ANSWERING REVIEWERS

April 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (8971-review.doc).

Title: Azathioprine does not reduce adenoma formation in a mouse model of sporadic intestinal tumorigenesis

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Name of Journal: World Journal of Gastroenterology

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

1 Format has been updated

2 Revision have been made according to the suggestions of the reviewer

Reviewer 00001391

-The reviewer asks for further discussion about the dual role of azathioprine on adenoma formation in the presence and in the absence of inflammation. We agree that this is a very important point. Azathioprine treatment reduces the risk of developing colorectal cancer in patients with IBD. Whether this association is the result of a better control of inflammation or if azathioprine has a direct effect on (sporadic) intestinal tumorigenesis was thus far not known. The results from current study suggest that azathioprine does not prevent sporadic intestinal tumorigenesis. This is further discussed in the manuscript.

-The reviewer questions the size of experimental groups. We reasoned that azathioprine could only be clinically applicable if polyp number would be reduced by at least 25%. We expected mice to develop a mean of 60 polyps (10% standard deviation). At a p of 0.05 (alpha 0,025) and power of 0.8 the sample size was calculated for 6 animals by group. This discussion is added to the method section.

-The reviewer asks for an updated bibliography, this has been done and we added the reference about the meta-analysis of thiopurines and risk of colorectal neoplasia in IBD.

-For readers convenience we changed all *C57B6* annotations for *wild type*.

-The reviewer suggests that the focus on azathioprine induced toxicity could be shifted more towards improved investigation on adenomas. In our experiments we do not find any differences in adenoma number. The size and location of the adenomas did not differ between azathioprine and vehicle treated mice as well. We therefore conclude that in the absence of inflammation azathioprine does not reduce adenoma formation and that azathioprine may have no clinical relevance in chemoprevention for sporadic colorectal cancer. The data about toxicity are highlighted as they rule out the possibility that azathioprine was administered in a too low dose and clearly show that even the high azathioprine dose does not display any effect on polyp formation.

-The reviewer asks for further discussion about the poor outcome of females compared to males. We agree that this is a very interesting observation and included in debt discussion of this finding. In short, azathioprine induced toxicitiy is known to be related to activity of the enzyme thiopurine S-methyltransferase (TPMT) which was recently shown to be lower in females than in males. This may explain why female mice were more vulnerable to azathioprine induced toxicity than males mice.

Reviewer 00009417

-Similarly to the reviewer 00001391, this reviewer suggests that in this manuscript the focus on azathioprine induced toxicity could be shifted towards improved investigation on adenomas. In our experiments we do not find any differences in adenoma number, size and location between azathioprine and vehicle treated mice. We conclude that in the absence of inflammation azathioprine does not reduce adenoma formation and that azathioprine may have no clinical relevance in chemoprevention for sporadic colorectal cancer. The data about toxicity are important because they rule out the possibility that azathioprine was administered in a too low dose.

-The reviewer comments that adenoma volumes should be estimated and compared between groups. We agree that although azathioprine does not influence adenoma initiation, it may still be important for the progression of small polyps into larger adenomas. However Figure 3B and 3C clearly demonstrates that both adenoma size as well as location does not differ between groups.

-The reviewer asks for discussion of the poor outcome of females compared to males. We agree this is an interesting observation and included in debt discussion of this finding. In short, azathioprine induced toxicity is related to activity of the enzyme thiopurine S-methyltransferase (TPMT) which was recently shown to be lower in females than in males. This may explain why female mice were more vulnerable to azathioprine induced toxicity than males mice.

-The reviewer asks for a table showing the number of mice under investigation at each point. We included this as figure 1B

Reviewer 01468173

-The reviewer remarks that there was no plan to check the dose of administration or adverse effects before carrying out this research. However the azathioprine dose was carefully decided based on previous publications (Reagan-Shaw et al). The estimated dose was 6–20 mg/kg which in humans has been proven to be clinically relevant. This is further discussed in the methods section.

-The reviewer asks about the plan that affects the results of this research. Our aim was to investigate if azathioprine could reduce adenoma formation in the absence of inflammation. Although azathioprine was clearly within the therapeutical range, it did not affect adenoma formation. Therefore we conclude that in the absence of inflammation, azathioprine can't be used for chemoprevention in patients with increased risk of developing colorectal cancer, such familial adenomatous polyposis (FAP).

3 References and typesetting were corrected

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

Sincerely yours,

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