

Reply to the Reviewer's Comments

We would like to thank to the reviewer for his/her valuable comments. Please find the responses below.

Abstract:

[1] The abstract is somewhat extensive..it can be summarized especially at material and methods
This is example: Authors:" The tumor tissues of study group were histopathologically re-examined for tumor grade, pathological stage, lymph node status, the presence of peri-intratumoral lymphocytic infiltrate and lymphatic invasion. The specimens were stained for semi-quantitative estimation of indoleamine-2,3-dioxygenase reactivity by immunohistochemistry. The total indoleamine-2,3-dioxygenase immunostaining score was calculated as the sum of the proportion score and the intensity score. Reviewer: This can be summarized as follow: Tissue sections from the studied tumors were re-examined histopathologically and were stained by immunohistochemistry with indoleamine-2,3-dioxygenase antibodies" All other details are not needed in the abstract..reader can find it in the material and methods.

Reply: The “methods” section in the abstract is revised.

[2] " compared to the control group" ...this is repeated in the abstract. It can be removed..it is understood when authors say "xxx is higher in cancer patients" that this is in comparison with control group.

Reply: The “results” section in the abstract is revised.

Introduction:

[1] page, 2nd paragraph line 11 Authors: " with lowered serum concentrations of Trp and increased Kyn" Comments: Although Kyn mentioned in abstract it should be written again here so that the reader knows what Kyn abbreviation stands for .

Reply: The word “kynurenine” is added.

[2] There is extensive explanation for the prediction of LN metastasis and other general points in the introduction..all this can be much summarized. There is no enough information about Trp and Kyn and the IDOThe whole manuscript is about IDO, please add some more information about its function, role in tissues in health and disease.....there is lack of information for ALL metabolites examined in the study

Reply: Information about tryptophan metabolites and IDO are added.

Material and methods:

[1]Page 8 , 3rd paragraph line 8:" fever" replaced with "fewer"

Reply: The word is corrected.

Results

[1] page 12 , 2nd paragraph, line 20 : " Evaluation of serum pteridin" Comment : this is the first time in the manuscript to mention "pteridin"...it should have been covered in the introduction...what is "pteridin" what is its relationship to other metabolites examined in the study..what is the importance to evaluate it?

Reply: Information about pteridines and their relation to tryptophan metabolism are added.

[2] Page 12, lines 22 and 23 " displayed serum IDO activities were strongly predictive for lymphatic invasion Table 2" Comment: This statement is not right , please see last comments in conclusion

Reply: The sentence is revised.

Figures:

[1] Generally in all figures: ? Nice figures and present well the findings of the study ? Magnification should be mentioned in the legends ? Arrows should be added

Reply: Magnifications and arrows are added.

[2] legend of figure 2 " Semi-quantitative indoleamine-2,3-dioxygenase (IDO) immunostaining. Brown stained cells: IDO expressing stained cells. Blue, unstained cells: Tumor cells " Comment : this is not scientifically proper legend...can be corrected like this " A tissue section of colorectal carcinoma stained with antibodies against IDO. Tumour cells show strong positive staining while normal mucosa show negative or very weak staining . magnification xxxxxx"

Reply: The legend is revised.

[3] figures 1 and 2 are repeated as figures 3c-1 and 3C-2... Comment: there is no need for this repetition, please remove figures 1 and 2...the other figures are nice and show all data

Reply: Figure 1 and 2 are deleted.

Discussion :

[1]: first paragraph is not understood...it need rewriting to make it clearer....what is exactly the relationship between

Reply: Discussion is revised.

[2] The discussion in general is hard to follow...the flow of ideas needs revision, more linking of ideas and some more clarification.

Reply: Discussion is revised.

Conclusion:

Authors" "As a conclusion, these evidences coupled with our data showed that high total IDO immunostaining score is a strong predictor for immune tolerance, lymphatic invasion and subsequent lymph node metastasis" Comment : the word" predictors"

Reply: Additional explanation is added into the last part of discussion.