

## **Point by point reply to the comments of Editor and Reviewers**

### Editor:

*For manuscripts submitted by non-native speakers of English, please provide language certificate by professional English language editing companies.*

**Reply: Amended.** An extensive English language correction has been done. We have provided the language editing certificate by Scribendi a professional English language editing company.

*Please offer the audio core tip*

**Reply: Amended.** We have uploaded the Audio Core tip.

*Please add PubMed citation numbers and DOI citation to the reference list and list all authors.*

**Reply: Amended.** We have added the missing PubMed citation numbers and DOI citation to the references.

*Table 1 and Table 2. Please offer legends for all abbreviation names.*

**Reply: Amended,** we have added the legends for all the abbreviation names.

*Please mark the figure 1b in this paper. Figure file names should identify the figure and panel. Avoid layering type directly over shaded or textured areas in the figure. Uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ..."*

*So please reoffer figures titles.*

**Reply: Amended.** The figure 1b (now figure 2b) has been marked in the paper. The Figure title has been changed as follow: "Figure 2. The nanoparticles. A: different types of nanoparticles for targeted drug delivery; B: specific nanoparticles for targeting pancreatic cancer stem cells".

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### Reviewer #1, Reviewer's code 03370303:

*A minor concern. The first sentence in the second paragraph of Introduction "Diagnosis followed by surgery and chemo or radiation therapy is the main treatment approach, however it doesn't present satisfactory results [2]." is hard to be understood. It should be rewritten in a clearer expression, for example, "Currently, surgery coupled with chemo or radiation therapy is the main treatment approach although it doesn't present satisfactory results [2]."*

**Reply: Amended.** We have corrected the sentence as suggested by the reviewer.

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### Reviewer #2, Reviewer's code 03670299:

*In this manuscript the author describe the potential use of Pancreatic cancer stem cells in the treatment of pancreas cancer. According to the manuscript description that indicates fundamentally as pancreatic cancer the pancreatic ductal adenocarcinoma (PDAC), it should be more appropriate change the title of the manuscript describing in the specific the treatment of PDAC.*

**Reply: Amended.** We changed the title as suggested by the reviewer in: "Pancreatic cancer stem cells: perspectives on potential therapeutic approaches of pancreatic ductal adenocarcinoma"

*The manuscript should be revised in its complex organization. The authors should add more recent literature regarding different themes treated in the manuscript. In addition, the characteristics of pancreatic ductal adenocarcinoma in the introduction should be described in more detail. Moreover, a recently reference should be added: Gallmeier E and Gress TM. Pancreatic ductal adenocarcinoma. Internist (Berl). 2018 Aug;59(8):805-822. doi: 10.1007/s00108-018-0460-z.*

**Reply: Amended. We have improved the description of characteristics of pancreatic ductal adenocarcinoma in the introduction and added more recent references, including the reference suggested by the reviewer.**

*Regarding the chapter relative to PANCREATIC CANCER STEM CELLS CHARACTERISTICS the authors should clarify the possible interaction between pancreatic cancer stem cells (PCSCs) and parental cells. A recently paper describe with clarity this interaction describing that “high local invasion of parenchymal cells into the CSC-derived vascular network suggests that a symbiotic relationship between the parenchymal cells and the CSCs underlies the initiation and maintenance of early PDAC infiltration and metastasis” (Biondani G. et al., FEBS Journal 285 (2018) 2104–2124).*

**Reply: Amended. We have clarified the cross talk between PCSCs and PDAC parental cells. In particular, we have described how their secretome is involved in the interaction and how it influences their growth. We have also added the reference suggested by the reviewer.**

*Regarding the paragraph on Chemoresistance related to EMT process, other interesting references should be added (Renz et al., 2017, Repurposing Established Compounds to Target Pancreatic Cancer Stem Cells (CSCs), Med. Sci. 2017, 5, 14; doi:10.3390). Moreover, some interesting data from Meidhof et al. demonstrated that the class I HDAC inhibitor mocetinostat acts as an epigenetic drug and interferes with Zeb1 function, restores miR-203 expression, and thereby represses EMT and stemness properties in PDAC and prostate cancer (Meidhof, S.; Brabletz, S.; Lehmann, W.; Preca, B.T.; Mock, K.; Ruh, M.; Schuler, J.; Berthold, M.; Weber, A.; Burk, U.; et al. ZEB1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. EMBO Mol. Med. 2015, 7, 831–847). Another interesting paper not cited by authors is: Zheng, X. et al., Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature 2015, 527, 525–530.*

**Reply: Amended. We have added and cited these references indicated by the reviewer.**

*Other the chapter on nanoparticles the authors should give results on the possible use of phytochemicals to targeting PCSCs (see references Aliebrahimi et al., 2018, Biomedicine & Pharmacotherapy 106 (2018) 1527–1536; Suvroma Gupta and Dipankar Pramanik. Phytochemicals and Cancer Stem Cells: A Pancreatic Cancer Overview, Current Chemical Biology, 10.2174/2212796810666160419152309).*

**Reply: Amended. We have added the possible use of phytochemicals to targeting PCSCs which would deserve to be analysed as nanoparticle formulations.**

*Minor Revision 1- There are some spelling/grammar/formatting errors in the current version of the manuscript. Please correct them carefully. 2- Abbreviations need to be described at first time in the text.*

**Reply: Amended. Spelling/grammar/formatting errors have been corrected and abbreviations have been described.**

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Reviewer #4, Reviewer's code: 03197771:

*Although the authors made an effort to tabulate the information, the fact that some compounds overlap both sections, together with the lack of an explanation of the mechanisms or levels of action of non-cancer drugs hinders an integrated understanding of the proposed novel therapies for pancreatic cancer to be explored and the justification behind these proposals. A summary figure is recommended.*

**Reply: Amended. A summary figure (new Figure 1) has been added as suggested by reviewer.**

*The following previous related reviews should be included: Ishiwata T, Matsuda Y, Yoshimura H, Sasaki N, Ishiwata S, Ishikawa N, Takubo K, Arai T, Aida J. Pancreatic cancer stem cells: features and detection methods. *Pathol Oncol Res.* 2018 Jun 8. doi: 10.1007/s12253-018-0420-x. Valle S, Martin-Hijano L, Alcalá S, Alonso-Nocelo M, Sainz B Jr. The Ever-Evolving Concept of the Cancer Stem Cell in Pancreatic Cancer. *Cancers(Basel).* 2018 Jan 26;10(2). pii: E33. doi: 10.3390/cancers10020033.*

**Reply: Amended. The reviews suggested have been included into the paragraph 2.1 PANCREATIC CANCER STEM CELLS CHARACTERISTICS**

*Testing of novel approaches should highlight how new treatments are expected to surpass conventional current treatments, if so, stating their possible limitations. This part is not clearly presented in the manuscript.*

**Reply: We have state the limitations of conventional treatments (in particular of gemcitabine) into the Introduction section.**

*Specific comments: The information provided in the abstract should be carefully chosen as it is limited in length. It has been noted a redundancy on the second sentence "Unfortunately, up to date the clinical efforts to fight this cancer have not yet resulted in improved long-term survival", which does not add any new information to the first.*

**Reply: Amended. We have removed the redundant sentence from abstract.**

*Page 4, lines 124 and following: "...other pathways such as autophagy", autophagy is a cellular process, not a pathway.*

**Reply: Amended.**

*Authors should review statements that lack references in the whole manuscript, for example: Page 4, lines 94 and following: "Recently, it has been demonstrated that cancer stem cells play critical roles in anticancer treatment resistance and are responsible for metastasis in several human malignancies, including PDAC" ; Page 8, lines 250 and following: ". In particular, Crocetinic acid decreases the number and size of the spheroids in a dose-dependent manner, and suppressed the expression of DclK1,*

*a PCSCs surface marker.”; Page 9, lines 264 and following: “This compound targets PCSCs by decreasing the expression of: Notch 1–4 receptors, their ligands Jagged1, Jagged2, DLL1, DLL3, DLL4, the downstream protein Hes-1, as well as of  $\gamma$ -secretase complex (required for Notch activation).”*

**Reply: Amended. The references have been added.**

*Page 12, lines 358 and following: it is not clear how NP approach could be integrated in pancreatic cancer prevention.*

**Reply: Amended. The sentence has been changed since actually the NPs described based on natural compounds refer to the treatment of pancreatic cancer.**

*Page 6, the sentence: “This observation suggests that it should be further investigated to verify if it is also effective for targeting PCSCs” should be relocated as the authors continue to describe additional antibiotics that impact CSCs.*

**Reply: Amended. This sentence has been relocated.**

*Authors should elaborate Figure 1 legend further.*

**Reply: Amended. The Figure 1 (now Figure 2) legend has been improved.**

*Extensive English language correction is needed. Examples are: Page 2, lines 35 and following: “Recent reports..” is fragmented, should be rewritten. Page 3, line 71: “..the major of patients..” Avoid subjective vague adjectives such as “the most conventional... (How is this measured? ); “only few months..” (Indicate range, otherwise the information provided is limited/incomplete); “very resistant..”; “the most mature...” etc*

**Reply: Amended. An extensive English language correction has been done. is Quality control of a manuscript’s language has been done by the language editing service of Scribendi (an elite editorial team since 1997). We have provided the language editing certificate**

*In general, the manuscript text needs to be edited to avoid unneeded terms or sentences. Explain acronyms in its first appearance, example: “GEMM model..” “Canadensis” should appear in lower case.*

**Reply: Amended. Unneeded terms or sentences have been corrected and acronyms explain in the first appearance.**