Manuscript Number 6957

Manuscript Title Pharmacogenetics of azathioprine in inflammatory bowel disease: a role for glutathione-S-transferase?

Comments To Authors	MANUSCRIPT TITLE: Pharmacogenetics of azathioprine in inflammatory bowel disease: a role for glutathione-S-transferase? AUTHORS: Stocco G et al MINOR POINTS: ABSTRACT: ? The abstract is contradictory to the discussion section. "deletion of GST-M1, which determines reduced enzymatic activity, was recently associated with increased sensitivity to azathioprine and increased production of azathioprine active metabolites". However, as outlined later, reduced GST-M1 leads to reduced conversion of AZA to 6-MP and therefore reduced active metabolites, reduced efficacy and reduced adverse events. Therefore the aforementioned sentence in the abstract is either an error, or there are too many double negatives grammatically, making it confusing. Clarifying or correcting this is important. **AUTHORS' RESPONSE: We appreciate the comment of the reviewer and have modified the abstract by changing, in the sentence pointed out, "increased" with "reduced".** Oxidative damage: ? Page 6, reference 18 – In the Chocair study, the nephrotoxocity was referring to organ rejection - presumably allopurinol lead to less nephrotoxicity (rejection) via increasing 6TGN levels and therefore the efficacy of azathioprine, as has subsequently been shown in IBD. **AUTHORS' RESPONSE: We appreciate the comment of the reviewer and have erased this sentence and citation. We have cited instead the paper by Blaker et al., Biochem Pharmacol. 2013 Aug 15;86(4):539-47, that demonstrates the inhibitory effect of a metabolite of allopurinol on TPMT, which should determine the increase in TGN concentration and reduction of MMPN concentration typically observed.** Effects of GST polymorphisms on azathioprine efficacy and metabolism in patients with IBD: ? Page 9, Ref 32 – in describing their own original work from 2007 there are several double-negatives that are used which make the results less clear. **AUTHORS' RESPONSE: As requested by the reviewer, we have eliminated some double negatives that are used in describing our own original work from 2007.**

MAJOR POINTS: ? The authors have produced a mini-review of the role of glutathione-S-transferase in azathioprine metabolism, highlighting their own work which demonstrates reduced efficacy, and adverse events, in patients with mutations in GSTM1. Given the paucity of important new data pertaining to the pharmacogenomics of thiopurines in the last few years this work is important, although as they acknowledge the sample sizes of their studies are small. ? As mentioned above, on several occasions double-negatives are used, making it confusing for the reader – this could be easily corrected. ?

AUTHORS' RESPONSE: We have checked for double negatives and removed some of them to improve clarity of the text.

The two studies by the authors are mentioned only relatively briefly towards the end of the manuscript, including the recent J Clinical Gastro Sept 2013 publication. I suggest that they are discussed earlier and in more detail as they represent the only studies in the IBD literature on this topic?

AUTORS' RESPONSE: We have moved the presentation of our two studies earlier in the manuscript, in particular as the second section, immediately after the introduction. More details about these studies have been added to the manuscript.

The last few paragraphs should be formatted into a separate "Conclusions" subheading to finish the manuscript.

AUTHORS' RESPONSE: We have formatted the last two paragraphs into a separate "conclusions" subheading to finish the manuscript, as requested by the reviewer.

Comments To Authors	The authors have constructed a well written and researched review of the role that glutathione-s-transferase plays in azathioprine metabolism and response. I have no major concerns about the content of the review. Minor points to address are as follows: 1.Page 4, paragraph 4. Please provide references for the final sentence. **AUTHORS' REPLY: to this sentence, we have added the references: 17 Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annual review of pharmacology and toxicology 2005; 45: 51-88.[15822171]; 18 Zhang ZJ, Hao K, Shi R, Zhao G, Jiang GX, Song Y, Xu X, Ma J. Glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1) null polymorphisms, smoking, and their interaction in oral cancer: a HuGE review and meta-analysis. American journal of epidemiology 2011 Apr 15; 173(8): 847-857.[21436184]; 19 Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Castano-Vinyals G, Yeager M, Welch R, Chanock S, Chatterjee N, Wacholder S, Samanic C, Tora M, Fernandez F, Real FX, Rothman N. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 2005 Aug 20-26; 366(9486): 649-659.[16112301]; 20 Chen CL, Liu Q, Pui CH, Rivera GK, Sandlund JT, Ribeiro R, Evans WE, Relling MV. Higher frequency of glutathione S-transferase deletions in black children with acute lymphoblastic leukemia. Blood 1997 Mar 1; 89(5): 1701-1707.[9057653].** 2. Page 4. The authors should consider providing a table which lists the relevant GST polymorphisms, functional effects, and frequencies in different races. This would be additive to the review. **AUTHORS' REPLY: a table listing relevant GST polymorphisms, functional effects and frequencies in different races has been added to the manuscript. Relevant text in the manuscript has been changed.** 3. Page 5, sentence 1. Please correct 'mercaptourine' to 'mercatopurine'. 5. page 6, paragraph 2. change '...thiopurines' metabolism, it is reasonable to suggest an ability of these agents to induce...' '...thiopurine metabolism, it is reasonable to suggest that

	these agents are able to induce...' 6. Page 6, paragraph 2, sentence 3. Change 'some evidences' to 'some evidence'. **AUTHOR'S REPLY: we have modified the manuscript as indicated by the reviewers.** 7. Figure 1. To aid readers who are not particularly familiar with the thiopurine pathway, it might be worthwhile including an additional figure which shows the pathway and where GST acts relative to other enzymes e.g. ITPA, TPMT, GMPS, IMPDH etc. **AUTHORS' REPLY: We have added a figure displaying the thiopurine pathway as requested by the reviewer.**
Comments To Authors	The authors accurately describe the role of glutathione-S-transferase in pharmacogenetics of azathioprine in inflammatory bowel disease. In my opinion this is a very interesting and important aspect to be considered for the management of thiopurine drugs in addition to TPMT genotyping. The manuscript offers a complete review on the argument and it is well written. Probably the authors should add more information on the actual methods for determining thiopurine metabolites concentration in erythrocyte and whole blood. In particular the authors should review the advantages of using mass spectrometry-based micromethods that allow the use of limited sample volumes for future studies involving pediatric patients. **AUTHORS' REPLY: We appreciate the reviewers' comment and have added a paragraph on methods for determining thiopurine metabolites, with a particular emphasis on the importance of mass spectrometry-based methods, that will allow the use of samples with micro volumes for studies involving pediatric patients.**