

### Answering to the Reviewers' comments

We thank all the reviewers for their valuable suggestions. We tried our best to answer them. Please note, all the corrections/ modifications suggested by the reviewer are mentioned in color in the text manuscript.

Reviewer #1:

Abbreviations in abstract and in the main text should be explained accordingly (e.g. OPD in abstract section).

Abbreviation 'OPD' has been explained in the abstract section.

There is no need to mention from statistical tests in the abstract section.

Abstract has been modified and the names of statistical tests have been removed from the abstract section.

-Complete and correct names of statistical tests must be used (e.g. Mann-Whitney U).

Correction has been done in the manuscript and shown in colored text.

-Test names should mainly be mentioned in statistical analysis section.

Names of statistical test are mentioned only in materials and methods under statistical analysis section and results/ tables as and when used during the analysis.

-The authors should mention about the limitations of the study preferably just before the conclusion in the "Discussion" section.

Following are the points mentioned in the discussion section which reflect the limitation of the study.

Page no. 15: Discussion paragraph no. 5: Nevertheless, in concordance with the results of the work by Bilim et al., 2000, current study failed to derive any independent prognostic relevance of  $\beta$ -catenin in bladder tumorigenesis<sup>[24]</sup>

Page no. 16: Discussion paragraph no. 7: Immunostaining of Cyclin D1 in tumors with altered levels of either pS9GSK-3 $\beta$  or  $\beta$ -catenin proteins and its association with tumor stage and grade may validate it as a marker of prognostic significance but does not explain its possible post translational activation by pS9GSK-3 $\beta$ /  $\beta$ -catenin/ TCF/ LEF transcriptional complex in bladder cancer progression.

Page no. 17: Discussion paragraph no. 8: In disagreement with other studies, immunostaining results fail to exhibit any possible correlation between increased nuclear Snail/ Slug and aberrant levels of pS9GSK-3 $\beta$ /  $\beta$ -catenin and their prognostic significance in a given cohort of bladder tumors. Conflicts in the results could be due to different sample size and their source and therefore, multicenter clinical studies are required for reliable conclusion.

-Discussion and conclusion sections should be shortened. A slight English correction is required.

Manuscript has been critically checked/ modified for any typological/ grammatical error(s).

Discussion and conclusion have been shortened as suggested.

Reviewer #2: Dr. Maurya and the other authors used bladder tumor tissues from 90 patients to study the relationships between the expressions of pS9GSK-3 $\beta$ ,  $\beta$ -catenin and its target genes and the prognosis of the patients. They found that aberrant (low or no membranous/ high nuclear/ high cytoplasmic) expression of pS9GSK-3 $\beta$  was significantly associated with tumor stage and tumor grade and shorter overall survival probabilities. This paper is interesting and may provide urologists a new method to classify the bladder cancer patients and predict their prognosis. However, a few points must be adequately addressed.

1. IHC score Please demonstrate different staining intensity on a scale of 0, 1, 2 and 3 where 0 was assigned as no expression, 1 as reduced expression, 2 as moderate expression and 3 as strong expression. The authors are recommended that they use a software to quantify the IHC scores.

Correction in terms of staining intensity scale has been incorporated in materials and method under the heading IHC score. This is to further mention that semiquantitative determination of

immunostaining results in tumor sections along with the controls (normal urothelium) was done by visual examination by the two pathologists independently.

2. On quantitative expression analysis at transcriptome level: RNA Extraction and Real Time-quantitative Polymerase Chain Reaction (RT-qPCR). Please show these results with images.

Representative images (amplification plot and melt curve) of RT-qPCR performed are incorporated as Fig.6.

3. Please show the Kaplan-Meier survival curves for these patients by age, gender, tumor type, stage, grade, etc for comparison.

Kaplan-Meier overall survival (OS) probability has been examined in a cohort of patients who aberrantly co-express pS9GSK-3 $\beta$  and  $\beta$ -catenin for tumor stage, grade, type, age and smoking/tobacco chewing status. The curves are incorporated in the manuscript as Fig. 5. OS probability studies were not possible with gender and Hematuria due to less number of patients in a comparing group.

4. Authors defined aberrant expression of pS9GSK-3 $\beta$  as low or no membranous/ high nuclear/ high cytoplasmic. Please sub-classify these types and their effects on the prognosis. Most importantly, clinicians need the information when a patient's age, gender, smoking status, tumor type, stage and grade are certain, how the expression patterns of pS9GSK-3 $\beta$  will influence the prognosis. This is the value of the manuscript.

Sub-classification of aberrant pS9GSK-3 $\beta$  expression as low or no membranous/ high nuclear/ cytoplasmic is done and the same has been examined with various clinicohistopathological variables and are shown in results section under the heading "*pS9GSK-3 $\beta$  and its diagnostic/ prognostic relevance in human bladder tumors*". Nevertheless, we failed to deduce any

diagnostic/ prognostic significance when low or no membranous/ high nuclear/ cytoplasmic expression of pS9GSK-3 $\beta$  was examined independently with patients' variables.

Reviewer #3: the paper is simple. the experiments are clear enough, the language must be correct.

Manuscript has been modified for any typological/ grammatical errors.

### Answering to Editor's comments

**Please find below the answers to the queries raised by the Editor. We tried to answer to each comment marked in the 44886-Review file (downloaded from the website) as well as to the queries asked through email by the Editor. All the possible changes (asked by the Editor) are been incorporated in colored text of the revised 44886-Review manuscript.**

Comment 1: For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies.

Language certificate has been obtained after extensive review of the manuscript for English language from the Prof. Nishi Pandey, (Professor in English), Department of English and Modern European Languages, University of Lucknow, Lucknow, U.P., INDIA.

Comment 2: Please provide the grant approval file.

The grant approval file has been uploaded during the submission of revised 44886-Review manuscript.

Comment 3: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039, Fax: +86-10-59080039.

Correction has been done.

Comment 4: Institutional Review Board Approval Form or Document

The Institutional Review Board Approval Form or Document has already been submitted in the required PDF format along with the manuscript submission. The statement has been added on the title page of the manuscript.

Comment 5: Signed Informed Consent Form(s) or Document(s)

Informed Consent Form signed by the corresponding author has been already submitted in PDF format along with the manuscript submission. Subjects were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each

patient agreed to treatment by written consent. The statement has been added on the title page of the manuscript.

Comment 6: Conflict-of-interest statement. Please offer signed pdf format.

The PDF file containing conflict-of-interest statement has been uploaded along with the manuscript. However, the declaration made by all the authors on any potential conflict-of-interest has been added on the title page of the manuscript.

Comment 7: Please offer the audio core tip.

The audio core tip in .mp3 format has been uploaded during the submission of revised 44886-Review manuscript.

Comment 8: Article Highlights

Article Highlights are incorporated in the revised 44886-Review manuscript.

Comment 9: Please do not use b1, b2. . . , please change them into A, B, C, D, E. . . .

Required change has been done in the revised 44886-Review manuscript.

Comment 10: Please do not use (i). . . , please change them into A, B, C, D, E. . . .

Required change has been done in the revised 44886-Review manuscript.

Comment 11-19: Please provide the decomposable figure, whose parts are movable and words can be edited.

The decomposable figures in power point file have been uploaded along with the revised 44886-Review manuscript.

About ref 3 and 19, please provide the web site for them.

Website addresses for ref 3, 18 and 19 are added in the revised 44886-Review manuscript.

We checked the paper by crosscheck, there are similar sentences (highlighted in the report) with other articles, please see details on the crosscheck report and rewrote these sentences.

Sentences showing high similarities during crosscheck have been rewritten in the text of the revised 44886-Review manuscript and are marked in colored text.