January 5, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14876-review.doc).

Title: Clinical predictors of thiopurine-related adverse events in Crohn's disease

Author: Gordon W. Moran, Marie-France Dubeau, Gilaad G. Kaplan, Hong Yang, Bertus Eksteen, Subrata Ghosh, Remo Panaccione.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14876

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.
2 Revision has been made according to the suggestions of the reviewer.

Reviewer 1

- Figure 1 has been altered as indicated.
- Table 1 has been aligned.
- As indicated in the caption at the bottom of table 2: the data is presented as median and interquartile range.
- References and typographic errors have been amended.

Reviewer 2

- We have now duly expanded on the effect of age on TPMT activity. The literature (Serpe L, et al. Pharmacogenomics. 2009 Nov;10(11):1753-65) that the reviewer is suggesting refers to a higher TPMT activity in infants and children when compared to adults. We have excluded the pediatric patient population from our study so this reference does not apply. The studies that we have referenced (references 30 and 31), including a large Spanish study analyzing TMPT activity in 14545 adult patients have identified no change in TPMT activity with age
- We have now amended our introduction to update the readers on the effects of 5-ASA co-administration with thiopurines on TPMT activity. We thank the reviewer to highlight this deficiency.
- Relevant ages of male and female participants in this study were not significantly different. Of all patients studied, 34.5% of women and 32.5% of men were over 40 when started a thiopurine.

Reviewer 3

We are sorry that this reviewer has not found our manuscript helpful. We hope that changes we have now made will improve the status of out manuscript. It would be very difficult to measure fatigue in a retrospective study. Our study as highlighted in the discussion is not large enough to assess the risk of discontinuing therapy due to a malignant complication.

The Reviewer is correct to highlight an important limitation of this paper, which is that we lacked data

on TPMT genotype, enzymes and metabolites that may have influenced the role of azathioprine/6-mercaptopurine. Due to this lack of data we are not able to: 1) evaluate whether metabolite levels affect rate of discontinuation; and 2) assess the effect of baseline TMPT activity on drug discontinuation. These are important limitations, which we have added to the discussion. However, our study population reflects "real-life" clinical practice whereby many regions do not routinely measure TPMT enzyme levels or metabolites. For example, in most regions in Canada our healthcare system does not cover routine measurement of thiopurine metabolites. In order to measure these levels patient have to pay out of pocket leading to most Canadian gastroenterologists making clinical decisions on discontinuing azathioprine/6-mercaptopurine without prior knowledge of TPMT genotype, TPMT enzyme levels, or metabolites. Like Canada, many countries outside of the USA (e.g. most European countries) do not routinely order azathioprine blood tests. Further, IBD is emerging in many countries in Asia, Africa and South America who may not routinely order these blood tests as well. Thus, we believe that the results of our cohort, which reflects clinical decision making in the absence of these blood tests (TPMT genotype, TPMT enzyme levels, or metabolites), will be highly relevant to gastroenterologists practicing in these countries. In fact, one of the reasons that we submitted our manuscript to the World Journal of Gastroenterology is that this journal is widely read by gastroenterologist from all continents, many of which will not order these tests.

Reviewer 4

- When discussing references 7 and 8 we have now made the following alteration: Thiopurines have been shown to reduce corticosteroid use and maintain remission in patients with CD[5, 6], but this evidence has been questioned by more recent data from two randomised controlled trials[7, 8] in CD patients with early disease, precluding a widespread usage of thiopurines in all patients with early CD.
- This retrospective study captured patients who have been prescribed 5-ASA therapy for their Crohn's disease several years ago when it was such practice to do so.
- The abstract conclusion has now been duly changed to: Thiopurine withdrawal due to adverse events is commoner in women over the age of 40 at prescription. These findings need to be replicated in other cohorts.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Dr Gordon W. Moran
Clinical Associate Professor and Honorary Consultant Gastroenterologist
NIHR Biomedical Research Unit in Gastrointestinal and Liver Diseases at Nottingham University Hospitals NHS Trust and The University of Nottingham
E floor, West Block, Queen's Medical Centre
Nottingham University Hospitals NHS Trust
Nottingham NG7 2UH
United Kingdom
Telephone: +44 (0)115 9249924 ext 70608
Fax: +44 (0) 115 82 31409
Email: Gordon.Moran@nottingham.ac.uk