

According to your comments:

1. The first submitted form of our article, the language, was edited by AJE, a company recommended on the journal's web page; following the journal's publication policy, a non-native English speaker certificate was sent. Because you requested a revision, it has been re-edited according to your request, and a new certificate has been provided.
2. There is only 1 Figure in our manuscript. In line with your criticism, it has been prepared so that all components are portable and editable, and arranged as a PowerPoint file. This figure is original, and in the lower right corner of the picture, copyright information is stated as "Copyright@Ünal B, Çelik MN, Gedik EO, Başsorgun CI, Elpek GO 2023". A new legend has been added according to your decision.
3. New references (Refs. 72-74, 85-87) have been added according to the comments of the referees. Consequently, the number of references has been revised throughout the text and tables.

Answers to the comments of the referees:

Referee I:

Comment 1: A Detailed description of Figure 1 in the manuscript is necessary. Is Figure 1 from the authors' experiment or not? The plotting scale should also be added in Figure 1.

- For a detailed description of Figure 1, the statements “ **Figure 1:** A: Tumor budding consisting of small clusters of 4 or fewer tumor cells present at the invasive edge in a case of hepatocellular carcinoma. B: The main processes and mechanisms involved in tumor budding” have been added to the legend.
- This figure is from our experiment.
- A scale bar is added.

Comment 2: The concept of EMT was first reported in the embryology area. EMT is a biological process that is of great importance in embryogenesis and organ development. I suggest that the research process and some discoveries of EMT could

be added to the discussion or introduction section. Some references could be cited, “EMT Transition States during Tumor Progression and Metastasis”, “Exosomes Regulate the Epithelial-Mesenchymal Transition in Cancer,” for example, or any other similar references.

- Therefore, we revised the last paragraph in the introduction section as “ This is not surprising, given the considerable roles of the EMT in tumor behavior and progression [72-74] Accordingly, the number of studies aiming to detect tumor aggression using comprehensive immunohistochemical and molecular methods far exceeds the number of studies focusing on TB, which can be easily detected as a simple, cost-effective morphological finding from resection materials.”
- We added three references (72-74) according to the comments.

Referee II:

Question 1: - all the published papers on the topic are reviewed in the manuscript? or did you perform a selection of them, and which were the criteria?

- Tumor budding (TB) is an important topic in many organ tumors, associated with many molecular pathways and EMT. However, we focused specifically on primary liver cancers, where tumor budding has yet to be studied. Our criterion for selecting studies was to include only articles in which tumor budding was evaluated microscopically. Because we aimed to reveal the current situation in applying this parameter, which is much easier and cheaper than other complicated methods in terms of primary cancers of the liver. Consequently, we included all the articles in which the morphological evaluation of TB was performed in HCC and CCC. Besides, your questions prompted us to re-examine whether there is another study that was overlooked during the publication process, and we identified another study in the ICC and included it in the manuscript (Reference no. 85). Significant data in this study are summarized in Tables 1 and 2. In addition, the findings, which are different from other studies, are included in the text in the cholangiocarcinoma section of TB as “ Moreover, when ICCs were analyzed according to growth patterns, it was noted that 80% of mass-forming

tumors had high TB. In contrast, this ratio was 16% and 2.3 in periductal infiltrating and intraductal growing subtypes, respectively [85].” (Page 9, 3rd Paragraph, Lines 16-19). Therefore, we thank you very much for your contribution to the evolution of our review.

Suggestion 1: The authors repeated several times that there are not so much papers in the literature about the topic, and I suggest to highlight instead that the literature suggest to further investigate the potential prognostic role of TB in these tumor types, which is a conclusion that could be stated more clearly.

- Therefore, in the first submitted form of the manuscript (FSFM), the statement “ However, regarding primary liver tumors, studies focusing on the relationship between TB and clinicopathological parameters and prognosis are relatively new and limited in number.” in the Introduction section (Page 3, 2nd Paragraph, Lines 21-22) has been changed as “ However, regarding primary liver tumors, studies focusing on the relationship between TB and clinicopathological parameters and prognosis are relatively new.”
- According to this comment, the statement in the last paragraph of the General overview of the mechanisms of TB (FSFM, page 6, Last paragraph, Lines 14-17) “ While these studies aimed to determine tumor aggression using comprehensive immunohistochemical and molecular methods, the number of studies focusing on TB, which can be easily detected from resection materials as a simple, cost-effective morphological finding, are very few.” has been revised as “ Accordingly, the number of studies aiming to detect tumor aggression using comprehensive immunohistochemical and molecular methods far exceeds the number of studies focusing on TB, which can be easily detected as a simple, cost-effective morphological finding from resection materials.” (Page 6, 3rd paragraph, lines: 21-24)
- In the section on TB in Cholangiocarcinoma (FSFM, pages 9, last line and page 10, lines1-2), the statement “ Nevertheless, although few in number, the findings of TB studies in ICC are similar and support the suggestion that TB is a relevant

prognostic factor in histopathological evaluation for these tumors.” has been revised as “ Nevertheless, the findings of TB studies in ICC are similar and support the suggestion that TB is a relevant prognostic factor in histopathological evaluation for these tumors.”(Page 10, 2nd paragraph, Lines 15-17)

- Finally, in the conclusions section (FSFM, Page 10, first paragraph, lines 22-24) the statement “ In this review, evidence from the published literature indicates that TB may be a promising prognostic factor for primary liver tumors.” has been changed as “This review highlights that TB may be a promising prognostic factor for primary liver tumors “. (Page 11, 1st paragraph of the conclusions section, Lines 18-19)

Question 2: do you believe that there is a need for a TB scoring method dedicated to primary liver cancer?

- Certain standards are essential for a parameter to be included in tumor reporting protocols in all pathology disciplines. This also applies to TB. The scoring we use in the colorectal cancer reporting protocol in daily practice has been determined according to the results of many studies for this type of cancer. On the other hand, it has been used in studies on many different cancer types, as we emphasized in the General overview of the mechanisms of TB section (FSFM, Page 6, second paragraph, Lines: 8-10) “Regardless of tumor type, buds in these areas are counted, and according to the recommendation of TBCC, TB is classified into three grades: low, intermediate, and high [7]. “

In our opinion, to determine which scoring method is more effective in primary liver cancer, many studies are required to compare different scoring methods.

Suggestion 2: I suggest discussing more that the assessing and scoring method are fundamental also to compare results from different studies. Anyway, despite different scoring methods TB seems to be a negative prognostic marker in these tumors.

- There is not enough data to discuss TB regarding scoring methods in HCC. In the evaluation of TB in cholangiocarcinomas, different methodologies are mentioned throughout the text (FSFM, Page 8, paragraph 2, lines 23-26; Page 9, Paragraph-2,

lines 7-11). However, as you mentioned, despite different scoring methods TB seems to be a negative prognostic marker in these tumors.

In addition, the importance of scoring in the cholangiocarcinoma section of TB (FSFM, page 10, paragraph 2, lines 3-9) was emphasized, and it was stated that different stratifications were applied in the studies. These methods are summarized in Table 2.

Suggestion 3: I suggest considering the recent paper in which TB0 has been proposed as new category for TB assessment in CRC.

- We also read with interest the article of Zlobec et al. refining the ITBCC tumor budding scoring system by adding a BD-0 category. According to your comment, we emphasized this study on the TB in cholangiocarcinoma section of the revised form of the manuscript “ More recently, in an elegant study, Zlobec et al.^[86] observed that CRC without TB (TB0) is relatively frequent and provided additional information on tumor behavior, suggesting a new “zero budding” category for TB. There is currently no evidence about the prognostic value of TB0 in cholangiocarcinomas, and it would be interesting to conduct further studies in which this category is addressed separately.” (Page 10, Last paragraph, Lines-24-29)

Suggestion 4 and Question 4: I suggest considering that intratumoral and peripheral assessment are not necessarily the same, despite this has been considered in CRC. Is there any paper on this topic in the liver?

- We agree entirely with you. We pointed out that the evidence for intratumoral TB is still weak (FSFM, Page 9, Paragraph 2, Line 27).

There is a paper on this topic in the liver that is included in the manuscript. In the study performed by Budau et al. ⁸⁴ TB is significantly independent of the area of investigation (intratumoral or peritumoral) (FSFM, page 9, lines 7-14)

Suggestion 5: There is no discussion about the reviewed despite their low number, which are other limitations? small number of cases? ethnicity? scoring method? etc....

- which are the potential therapeutic implication of TB assessment and inclusion in pathology report?

- The number of cases, ethnicity, and scoring methods are presented throughout the text and presented in Tables 1 and 2. Our review is not a systematic meta-analysis, and we did not perform any statistical analysis. According to your comments, we added the statements “ In CRC, TB, combined with other established biomarkers, may allow us to discriminate between patients who would benefit from oncological resection and patients who will receive adjuvant therapy and to classify different therapeutic options, especially in advanced-stage patients^[87]. Thus, TB can predict prognosis and regulate treatment options in primary liver cancers. However, the role of TB in the treatment of these tumors remains to be investigated.”(Page 11, 2nd paragraph, Lines 11-16)