August 14, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3491-review.doc).

Title: Inhibitor of differentiation (ID) proteins do not influence prognosis of biliary tract cancer

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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 has been made according to the suggestions of the reviewers:

Reviewer 1

1. Title page: order the numbers of centers. It sounds a little bit strange 1,4,2,3,5.
    a. Authors and centers have been rearranged
2. Abstract: First time you say ID should be explained (first line (AIM)).
    a. This has been added accordingly
3. Material and Methods: As a surgeon I would like to know the criteria for deciding the type of treatment done (see explantion in results).
    a. All patients were treated in advanced stage at our institution. While patients had been treated surgically early in there disease, they had become unresectable at time of treatment in our institution. Most cases were discussed in the institutional tumor board.
4. Main problem: I think that extrahepatic cholangiocarcinoma, intrahepatic cholangicoarcinoma and galbaldder cancer are not the same disease in survcal rates and prognostic (you demonstrate nicely) so to mix cases is not a good idea to extract conclusions about survival.
    a. Based on the additional analyses we performed, your statement was further confirmed, as tumor localization is one of the prognostic factors for survival. The relevant sections have been rewritten.
5. Results: Main problem: 1. As you explained in Table 1 and inclusion criteria. The study was performed in unresectable cases but there are 30 stage I and II cases that usually (not always) are good candidates for surgical resection but only 9 cases are operated on after downstaging with chemotherapy (which initial stage were these patients?).
    a. All patients were treated in advanced stage at our institution. While patients had been treated surgically early in there disease, they had become unresectable at time of

treatment in our institution. Treatment in patients where a downstaging by chemotherapy had been achieved is to heterogeneous to draw any conclusion.

6. German surgeons are always very aggresive with cholangiocarcinoma I repeat I would like to know an explanation for the very low rate of surgery in this serie.
    a. All patients were treated in advanced stage at our institution. While patients had been treated surgically early in there disease, most had become unresectable at time of treatment in our institution.
7. I think that results should be exposed in other way. I think that explanation about figures in the middle of results is not the "usual" way to expose results.
    a. This has been rearranged.
8. The surprise of different survivals I suppose that only refers to good results obtained in Klatskin tumor, the other survical rates are similar to the published before. I think that a statistical relationship with stage is mandatory. As I said before one of the weakness of the paper is mixing cases.
    a. Data on stage and grade were added as Table 2. In addition, both parameters were included in the multivariate analysis but failed to reach significance levels. Case numbers for subgroup analyses in GBC, IHC and EHC and stage/grade would be merely descriptive and are not focus of this research.
9. Discussion: Too much dogmatic. I think that a better statistical analysis is needed to write that EHC has better progosis than IHC.
    a. A multivariate analysis has been integrated and the results and discussion parts have been rewritten.
10. Figures: a legend is needed shorter than included in the middle of results.
    a. This has been corrected
11. References: some mistakes. Not always in Vancouver style. perhaps too much references in a original research paper.
    a. This has been corrected

Reviewer 2

1. Minor comments: In Abstract, the terminology “ID protein” should be full spelled out as “the inhibitor of DNA-binding (ID) protein”.
    a. This has been added accordingly
2. The abbreviation “CC” is not appropriate for biliary tract cancer.
    a. We have been using the term cholangiocarcinoma as the common term, abbreviated with “CC.” As this might be the German equivalent, “CC” has been rephrased to “BTC”.
3. My concern is how to get the tumor samples from 129 patients (56 receiving best supportive care, 64 receiving chemotherapy, and only 9 undergoing surgical resection). The authors should clarify this and describe the archival tumor samples in detail.
    a. All patients were treated in advanced stage at our institution. While patients had been treated surgically early in there disease, they had become unresectable at time of treatment in our institution.

4. The authors investigated the prognostic values of ID proteins using univariate analysis. The authors should perform multivariate analysis using the Cox proportional hazards regression model.
    a. We appreciate this comment. Indeed, we performed a multivariate analysis. Data generated influenced the message of our paper, which has been modified accordingly.
5. Page 14, the description of a tumor suppressive role of ID4 is not clear.
    a. This has been rewritten to provide more detail.
6. Page 10, “As for ID1 and ID2, ID3 expression in the overall study population did not correlate with OS (p=0.28 for cytoplasmic, and p=0.44 for nuclear ID3 expression) (Figure 4D)” does not fit Figure 4D. The p value is 0.037 in Figure 4D (OS 1.1 vs 1.0 years). Is it correct? The authors should clarify this.
    a. This has been corrected
7. Minor comments: The authors should rewrite the submitted manuscript according to the Instructions for Authors, World Journal of Gastroenterology.
    a. This has been corrected

Reviewer 3

1. The results of this manuscript could not support the utility of these proteins as predictive or prognostic markers for CC. This was due to that the major confounding factors such as location of the tumor and tumor stage were not well adjusted in calculation of overall survival. Only divided all patients into two subgroups (patients treated with and without chemotherapy) for calculation of overall survival may cause bias.
    a. Performing a multivariate analysis decreased the value of ID expression The paper has been rewritten accordingly.
2. The manuscript should be rewritten to fit the style of this journal and shorten in a more concise form.
    a. This has been corrected
3. In section of Results, the paragraph of [ID protein expression could serve as negative prognostic factor in subgroups of patients with CC] To investigate the clinical relevance of the above findings, results from the ID protein expression analysis were correlated with overall survival (OS), tumor grade, tumor stage, prior chemotherapy, and response to chemotherapy. There was no data to show the results of correlation between ID protein expression and tumor grade or tumor stage.
    a. This information has been added as Table 2 and was included in the multivariate analysis.
4. In section of Results, the paragraph of [ID protein expression could serve as negative prognostic factor in subgroups of patients with CC] As for ID1 and ID2, ID3 expression in the overall study population did not correlate with OS (p=0.28 for cytoplasmic, and p=0.44 for nuclear ID3 expression) (Figure 4D). This description was not match to the figure and the figure legend.
    a. Figures and legends have been revised.
5. The staging and grading systems for CC applied in this manuscript should be list in the reference.
    a. Staging was performed using the UICC/AJCC staging system

6. Discussion should not duplicate the sentences already described in section of Results.
    a. Results and discussion haven been rewritten
7. Figure 1 did not show the P value in either the figure or figure legend.
    a. P-values have been added.
8. Was the picture in Figure 2 low CC ID3 correct?
    a. Yes, this was correct
9. The legend in Figure 2 was too redundant. “Cytoplasmic staining intensity was scored ranging from 0, negative; 1, low; 2, moderate to 3, strong. Nuclear expression was scored according to the percentage of positive nuclei (0: 0-10%; 1: 11-50%; 2: 51-80%; and 3: 81-100%). Overall ID protein expression was scored as low (0+1) or high (2+3).” should move to Figure 3 and the Figure 3 legend should be revised.
    a. Figures and legends have been reworked.

Reviewer 4

1. First of all, the authors affirm that this is a series of advanced, primarily unresectable cancers. However, according to table 1, more than 20% of the cases appear to be in Stage I or II and 30% in Stage III, and do therefore not qualify for irresectability.
    a. All patients were treated in advanced stage at our institution. While patients had been treated surgically early in there disease, they had become unresectable at time of treatment in our institution.
2. Again, in the same table it appears that 7% of the patients received surgical treatment, but there is no mention of a neoadjuvant therapy performed in some of the patients in the text. These contradictory issues should be explained and revised.
    a. All patients were treated in advanced stage at our institution. While patients had been treated surgically early in there disease, they had become unresectable at time of treatment in our institution.
3. Some of the panels in figure 2 (ID 2 and 4 CC low and CC high) do not convincingly show cholangiocarcinoma: other portions of the tumor should be shown.
    a. Tumor type and localization have ben verified by our pathologists.
4. Moreover, since this is mostly an expression analysis, it would be good to see more pictures of tumors of different localization (intra- and extrahepatic, gallbladder) and grading.
    a. We chose to not show this data as no difference was observed in the different tumor types.
5. Adenocarcinoma of the bile duct shows different epidemiological and pathogenetic characteristics compared to gallbladder cancer. It would be better to keep these two entities separated throughout the manuscript.
    a. We have rephrased the relevant sections to address this comment
6. The authors should then clearly define the focus of their study. Since this seems to be the expression and the prognostic relevance of Id-protein expression, the structure of the manuscript should be changed accordingly and the paragraph concerning the survival analysis according to tumor site should be outshined.
    a. The manuscript has been rewritten to clarify the focus on ID-protein expression

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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