

**Dear Editor and Reviewers:**

Thank you a lot for helping us to develop our work and giving us the chance to revise our manuscript.

In our manuscript formerly submitted, we aim to explore the the relationships between clinicopathological parameters and circulating tumor cell (CTC) enumeration and classification in pancreatic cancer. We appreciate the thorough reviews provided by the journal and the positive response of reviewers that found the research of this manuscript was interesting and of scientific importance. We have studied comments carefully and made corresponding revision which we hope to meet the rigorous standards. Revised portions are marked in red front in this revised manuscript. The responses to the editor's and reviewer's comments and major revisions in the manuscript are listed as follow:

**Reviewer (Number ID:00070509):**

**Comments:**

The authors hypothesize that both total CTC number and CTC EMT phenotype may act as potential biomarkers for PDAC prognosis. They conclude that CTCs are highly associated with PDAC clinicopathological features and are a novel prognostic factor for this disease through their experiments. The paper is well written. Conclusions drawn from the experiments are adequate. I suggest the acceptance of the paper without further changes. However, there is one

thing to be corrected in Figure 1. (B) Biophenotypic epithelial/mesenchymal CTCs. --> Mesenchymal CTCs. (C) Mesenchymal CTCs. --> Biophenotypic epithelial/mesenchymal CTCs.

**Response:**

We thank the reviewer for pointing this out and apologize for our mistakes. We have corrected the mistakes in Figure1.

**Reviewer (Number ID:00505755):**

**Comments.** The background and introduction describing that CTCs have a property as prognostic indicator may be revised to include the detailed information about the interaction between CTCs and PDAC. Please check abbreviations carefully. Proofreading is needed.

**Response**

We are appreciated with this suggestion by the reviewer. We have included the detailed information about the interaction between CTCs and PDAC in the background and introduction. We have defined all of the abbreviations and the present article has been proofread by a professional English language editing company.

**Reviewer (Number ID:00505755):**

**Comments 1. This study suffers from a small sample size.**

**Response:** We have expanded the data sets by selecting a further 27 PDAC patients and analyzed the relationships between clinicopathological parameters and the relative abundance of three circulating EMT-CTC subpopulations.

**Comments 2.** It was performed despite the known disconnect between EMT and metastasis in pancreatic cancer (Martin C. Whittle, Sunil R. Hingorani, Oncotarget 2015), a paper that the authors do not cite. The implications of this disconnect in light of the current findings should be presented and discussed

**Response:** Thank you very much for reminding us to cite the research mentioned above. We have included it in the introduction.

**Comments 3.** The general information on human subjects and the correlation of total CTC status with clinicopathological factors (Table 2) could be made more detailed (for example it would be interesting to show mean age, median age, ethnic group)

**Response:** We appreciate the careful reading of our manuscript and valuable suggestions of the reviewer. We have made the general information on human subjects more detailed and shown median age and ethnic group in Table 2.

**Comments 4.** On page 11/26 last line of paragraph of Results, it is not clear where the numbers 67.5%, 42.5%, 32.5% are derived. Data should be presented more clearly in Table 1 to align with text.

**Response:** We thank the reviewer for pointing this out and apologize for our mistakes. In the present study, we expanded the data sets and the positive rates of epithelial, hybrid, and mesenchymal CTCs were 60.7%, 36.4% and 45.8%, respectively.

**Comments 5.** Table 1 and Figure 1 should be combined.

**Response:** We have combined Table 1 and Figure 1.

**Comments 6.** Table 3 results are not mentioned in text nor are data in this table discussed. This is also the case for Figures 3D and 3F

**Response:** We thank the reviewer for pointing this out and apologize for our mistakes. Table 3 (Page 13), Figure 3D and 3F (Page 15) was mentioned in the present result part.

**Comments 7.** For ease of understanding the results, it is better to have different labels on the y-axis of figure 2E and F to reflect overall survival and progression free survival respectively rather than just mention it in legend.

**Response:** Thank you very much for reminding us this. We have used different labels on the y-axis of figure 2E and F to reflect overall survival and progression free survival respectively.

**Comments 8.** In figure 2G, the CTC negative and positive lymphocytes results appear to be overlapping yet they are statistically significant?

**Response:** In the present study, we found that the lymphocyte counts for the CTC-positive group was less than that of the CTC-negative group ( $1.6 \pm 0.2 \times 10^9/L$  and  $1.9 \pm 0.3 \times 10^9/L$ , respectively.  $P < 0.01$ ).

**Comments 9.** Delete all empty pages in the manuscript.

**Response:** We thank the reviewer for pointing this out and apologize for our mistakes. All empty pages have been deleted.

**Comments 10.** Tables 4-1 and 4-3 are the same!

**Response:** Thank you very much for reminding us this. We apologize for our mistakes. We provided the correct Table4-3 in the present manuscript.

**Comments 11.** Figures 2 and 3 labels, what is AJCC stages? What is CTM on page 19? CK20 on page 16? Etc. Improve write up of paper, have a native English speaker check it for errors and define all abbreviations upon first use. Even abstract has too many abbreviations!

**Response:** We appreciate the careful reading of our manuscript and valuable suggestions of the reviewer. We have defined all abbreviations upon first use. Moreover, the present article has been proofread by a professional English language editing company.

**Reviewer (Number ID:02441100):**

**Comments 1.** In “Introduction” (page 5) the sentence “...(EUS-FNA) is the current gold standard technique” should be clarified.

**Response:** Thank you very much for this review’s positive remarks. We have clarified the sentence mentioned above in the introduction part.

**Comments 2.** epithelia-to-mesenchymal transition (EMT)” (pages 6-7) is internationally named “epithelial- mesenchymal transistion”

**Response:** We appreciate the careful reading of our manuscript and have corrected our mistakes.

**Comments 3.**“preoperative chemotherapy “(page 8) should be checked.

**Response:** We apologize for our mistake and have corrected the spelling.

**Comments 4.** In “Results- Correlation of CTCs with clinicopathological feature (page12) “(Tab. 3)” should be added at the end of the sentence “Multivariate analysis revealed that ...for overall survival in patients with PDAC. Moreover

“ (Figure 2F and G)” on page 13 should be changed to “(Figure 2G and H)” according to the legend of Figure 2.

**Response:** We thank the reviewer for pointing this out and we have added (Table 3) at the end of the sentence mentioned above. Meanwhile, we have corrected the sequence error or the figures mentioned above.

**Comments 5.** In “Results- Association between CTC- EMT subpopulation and clinicopathological features” is reported that “The presence of E-CTCs was positively correlated with TMN stage( $P \leq 0,01$ ) and distant metastasis ( $P \leq 0,01$ ). ” However in Table 4 distant metastasis is not indicated as clinicopathological factor (pay attention: Table 4.3 is analogous to Table 4.1!!)

**Response:** We apologize for our mistakes. M-CTCs but not E-CTCs was positively correlated with TNM stage and distant metastasis. We provided the correct Table4-3 in the present manuscript.

**Comments 6.** The acronym CTMs in “Discussion” (Pag.20 ) should be defined.

**Response:** We have defined CTMs as circulating tumor microembolis in the discussion part.

**Comments 7.** At last the legend of table 1 should be checked : why “ total and vim+ CTC status”? Vim+ is not included in “total CTCs status”?

**Response:** We apologize for our mistakes. The legend of table 1 has been changed to “Baseline CTC characteristics of treatment-naïve patients with advanced PDAC according to CTC status.”