase). TPMT converts 6-mercaptopurine into an inactive form, 6-methylmercaptopurine, while hypoxanthine guanine phosphoribosyltransferase converts 6-mercaptopurine into an active form, 6-thioguanine (6-TGN)[1]. TPMT activity correlates inversely with 6-TGN levels in erythrocytes and presumably other hematopoietic tissues, since these cells have negligible xanthine oxidase (involved in the other inactivation pathway) activities, leaving TPMT methylation as the only inactivation pathway. The cytotoxicity of azathioprine is due, in part, to the incorporation of 6-TGN into DNA[2].

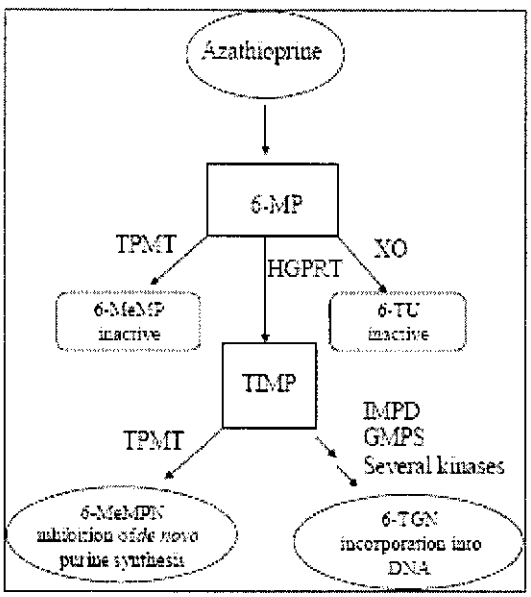

Figure 1: Metabolism pathway of azathioprine: competing pathways result in inactivation by TPMT or XO, or incorporation of cytotoxic nucleotides into DNA.

TPMT activity is highly variable: in a study performed by Lennard et. al. in the Children's Hospital, Sheffield, United Kingdom, in acute lymphoblastic leukaemia children, 90% of individuals had high/normal activity; approximately 10% have intermediate activity and 0.3% low/absent activity. The activity is largely influenced by polymorphisms in the TPMT gene[3].

Thiopurines are very useful medications for the treatment of autoimmune disease (e.g. Crohn's disease and rheumatoid arthritis), leukaemia, Hodgkin's lymphoma, multiple myeloma and after renal transplantation[2]. They have a relatively narrow therapeutic index, with life-threatening myelosuppression as a major toxicity[4]. There is some evidence that suggests that pre-treatment TPMT testing may be effective in reducing the number of profound neutropenic episodes experienced by patients prescribed azathioprine[3]. Recording of myelosuppression adverse events indicate that in a number of cases the outcome is death[5], therefore it is imperative to have the enzyme activity level before starting with treatment.

Over 23 variants in the TPMT gene, associated with decreased TPMT activity, have been identified. Three variant alleles, TPMT*2, TPMT*3A and TPMT*3C account for 80 - 95% of intermediate or low activity cases[3].

There are many differences in polymorphism in different races. These differences were determined in a number of studies in various areas in the world.

In a study done by Hon et. al. to investigate the polymorphism in TPMT in American Caucasians and African Americans, there was a difference in prevalence of the 3 main polymorphisms between the two racial groups. In this study the TPMT*3C allele was the most prevalent mutant allele in the African-Americans, whereas the TPMT*3A allele was the most prevalent mutant allele in the American Caucasian population[6].

A study was done by Spire-Vayron de la Moureyre et. al. in 191 European individuals. Fourteen mutations (of which one was novel) were detected. Four of them were mutations characterizing allelic variants TPMT *2, *3A, *3C and *7. Six were mutations characterizing different variable number of tandem repeats (VNTR) in the promoter region and 4 were silent mutations. Allele *3A was determined to be the most detrimental mutation[7].

In another study done by Bosson et. al. in a heterogeneous Brazilian population, it was determined that TPMT *2, *3A and *3C alleles were present at a 2.2%. 1.5% and 1.0% frequency respectively[8]. The ethnicity of the population with polymorphism was as follows:

Table 1: Ethnicity of TPMT alleles in a Brazilian population[8]

	TPMT *2	TPMT *3A	TPMT *3B	TPMT *3C
White	3	1	1	4
Non-white	4	2	3	3

A study by Hakooz et. al. in the Jordanian population determined that TPMT*3C (0.3%) and TPMT*3A (0.89%) were the only mutant alleles found in a sample of 169 individuals[9].

4. HYPOTHESIS

It was expected that the prevalence of TPMT polymorphism will be the same in Central South Africa as in the literature in terms of most prevalent polymorphisms as well as differences in Caucasians, Africans and Mixed Race healthy male subjects.

5. RESEARCH AIMS AND OBJECTIVES

Research Aim: Determine the prevalence of TPMT polymorphism in healthy male subjects in Central South Africa. Only male subjects were included in the clinical trials, therefore only male subjects were included in this study.

6. INVESTIGATIONAL PLAN

6.1. Study design

This is a descriptive retrospective study to determine the prevalence of TPMT polymorphisms in healthy male subjects in Central South Africa.

6.2. Objectives (Endpoints)

6.2.1. Primary Objective (Endpoint)

- to determine the prevalence of TPMT polymorphisms in healthy male subjects in Central South Africa
- to determine the different alleles that are most prevalent in different racial groups in the healthy male subjects in Central South Africa
- to compare the prevalence of mutant alleles in healthy male subjects in Central South Africa with data from the literature

6.3. METHODOLOGY

6.3.1. Study Plan

This is a descriptive retrospective study that investigated the prevalence of TPMT polymorphism in healthy male subjects in Central South Africa.

TPMT polymorphisms were determined in 540 healthy male subjects during 4 clinical trials performed between 23 February 2009 and 29 February 2012 at the PAREXEL Bloemfontein Early Phase Unit. The results were used during these clinical trials to exclude subjects that were either intermediate or poor TPMT metabolizers to prevent severe adverse events in certain trials. Only males were included in these studies and therefore the results of TPMT genotyping are only available for males.

Genotyping tests were performed at the Chemical Pathology Department of the University of Cape Town, as well as Synexa Life Sciences in Cape Town.

Genotyping tests at the University of Cape Town evaluated the following gene mutations:

- TPMT*3C
- TPMT*3B

The evaluation of poor and intermediate metabolisers was performed as follows:

- Point mutations at nucleotide 719 with a transition of purine A for purine G which constitutes to TPMT*3C allele
- If the above tested positive, point mutations at nucleotide 460 with a transition of purine G for purine A were tested, which constitutes to TPMT*3B
- If both tested positive, allele TPMT*3A was present and the subject was classified as a poor metabolizer
- If only A719G tested positive and G460A tested normal, the subject was classified as an intermediate metabolizer

Genotyping tests at Synexa Life Sciences, Cape Town evaluated the following gene mutations:

- TPMT*2
- TPMT*3B
- TPMT*3C
- TPMT*4
- TPMT*3A

Results were classified as homozygous wild type, heterozygous or homozygous mutant.

Study data were collected from 02 December 2013. The test results were listed in Excel format and used to evaluate the data relating to the study objectives. It was ensured that there were no duplicate tests and that subjects only had one test in the listings.

6.3.2. Randomisation and Blinding (if applicable)

Not Applicable

6.4. STUDY POPULATION

The criteria were set to ensure a homogeneous study population. To prevent bias, strict adherence to inclusion and exclusion criteria were maintained.

6.4.1. Sampling/ Number of Subjects

All valid genotyping TPMT tests that were performed were included in the analysis.

6.4.2. Selection Criteria - Inclusion

1. Tests had to be a genotyping TPMT test
2. If a repeat test was performed only the first valid test result was included.

6.4.3. Selection Criteria - Exclusion

1. Inconclusive test results were excluded.

6.5. Study Procedures

6.5.1. Informed Consent Process

All subjects screened for studies at the PAREXEL Bloemfontein Early Phase Unit gave consent for the specific study and it was explained that TPMT data would be collected and analysed. Therefore, a waiver of informed consent for the current study was received from the Health Research Ethics Committee.

6.6. Procedures and Data to be collected

The following data were captured to perform the analysis.

- Subject unique identification code
- Subject race
- Different TPMT alleles tested and the result of the test
- Classification as normal, intermediate or poor metabolizer

The selected data were entered into an Excel spread sheet which was then used for data analysis.

6.7. Intervention

Not applicable.

6.8. CHANGES TO THE STUDY CONDUCT

No changes were made to the final protocol (dated 16 October 2013).

The following deviations occurred relating to the data collection and analysis.

1. The first statistical analysis (done on 05 December 2013 by the Centre for Statistical Consultation, Stellenbosch University) did not include One Way Frequency analysis of all the different races and types of mutations. This was done by the PAREXEL Early Phase Bloemfontein Biostatistics and Programming Division, as the statistician mentioned in the protocol was not available over this period. The One Way Frequencies were performed on 02 January 2014. The comparison data analysis for the literature and the study results was completed by 07 January 2014.
2. The versions of SAS used for the analysis of data were 9.2 (PAREXEL Bloemfontein Early Phase Biostatistics and Programming division) and 9.3 (Centre for Statistical Consultation, Stellenbosch University) and not 9.1.3 as stated in the protocol. This was due to updated software after the protocol was compiled.
3. The data from the literature mentioned in the protocol were not USA data, but UK data. The study data were compared to this literature data.

7. DATA ANALYSIS AND STATISTICAL METHODS

7.1. Analysis Populations

The results from all subjects who qualified based on the eligibility criteria were included in this study.

7.2. Sample Size

No formal sample size calculation was performed; all subjects for whom TPMT results were available for clinical trials performed between 23 February 2009 to 29 February 2012 were used in the study.

7.3. Analyses of Data

The statistical evaluation of the study data were performed by the Center of Statistical Consultation at the University of Stellenbosch and the Division of Biostatistics and Programming, PAREXEL Early Phase Bloemfontein. Data were analyzed using SAS 9.2 (PAREXEL) and 9.3 (University of Stellenbosch).

The 95% confidence intervals for the binary proportions were used to estimate the study population prevalence. If small prevalence rates were observed, exact methods were used to calculate these confidence limits.

In comparing the prevalence across different ethnic groups, a Chi-squared test was used. When small expected frequencies were observed a Fishers exact test for 2x2 tables and an exact test for rxc tables were used to determine the association.

To compare the prevalence rates to literature data, the assumption that the literature data are described as a constant was made; the analysis was a single sample T-test for proportions as well as a Chi-Squared test. Literature data used for the comparison of total prevalence were UK results and not USA results as stated in the study protocol.

A 5% significance level was assumed throughout.

8. ADMINISTRATIVE OBLIGATIONS

8.1. Source Data

All applicable source data are stored at the PAREXEL Early Phase Unit, Bloemfontein for a period of at least 15 years or according to the applicable study protocol and contract.

8.2. Data Collection and Management

All results were entered into the Excel spread sheet by the researcher. A quality check was performed by a second person to ensure that all data were entered correctly.

9. RESULTS

9.1. Study Subject Disposition

Altogether, results for 539 subjects were available for the subject disposition. The following racial groups were included in this study: Black (African), Caucasian and Mixed Race.

Table 2: Study Subject Disposition

Race	Frequency	Percent
Black	251	46.57
Caucasian	261	48.42
Mixed Race	27	5.01
Total	539*	100.00

* The actual total number is 539 due to 1 missing frequency in the data. One participant was not allocated a race.

The number of Black and Caucasian subjects was similar. Mixed Race subjects only made up 5.01% of the total study population.

9.2. Prevalence of TPMT polymorphism

The total prevalence of normal enzyme activity and TPMT polymorphism are presented in Table 3.

Table 3: Prevalence of TPMT polymorphism

Result Type	Frequency	Percent
intermediate	33	6.11
normal	484	89.63
poor	23	4.26
Total	540*	100.00

The prevalence of normal TPMT enzyme activity was 89.63%. TPMT polymorphism in the Central South African region was 10.37%. Of this total, 6.11% were intermediate metabolizers and 4.26% were poor metabolizers.

12.3 Prevalence of *2 allele abnormalities

The prevalence of *2 allele abnormalities were as follows:

Table 4: Prevalence of *2 allele abnormalities#

		Race			Total
		Black	Caucasian	Mixed Race	
Value heterozygous	Frequency	2	5	0	7
	Col Pct*	0.89	2.43	0.00	
Total Percentage		0.89	2.43	0.00	

#for *2 allele there were 84 missing frequencies, as not all studies tested for this allele.

*Col Pct: Column Percent

Of the total number of subjects, 2 Black subjects and 5 Caucasian subjects were heterozygous. This constitutes 0.89% and 2.43% respectively of the total study population. None of the Mixed Race subjects tested heterozygous for *2 allele. There were no clinical significant differences (p=0.5071) between the different races, although this could be largely due to the low sample size in these groups.

12.4 Prevalence of *3A allele abnormalities

The prevalence of *3A allele abnormalities were as follows:

Table 5: Prevalence of *3A allele abnormalities#

		Race			Total
		Black	Caucasian	Mixed Race	
Value heterozygous	Frequency	0	9	1	10
	Col Pct*	0.00	4.81	4.76	
homozygous mutant	Frequency	0	2	2	4
	Col Pct*	0.00	1.07	9.52	
Total Percentage		0.00	5.88	14.28	

#for *3A allele there were 122 missing frequencies, as not all studies tested for this allele.

*Col Pct: Column Percent

Of the total number of subjects, 9 Caucasian and 1 Mixed Race subject were heterozygous. This constitutes 4.81% and 4.76% of the total study population per race. Two (2) Caucasian and 2 Mixed Race subjects were homozygous mutant for *3A allele. This reflects 1.07% and 9.52% of the total study population per racial group. None of the Black subjects tested heterozygous or homozygous mutant for *3A allele. There was a statistical significant difference between the different races (p=4.11E-05), possibly due to no subjects with mutations for this allele.

12.5 Prevalence of *3B allele abnormalities

The prevalence of *3B allele abnormalities were as follows:

Table 6: Prevalence of *3B allele abnormalities#

		Race			Total
		Black	Caucasian	Mixed Race	
Value					
heterozygous	Frequency	13	16	5	34
	Col Pct*	5.65	7.51	20.00	
homozygous mutant	Frequency	2	0	0	2
	Col Pct*	0.87	0.00	0.00	
positive	Frequency	0	6	0	6
	Col Pct*	0.00	2.82	0.00	
Total Percentage		6.52	10.33	20.00	

#for *3B allele there were 72 missing frequencies, as not all studies tested for this allele.

*Col Pct: Column Percent

Of the total number of subjects, 13 Black, 16 Caucasian and 5 Mixed Race subjects were heterozygous for *3B. This constitutes 5.61%, 7.51% and 20.00% of the total study population per race respectively. Two (2) Black subjects were homozygous mutant for *3B allele. This constitutes 0.87% of the study population per racial group. None of the Caucasian and Mixed Race subjects tested homozygous mutant for *3B allele. Six (6) Caucasian subjects tested positive for *3B, it was not confirmed by the applicable laboratory if these were heterozygous or homozygous mutant abnormalities. There is a statistical significant difference between the different races for this allele (p=0.0053), possibly due to the high prevalence in Mixed Race subjects.

12.6 Prevalence of *3C allele abnormalities

The prevalence of *3C allele abnormalities were as follows:

Table 7: Prevalence of *3C allele abnormalities#

		Race			Total
		Black	Caucasian	Mixed Race	
Value					
heterozygous	Frequency	4	4	2	10

	Col Pct*	1.59	1.53	7.41	
homozygous mutant	Frequency	0	9	1	10
	Col Pct*	0.00	3.45	3.70	
positive	Frequency	5	6	0	11
	Col Pct*	1.99	2.30	0.00	
Total Percentage		3.58	7.28	11.11	

#for *3C allele there was 1 missing frequency, as one subject were not allocated a race.

*Col Pct: Column Percent

Of the total number of subjects, 4 Black, 4 Caucasian and 2 Mixed Race subjects were heterozygous for *3C. This constitutes 1.59%, 1.53% and 7.41% of the study population per race respectively. Nine (9) Caucasian and 1 Mixed Race subjects were homozygous mutant for *3C allele. This constitutes 3.45% and 3.70% of the total study population per racial group respectively. None of the Black subjects tested homozygous mutant for *3C allele. Five (5) Black and 6 Caucasian subjects tested positive for *3C. It was not reported by the applicable laboratory if these were heterozygous or homozygous mutant abnormalities. None of the Mixed Race subjects tested positive for *3C. There was a statistical significant difference between the different races (p=0.0023).

12.7 Prevalence of *4 allele

None of the subjects tested positive or abnormal for *4 allele. All were homozygous wild type.

Note: For *4 allele, there were 83 missing frequencies as not all studies tested for this allele.

10. DISCUSSION

In 4 clinical trials performed at the Early Phase unit PAREXEL Bloemfontein, 540 subjects were tested for TPMT polymorphism as part of the entry criteria for these trials. They all received a genotyping test for TPMT polymorphism and all subjects were included into this research study.

Of the total study population, 89.63% tested normal (homozygous wild type) for TPMT enzyme activity. The rest had polymorphisms for the TPMT enzyme activity. Of this approximate 10%, 6.11% were intermediate metabolizers and 4.26% were poor metabolizers. When this was compared to the literature, there was a difference for the intermediate and poor metabolizer prevalence. This difference was 4.26% for poor metabolizers compared to 0.3% according to the literature. There were 6.11% intermediate metabolizers in this study, compared to approximate 10% according to the literature.

This difference may be clinically important as poor metabolizers can present with severe adverse events, even life-threatening, when treated with thiopurine medications.

When comparing the most prevalent allele polymorphism, differences to the literature were found. In Central South Africa exon *3B was most prevalent for Black (6.52%), Caucasian (10.33%) and Mixed Race (20.00) subjects. In the literature, exon *3C was most prevalent in Africans, Asians and South-Americans and exon *3A was most prevalent for Caucasians[6,7,8,9,10]. No data were available for Mixed Race subjects that

can be compared to the South African population. One of the clinical trials included in this study only tested exon *3B if exon *3C tested abnormal. It was considered that *3B will not be abnormal if *3C is normal. However, from the data collected from the other 3 clinical trials used for this study, 19 subjects had an abnormal *3B exon with a normal *3C exon. Therefore it is a possibility that the percentages for exon *3B might be even higher than what is stated above, but still it will be the most prevalent abnormality. This one clinical trial also did not test for *2, *3A and *4 routinely, therefore data may be incomplete.

Table 8 compares different populations of different areas in the world and the most prevalent mutations.

Table 8: Most prevalent allele mutations in different areas in the world per race (where available)[10]

Country/Population	Race	Most prevalent mutation	Prevalence (%)
South Africa	Black	*3B	6.52
	Caucasian	*3B	10.33
	Mixed Raced	*3B	20.00
Europe[7]	Caucasian	*3A	NA
United States of America[6]	Caucasian	*3A	3.2
	African American	*3C	2.4
South East Asia	NA	*3C	5.0
Argentina	NA	*3A	3.1
Belgium	NA	*3A	8.0
Brazil[8]	White	*3C	NA (4 individuals)
	Non-white	*2	NA (4 individuals)
	Mixed[10]	*3C	2.1
China	NA	*3C	2.3
	Han	*3C	2.2 - 2.6
	Jing	*3C	1.9
	Yao	*3C	3.7
	Uygur	*3C	3.1
Colombia	NA	*3A	3.6
Egypt	NA	*3C	1.3
France	Caucasian	*3A	5.7 - 5.9
Germany	NA	*3A	8.0
Ghana	NA	*3C	7.6
Italy	NA	*3A	3.9
India	NA	*3C	0.8
Japan	NA	*3C	0.8 - 2.2
Jordan[9]	NA	*3A	0.59
Kenya	NA	*3C	5.4
Malaysia	NA	*3C	2.3
New Zeeland	Caucasians	*3A	5.0
Poland	NA	*3A	2.7
Norway	Saami	*3C	3.3
Sweden	NA	*3A	3.75
Taiwan	NA	*3C	1.4 - 5.0

NA=Not available
South African data from this current study were included.

The article did not include TPMT*3B and it is not clear if it was not tested, or not detected in the mentioned studies. Therefore one should be cautious to conclude that it is only in South Africa that *3B is most prevalent.

When evaluating the different polymorphisms in the current study, the following were seen. For exon *2 0.89% Black and 2.43% Caucasian subjects tested heterozygous.

For exon *3A 4.81% Caucasians and 4.76% Mixed Race subjects tested heterozygous. Caucasians tested homozygous mutant in only 1.07% of the total study population, but 9.52% Mixed Race subjects tested homozygous mutant. None of the Black subjects tested either heterozygous or homozygous mutant for exon *3A which is expected from the literature.

For exon *3B, 5.65% Black, 7.51% Caucasian and 20.00% Mixed Race subjects tested heterozygous. Homozygous mutant results were only seen in 0.87% Black subjects, but not at all in Caucasian and Mixed Race subjects. In Caucasian subjects 2.82% tested positive for *3B, but a breakdown of the different mutations were not done by the applicable laboratory, so it can be either heterozygous or homozygous mutant.

With exon *3C, 1.59% Black, 1.53% Caucasian and 7.41% Mixed Race subjects tested heterozygous. No Black subjects tested homozygous mutant, but 3.45% Caucasians and 3.7% Mixed Race subjects tested homozygous mutant. There were no Mixed Race subjects testing positive for exon *3C, but 1.99% Black and 2.3% Caucasian subjects tested positive. Again, no breakdown of the different mutations was done by the applicable laboratory for this exon. It can be expected that the Black and Mixed Race subjects have polymorphism for *3C, but a number of Caucasians also had polymorphism, which is different from the literature. A very low percentage of Black subjects were polymorphic for this allele, and none were homozygous mutant, which is unexpected if compared to the literature.

None of the subjects tested polymorphic for exon *4.

From these results, it may be possible that Black patients do not have to be tested for exon *3A when started on thiopurine medications. Also, no patient may need to be tested for exon *4, as none of the 540 subjects tested abnormal for this exon. However, if a polymorphism is expected and none of the other exons stated here are abnormal, this amongst others can be considered.

It is interesting that about 20% of the Mixed Race study population had polymorphism for exon *3B, therefore it may be possible that a 5th of the Mixed Race population may be intermediate or poor metabolizers. However, the sample size was very small and this result should be viewed with caution. Research is required on a larger sample size to come to a more reliable conclusion.

Of importance is that in Central South Africa, 4.26% of persons are poor metabolizers of thiopurine medications compared to the 0.3% poor metabolizers documented in literature. Although this difference was not statistically significantly (p=0.9689), it may be clinically meaningful. It might be necessary to test patients with adverse effects when using thiopurine medications to establish if they are poor or intermediate metabolizers. This can influence the specialist's decision to lower the dose, or to stop the medication. When considering the literature, it will only be necessary to stop the medication in a very small number of patients, however, in Central South Africa this number may be higher due to the higher prevalence of poor metabolizers.

Currently TPMT enzyme activity is only tested in certain patient populations in South Africa due to cost constraints. As the prevalence of poor metabolizers were higher

than reported in the literature, it might be necessary to investigate the feasibility of testing for TPMT activity on a routine basis before starting treatment. Additional research is necessary in a broader population of the country and also to determine the health economic impact for these routine tests. The cost of treating severe adverse events such as bone marrow suppression may be more costly than to perform a routine TPMT activity test before starting with treatment. The specialist would be able to start at a lower dose if the enzyme activity is intermediate or can make use of another treatment option if the activity is poor.

One should also consider which type of enzyme activity test should be performed. It is known that there is a high degree of concordance between TPMT genotype and phenotype in Caucasians. Africans are known to have around 20% less TPMT activity than Caucasians for the same allelic distribution[8]. Therefore it is clear that there are a number of considerations when treating the patient in the clinical setting. Genotyping was done for these clinical trials, but it might be more feasible and clinically relevant to perform a phenotype test in the clinical setting. This might also result in lower expenses and prevent any ethical issues associated with genotype testing.

The shortcomings of this research study were that only 2 of the 4 clinical trials included in this study, had the full panel (*2, *3A, *3B, *3C and *4) of genotype tests. One study only tested for exon *3C, and if this was positive exon *3B was tested. The test also did not differentiate between the different abnormalities (heterozygous or homozygous mutant). Therefore 84 subjects only had limited results. Another 38 subjects from a second study did not have *3A results. There were missing frequencies due to this for all the analysis and the influence is difficult to determine.

Another factor that should caution the generalisation of the results is the low number of Mixed Race subjects in these 4 clinical trials. Only 5.01% of the total study population was Mixed Race, and therefore the results should be viewed with caution and need to be confirmed in a larger sample size.

11. CONCLUSION

Although it was expected that the number of poor and intermediate metabolizers would be similar to data that are available in the literature, it was confirmed that the percentage of poor metabolizers is more than what is documented in the literature. The percentage of normal metabolizers and total polymorphisms are similar to what is seen in the literature.

The most prevalent polymorphism was in exon *3B for all the racial groups. This differs from what is available in the literature for the rest of the world, where exon *3A is most prevalent in Caucasian and *3C is most prevalent in Africans, Asians and South Americans. No data was available for Mixed Race persons that could be compared in this study.

Twenty (20) percent of Mixed Race subjects tested heterozygous for exon *3B, which is significantly more than any other race and for any other abnormality, but as the sample size for Mixed Race subjects was very small these results should be viewed with caution.

Exon *4 is most likely not present in South Africans and could be omitted from standard tests. However, if a polymorphism is expected and none of the other exons stated here are abnormal, this amongst others can be considered.

12. REFERENCES

1. Cambell, S., Kingstone, K. and Ghosh, S. (2002) 'Relevance of thiopurine methyltransferase activity in inflammatory bowel disease patients maintained on low-dose azathioprine', *Alimentary Pharmacology and Therapeutics*, vol. 16, pp. 389 - 398.

2. RxList, [Online], Available: http://www.rxlist.com/imuran-drug/indications-dosage.htm, http://www.rxlist.com/purinethol-drug/indications-dosage.htm , http://www.rxlist.com/tabloid-drug.htm [accessed 07 January 2014]

3. Payne, K., Newman, W., Fargher, E., Tricker, K., Bruce, I.N. and Ollier, W.E.R. (2007) 'TPMT testing in rheumatology: any better than routine monitoring?' *Rheumatology*, vol. 46, pp. 727 - 729.

4. Weinshilboum, T. (2001) 'Thiopurine Pharmacogenetics: Clinical and Molecular Studies of Thiopurine Methyltransferase', *Drug Metabolism and Disposition*, vol. 29, pp. 601 - 605.

5. Anstey, A., Lennard, L., Mayou, S.C. and Kirby, J.D. (1992) 'Pancytopenia Related to Azathioprine - An Enzyme Deficiency Caused by a Common Genetic Polymorphism: A Review', *Journal of the Royal Society of Medicine*, vol. 85, pp. 752 - 756.

6. Hon, Y.Y., Fessing, M.Y., Pui, C., Relling, M.V., Krynetski, E.Y. and Evans, W.E. (1999) 'Polymorphism of the thiopurine S-methyltransferase gene in African-Americans', *Human Molecular Genetics*, vol. 8, no. 2, pp. 371 - 376.

7. Spire-Vayron de la Moureyre, C.,Debuysere, H., Mastain, B., Vinner, E., Marez, D., Lo Guidice, J., Chevalier, D., Brique, S., Motte, K., Colombel, J., Turck, D., Noel, C., Flip, R., Pol, A., Lhermitte, M., Lafitte, J., Libersa, C. and Broly, F. (1998) 'Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene *(TPMT)* in a European population', *British Journal of Pharmacology*, vol. 125, pp879 - 887.

8. Boson, W.L., Romano-Silva, M.A., Correa, H., Falcâo, R.P., Teixiera-Vidigal, P.V. and De Marco, L. (2003) 'Thiopurine methyltranferase polymorphisms in a Brazilian population', *The Pharmacogenomics Journal*, vol. 3, pp 178 0 182.

9. Hakooz, N., Arafat, T., Payne, D., Ollier, W., Pushpakom, S., Andrews, J. and Newman, W. (2010) 'Genetic analysis of thiopurine methyltransferase polymorphism in Jordanian population', *European Journal of Clinical Pharmacology* vol. 66, pp 999 - 1003.

10. Katsanos, K.H. and Tsianos, E.B. (2007) 'Azathiopine/6-mercaptopurine toxicity: the role of the TPMT gene', *Annals of Gastroenterology* vol. 20(4), pp251 - 264.

List of Figures

List of Tables

SIGNATURE INVESTIGATOR

The signature below constitutes approval of this report by the signatory and provides the necessary assurances that this study was conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Signed: _____

Name: Maria Magdalena Ferreira, M.B.,Ch.B.; M.Fam.Med.
Title: Medical Director, PAREXEL Bloemfontein

Date: 09 Jan 2014