200 First Street SW
Rochester, Minnesota 55905
Phone 507-538-4877, Fax 507-284-0762

Lewis R. Roberts, M.B. Ch.B., Ph.D.
Center for Basic Research in Digestive Diseases

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Dear Editorial Committee of the World Journal of Gastroenterology,

Thank you very much for considering publication of our manuscript entitled "**Metformin Does Not Improve Survival in patients with Hepatocellular Carcinoma**" in your journal. Please find below our responses to the reviewers' comments. We have also updated our manuscript according to the responses (underlined). Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO: 8095-edited).

Title: Metformin Does Not Improve Survival in patients with Hepatocellular Carcinoma

Authors: Mamatha Bhat, Roongruedee Chaiteerakij, William S. Harmsen, Terry M. Therneau, Cathy D. Schleck, Gregory J. Gores, Lewis R. Roberts

Name of Journal: *World Journal of Gastroenterology*

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Response to reviewers

Reviewer *00068278*

In the presented study, the authors assessed the effect of metformin on the survival of 701 patients with newly diagnosed HCC. They did not find a statistically significant survival advantage of metformin among 3 groups (diabetic patients on metformin, not on metformin and non-diabetic patients).
Minor points:
1) Page 4, paragraph 2; organic cation transporter 1 (OCT1) transporter must be deleted:

Response:
Thank you for the comment, the repeated word has been accordingly deleted.

2) Some abbreviations were not defined on first mention in the text (mTOR, VEGF, etc).

Response:
These abbreviations have been fully defined as vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR), please see highlighted changes.

3-Discussion section, paragraph 2; the reference number of Chen et al must be corrected as "11".

Response:
The reference number has accordingly been corrected.

4- The manuscript has not been written in accordance with the Journal's rules (references within the text and in the references, style, tables, etc).

Response:
These have been accordingly revised and highlighted in the edited manuscript version.

Major points:

1-Although it has been stated that metformin decreased HCC risk among diabetic patients in a dose-dependent manner (reference #11, and Yi G, et al. Int J Oncol. 2013 Nov;43(5):1503-10. doi: 10.3892/ijo.2013.2077), no data given about the dose of metformin.

Response:

Thank you for this comment. The lack of data regarding dosing is a limitation of this retrospective study of a large number of patients, many of whom had passed away, making it impossible to trace back doses of metformin. Nonetheless, many large-scale studies develop conclusions regarding efficacy of medications without doses being available, and this is accepted in the statistical literature.

A sentence has accordingly been added in the paragraph on limitations in the Discussion section on page 9 of the revised manuscript as follows:
"Due to a retrospective study design, we were not able to obtain the information on dose and duration of metformin use. Although the dose of metformin was not available, we did obtain the definitive finding that the use of metformin did not have any effect on survival among HCC patients."

2- On Table 1, the BCLC stage distribution shows only 514 patients (no information about 187 patients).

Response:
There were 187 missing data for BCLC, given that this was a retrospective study, and not all information was available in the medical record.

We have added sentences to clarify the missing data for BCLC in the Result section on page 6 of the revised manuscript as follows:

"Given that BCLC stage is known predictor for survival in HCC patients, only 514 patients whom data on the BCLC stage was available were included in the Cox Proportional Hazards analysis."

Additionally, this has been added as a limitation of our study in the Discussion section on page 9 of the revised manuscript) as follows:

"Additionally, there were some missing data for BCLC, given that this was a retrospective study, and not all information was available in the medical record. "

3- (a) Overall the length of the discussion is short. (b) It would be helpful to state that the failure to find a difference may be secondary to a Type II statistical error. (c) It has been found that age and BCLC stage were associated with increased risk of death (HR>1.0). It would be helpful to present data and comment on age groups and BCLC stage differences between the groups.

3a Response:

We have expanded the discussion on pages 7-8 of the revised manuscript as follows:
"Recently, research into therapeutics for malignancy has turned towards agents that could modify aspects of metabolism. The mTOR pathway as a pro-survival pathway is particularly affected by cellular energetics. AMP-activated protein kinase (AMPK), an intracellular sensor serving to maintain energy balance, is stimulated by increased energy consumption as reflected by an elevated AMP/ATP ratio. AMPK activation in turn inhibits the mTOR pathway and anabolic processes, including the energy-consuming process of protein synthesis. Metformin is thought to affect tumor growth by two mechanisms: 1) through inhibition of mitochondrial oxidative phosphorylation which activates AMPK thereby resulting in mTOR pathway inhibition and 2) through decreased serum glucose, which inhibits IGF-R, thereby preventing downstream mTOR pathway activation in insulin-responsive cancers[17]. Over the last few years, metformin has demonstrated promising results in various malignancies, including breast[7], lung[8] and melanoma[9]. Retrospective studies have suggested that metformin prevents development of HCC among patients with diabetes[12] and those with chronic liver disease[11]. A greater effect on HCC could be anticipated, given that the OCT1 transporter is most highly expressed in hepatocytes, enabling increased uptake of metformin in the liver[10]. Therefore, the reality of *in vivo* pharmacokinetics favours accumulation of metformin in the liver. Metformin is absorbed from the gut into the portal vein circulation, which drains directly into the liver. In vitro, by inhibiting the mTOR pathway, rather than activating it as insulin would, metformin arrests the cell cycle and induces cancer cell apoptosis[18]. In vivo, metformin has been shown to inhibit DEN-induced liver tumorigenesis by affecting lipogenesis[19] and inhibit tumor growth in mouse xenograft models of HCC[20]. Induction of cancer cell apoptosis following cell cycle arrest appears to be the mechanism underlying tumor growth inhibition in these xenograft models[21]."

3b Response:

Thank you for this comment. Type II errors occur when a result is interesting but the confidence interval is too wide. In this study the confidence interval is short (0.8-1.3) and centered at 1.0. In such a grave disease as HCC any benefit less than 50% reduction in death rate is of little clinical merit, and this is well outside the confidence interval. It is therefore highly unlikely that a Type II error accounts for the failure to find a difference.

3c Response:

We thank the reviewer for this comment. We have added Table 1 to present baseline characteristics of patients, including mean age, age groups, gender, etiology of chronic liver disease, BCLC stages and treatment type. These baseline characteristics were compared among the 3 groups, i.e. non-diabetics, diabetics not on metformin and diabetics on metformin.

Table 1 was shown on page 13 of the revised manuscript.

4- Is there any explanation about higher mortality rate and shorter median survival in patients with stage C compared to those with stage D?

Response:
The total number of stage D subjects for whom ongoing metformin treatment could be established (> 90 days) is small (n=59). The median survival and 95% confidence intervals for the four groups are:

A: median = not reached; 95% confidence interval = (1657, not reached)
B: median = 1140; 95% confidence interval = (592, not reached)
C: median = 366; 95% confidence interval = (296, 464)
D: median = 827; 95% confidence interval = (337, not reached)

So although the median survival for stage D patients appears much larger than for stage C, the confidence intervals for the two median survivals have substantial overlap. The fact that an upper confidence interval for the median could not be computed for 3 of the 4 groups further highlights that the median values for all of the groups remain quite uncertain without further long term follow up, and any difference in that value for a specific BCLC stage should be treated as preliminary.

We reviewed our data, and noted that this apparently paradoxical difference in median survival is due to our having started the clock at day 91 after HCC diagnosis, with a high proportion of the stage D patients died within 90 days after HCC diagnosis. These patients were therefore excluded from the survival analysis, making the survival of stage D patients appear longer than it should be. Nonetheless, the confidence intervals for the stage C and D patients overlapped significantly; hence, the median survival is no different between the two groups.

We have added sentences for the explanation in the Discussion section on page 9 of the revised manuscript) as follows:

"An important point to note is our definition of significant exposure, wherein only those patients who had continued metformin beyond 90 days after the diagnosis of HCC were considered as having been significantly exposed. The censoring of patients who passed away within 90 days of diagnosis accounts for the paradoxically increased median survival of the remaining stage D patients as compared to that of stage C patients. This is due to a significant proportion of the stage D patients died within 90 days after HCC diagnosis. Nonetheless, the

confidence intervals for the two median survivals have substantial overlap, indicating that the median survivals were in fact similar. ”

5-Metformin may act differently in HCC patients who have different causes (Yilmaz Y, et al. Tumori. 2013 Jan-Feb;99(1):10-6. doi: 10.1700/1248.13781.). It would be helpful to present data on etiologic groups.

Response:

We have analyzed the effect of metformin on survival of HCC patients with nonalcoholic fatty liver disease (n=102), given that the patient number distribution allowed for this kind of analysis and that this is particularly of interest in those patients who developed HCC in the context of fatty liver disease. Those patients who continued metformin did not have a survival advantage as compared to non-diabetics or those diabetics not on metformin/had discontinued metformin. These results are presented in the Results section in Table 2 on p. 9.

This is also presented in the Results section on p.7 as follows:

“Assessment of survival in patients with HCC in the context of fatty liver disease also showed no benefit to the use of metformin. Although NAFLD-related HCC patients with diabetes who were on metformin had longer median survival than those not on metformin (36.5 vs. 16.3 months), the survival difference was not statistically different (P = 0.25), likely due to the small number of NAFLD-related HCC patients who were on metformin (n=14)**.”**

Additionally, a sentence has been added to this effect in the Discussion section on p.8.
“Additionally, patients with HCC caused by fatty liver disease had no greater survival advantage on metformin as compared to those patients not on metformin. **”**

Reviewer 00742517

The article is a retrospective study of metformin effect on HCC patients between Jan 2005 and June 2011 and shows no surival benefit to the use of metformin in DM patients with HCC. Overall the paper is well designed. My specific comments are as follows. Virus, alcoholic and fat are main etiolgies of HCC. As we know, metformin works in overweighted patients who suffered insulin resistence. It is common used in fatty liver disease.

1) So, it will be better if author could a) add BMI/HOMA-IR result in Table 1 and b) divide HCC into fatty liver group and non-fatty liver group. Metformin might affect more in HCC induced by fatty liver disease.

Response 1a:

Although this is a very interesting point by the reviewer, the HOMA-IR is not a variable available in our BRIDGE database.

Response 1b:

Regarding HCC in fatty liver disease, please see the response above to Reviewer *00068278's* question.

Reviewer 02855671

1) Some minor points to be addressed: a) to add one or two phrases in the "Background and aim" of the Abstract section b) to expand the "Results" section - the fifth reference number in the " Discussion" is 11 and not 1 c) what kind of HCC therapies did the patients undergo? May this have some relevance in the discussion (see paper by Chen TM 2011 J Gastroenterol Hepatol on metformin after RFA)? d) in table 1 the cumulative number of BCLC patients is 514 and not 701. Please address

Response to 1a:
We had kept the aim to a limit of 20 words, given that this was the limit stated in the Instructions to Authors.

Response to 1b:
Thank you for the correction, this has been accordingly revised.

Response to 1c:
We have added data on treatment types in in Table 1 on page 8 of the revised manuscript.

Response to 1d:
We apologize for not clearly stating that there were 187 missing data for BCLC.

We have added sentences to clarify the missing data for BCLC in the Result section on page 6 of the revised manuscript as follows:

"Given that BCLC stage is known predictor for survival in HCC patients, only 514 patients whom data on the BCLC stage was available were included in the Cox Proportional Hazards analysis."

The major points that authors must be clarified are listed below:
1. The main factors related to the HCC survival are treatment strategies, including surgery, radiofrequency ablation (RFA), liver transplant, TACE and chemotherapeutic agents (Figure 1). The aim of this article is to evaluate the metformin exposure at least 90 days for newly diagnosed HCC patients and the result is that metformin exposure does not improve one –year survival. The result is not persuasive, because of the authors neglect the effects of the aforementioned treatments play a critical role in HCC survival than a single drug exposure.

Response
We appreciate this point made by the reviewer. We were not able to examine the effect of treatment strategies on survival outcome of HCC patient in this study. Some patients received treatment prior to first visit at Mayo. However, the information on treatment type prior to first visit at Mayo was not available in the BRIDGE database. We discussed this comment with our statistical experts. Given that BCLC Stage itself dictates the type of treatments

patients undergo, adjusting for BCLC Stage accounts for the type of treatment the patients have undergone.

2. The methodology of this article is not rational. Case-matched method is suitable for this study and the outcome is capable of convincing the readers.

Response:
We thank the reviewer for this comment. However, if there are other important predictors of outcome, then either matching the subjects on those factors (case/control) or adjusting for those factors in the regression model are both well used and well regarded analysis strategies. This is the opinion of our co-author Dr. Terry Therneau, who is the author of one of the major textbooks on the application of survival models to medical data (Therneau and Grambsch, Springer, 2000). If a factor is a particularly strong predictor of mortality, then matching (or equivalently, a stratified Cox model) may have an advantage over covariate adjustment. No such factors are available in this disease setting.

3. The total numbers of BCLC stage is incorrect to overall patients (Table 1)

Response:
Again, we apologize for not clearly stating the number of missing data for BCLC stage. We have added sentences to clarify the missing data for BCLC in the Result section on page xxx of the revised manuscript as follows:

"Given that BCLC stage is known predictor for survival in HCC patients, only 514 patients whom data on the BCLC stage was available were included in the Cox Proportional Hazards analysis."

We hope the above adequately addresses the comments by the editorial committee of the *World Journal of Gastroenterology,* and thank you for considering our manuscript.

Sincerely yours,

Mamatha Bhat, MD, FRCPC
Lewis Roberts, MBBS PhD
Mayo Clinic, Division of Gastroenterology
Rochester, Minnesota
U.S.A.
55905