RESPONSE TO REVIEWERS

Manuscript number: 80875

Manuscript Title: Recent advances in Targeted Therapy for Pancreatic Adenocarcinoma

Dear Editor,

Thank you for reading our manuscript and reviewing it, which will help us improve it to a better scientific and language level. We revised our manuscript, and quite a lot of changes have taken place. So we have sent the revised manuscript, and a version containing all the changes visible.

At the following, the points mentioned by reviewers will be discussed:

Comments to author

Reviewer #1:

Comments: Consider general English revision, grammar and style Title A reformulation of the title as "Recent advances in Targeted Therapy for Pancreatic Adenocarcinoma" is more accurate.

Response: The authors thank the Reviewer for his kind suggestion. The authors agreed with the Reviewer and revised the Title "Recent Advances for Targeted Therapy in Pancreatic Adenocarcinoma" to "Recent advances in Targeted Therapy for Pancreatic Adenocarcinoma".

Comments: Abstract It describes the landscape of PDAC, including the most characteristic genetic alterations, but refers to "tumor microenvironment, chemoresistant cancer stem cells, and the desmoplastic stroma" merely as targets for therapy, not as constitutive and very relevant elements of PDAC, the true reason of its relevancy as targets.

Response: The authors thank the Reviewer for his kind suggestion. The authors think that the reviewer's opinion makes sense. The reason why tumor microenvironment, chemoresistant cancer stem cells, and the desmoplastic stroma are targeted is that they are constitutive and very relevant elements of PDAC. Therefore, the authors revised the sentence "In addition, the tumor microenvironment, chemoresistant cancer stem cells, and the desmoplastic stroma have been the target of some promising clinical investigations." to "In addition, the self-preserving cancer stem cells, dense tumor microenvironment (fibrous accounting for 90% of the tumor volume), and suppressive and relatively depleted immune niche of PDAC are also constitutive and relevant elements of PDAC."

Comments: In the final lines, "analyze possible reasons for the lack of positive results in clinical trials and ways to improve them" could be substituted by "analyze possible

reasons for the lack of positive results in clinical trials and suggest ways to improve them."
Response: The authors thank the Reviewer for his kind suggestion. The authors revised the sentence "analyze possible reasons for the lack of positive results in clinical trials and ways to improve them" to "analyze possible reasons for the lack of positive results in clinical trials and suggest ways to improve them."

Comments: The sentence "also discuss emerging trends in targeted therapies as the most promising approach." has to be reformulated. The emerging trends by themselves, without any other reason, do not constitute a promising approach.
Response: The authors thank the Reviewer for his kind suggestion. Thanks to the Reviewer's reminder, the authors have re-reviewed the sentence and found that it is unclear and has some semantic repetitio, and the original meaning is to express "some new trends of targeted treatment of PDAC at present". Therefore, the authors revised the sentence "also discuss emerging trends in targeted therapies as the most promising approach." to "also discuss emerging trends in targeted therapies for PDAC."

Comments: As the authors properly remark in the summary "This suggests to us that, in fact, most clinical trials have also demonstrated that monotherapy of targeted drugs is not feasible. Therefore, combining targeted inhibitors of multiple pathways may be the future targeted therapy research's primary direction." This idea has to be incorporated in the abstract, as reflects the current paradigm in the development of effective PDAC's treatment strategies.
Response: The authors thank the Reviewer for his kind suggestion. The authors agreed with the Reviewer and added a sentence ---"We also discuss emerging trends in targeted therapies for PDAC: combining targeted inhibitors of multiple pathways. "to Abstract.

Comments: Keywords Targeted therapy and Cancer stem cell are MeSH terms. Pancreatic adenocarcinoma is not a MeSH term, can be replaced by pancreatic carcinoma. Stroma targets is not a MeSH term, is poorly descriptive and does not add to "targeted therapy" whereby could be omitted. Tyrosine kinase inhibitors, again not MeSH term, could be omitted, because are not the only class of agents to be used as targeted therapy. As described in the text there are monoclonal antibodies, epigenetic modifiers…
Response: The authors thank the Reviewer for his kind suggestion. The authors revised the Keywords "Pancreatic adenocarcinoma; Targeted therapy; Stroma targets; Tyrosine kinase inhibitors; Cancer stem cell." to "Pancreatic carcinoma; Targeted therapy; Cancer stem cell; Monoclonal antibody; Epigenetic modifier."

Comments: Core Tip Please consider the observations formulated for "Abstract".
Response: The authors thank the Reviewer for his kind suggestion. Based on the suggestions on Abstract, the authors revised the Core Tip to "Pancreatic adenocarcinoma (PDAC) is a fatal and rare disease with a 5-year survival rate of 8% and a median survival of 6 months. In pancreatic adenocarcinoma, targeted therapy has been extensively evaluated, however, survival improvement of this aggressive disease using a targeted strategy has been minimal. This manuscript summarizes current targeted therapies and

clinical trials targeting dysregulated signaling pathways and components of the PDAC oncogenic process, analyzes possible reasons for the lack of positive results in clinical trials and suggest ways to improve them. We also discuss emerging trends in targeted therapies for PDAC: combining targeted inhibitors of multiple pathways."

Comments: Introduction In this section, there is not a general description of the structural and biological characteristics of PDAC, essential to understand the lack of response to the common treatments, the bad prognosis and the reason for exploring the subsequently reported therapeutic strategies. Also is essential to consider this before to state, "The development of novel and effective therapeutic strategies is therefore vital to improving treatments that are both targeted and personalized.", because the necessity of targeted and personalized treatments is derived from the complexity and particularities of PDAC's structure and biology. In order to improve the above mentioned, some of the paragraphs used in other sections could be transferred here, as 4. Stroma targets or the first and third paragraphs from Summary.

Response: The authors thank the Reviewer for his kind suggestion. The authors think that the reviewer's opinion makes sense. There should be a general description of the structural and biological characteristics of PDAC, which is necessary for the readers to understand the lack of response to the common treatments, the bad prognosis and the reason for exploring the subsequently reported therapeutic strategies. What's more, the necessity of targeted and personalized treatments is derived from the complexity and particularities of PDAC's structure and biology. The authors thank the Reviewer not only for pointing out the authors' negligence, but also for telling the authors about the methods of modification. The authors have added some content in Introduction. However, since there is a detailed description of the mechanisms that cause PDAC and their complexity when talking about a specific pathway, such as stroma targets, in the Introduction section the authors just summarize the complexity and particularities of PDAC's structure and biology. The authors revised the paragraph 1st of Introduction to "Pancreatic adenocarcinoma (PDAC) is a fatal disease with a 5-year survival rate of 8% and a median survival of 6 months. It ranks fourth among cancer-related deaths in the U.S., and it will become the number two cause within a decade. In PDAC, several mutations in the genes are involved, with Kirsten rat sarcoma oncogene (KRAS) (90%), Cyclin dependent kinase inhibitor 2A(CDKN2A) (90%) and Tumor suppressor 53(TP53) (75%–90%) being the most common. Mothers against decapentaplegic homolog 4(SMAD4) represents 50% (Table1). In addition, the self-preserving cancer stem cells, dense tumor microenvironment (fibrous accounting for 90% of the tumor volume), and suppressive and relatively depleted immune niche of PDAC are also constitutive and relevant elements of PDAC. They are considered significant clinical barriers to successful therapy development, making it one of the most challenging diseases to treat. At present, only surgical resection is a potentially curative treatment for this refractory disease, which shows an improvement in survival rates."

Comments: Material and methods No mention of any method. To be corrected. At least the time interval, keywords and database/s consulted have to be referred.

Response: The authors thank the Reviewer for his kind suggestion. Methods are not

required in the structure guidelines of review provided by WJGO journal. If methods were added to the text as a large heading, it would detract from the overall structure and appear abrupt. So the authors added the Method to the abstract and the last paragraph of the introduction. Methods: The NCDI clinical trial website (www.clinicaltrials.gov) and the PubMed database were queried to identify completed and published (PubMed) and ongoing (clinicaltrials.gov) clinical trials (from 2003-2022) using the keywords: pancreatic cancer and targeted therapy. Time interval are shown in table2 as well. And the PubMed database were also queried to search for information about the pathogenesis and molecular pathways of PDAC using the keywords pancrearic cancer and molecular pathways.

Comments: Results In this type of review, there is no place for Results as such, but for a structured exposition of the findings in literature.

Response: The authors thank the Reviewer for his kind suggestion. The authors have carefully read the structure guidelines of review provided by the WJGO journal and it does not require RESULTS as a separate structural exposition. Thanks again for the reminder. Results related content is in Table2 and the author's reflections are in Summary.

Comments: As general observation and to be corrected, several of the acronyms used are not adequately explained in their first mention (PanIN, page 3;gBRCAm, page 9;HA, page 12;GA, page 13;MMB, page 15;nab-P+G, page 15;A2AR, page 16).

Response: The authors thank the Reviewer for his kind suggestion. Thank the Reviewer for being so attentive and for the reminder. The authors have corrected all the abbreviations in the text that do not conform to the rules. As in these examples that the Reviewer have presented: PanIN, page3 was revised to “pancreatic intraepithelial neoplasia (PanIN)”; gBRCAm, page9 was revised to “germline BRCA mutations (gBRCAm)”; HA, page12 was revised to “Hyaluronic acid (HA)”; GA, page13 was revised to ”gemcitabine”; MMB, page15 was revised to ”momelotinib”; nab-P+G, page15 was revised to “gemcitabine plus nab-paclitaxel”; A2AR, page16 was revised to “adenosine A2 receptor”.

Comments: The authors employed “PSC” referred to two different subjects: page 11, section 4., for pancreatic stellate cells and page 14, section 5., for pancreatic cancer stem cells. Confusing and to be corrected.

Response: The authors thank the Reviewer for his kind suggestion. This is a clerical error by the author. page 14, section 5. The correct abbreviation for pancreatic cancer stem cells is CSCs.

Comments: In page 6, paragraphs 3rd and 4th, there is no indication about the mutational status of EGFR and KRAS in the referred studies, key for evaluating the context of efficacy of the treatments exposed. Consider the same in the 6th, referred to trastuzumab and HER2 expression or afatinib and EGFR mutations.

Response: The authors thank the Reviewer for his kind suggestion. The authors have added two references in In page 6, paragraphs 3rd: “Walsh N, Kennedy S, Larkin A,

Corkery B, O'Driscoll L, Clynes M, Crown J and O'Donovan N. EGFR and HER2 inhibition in pancreatic cancer. Invest New Drugs 2013; 31: 558-566 [PMID: 23076814 DOI: 10.1007/s10637-012-9891-x] "and "Einama T, Ueda S, Tsuda H, Ogasawara K, Hatsuse K, Matsubara O, Todo S and Yamamoto J. Membranous and cytoplasmic expression of epidermal growth factor receptor in metastatic pancreatic ductal adenocarcinoma. Exp Ther Med 2012; 3: 931-936 [PMID: 22969995]" to supplement the mutational status of EGFR and KRAS in PDAC. The clinical trail of erlotinib in Paragraph 3rd did not examine the mutational status of EGFR in each PDAC patient.569 patients were randomly assigned (285 erlotinib and gemcitabine and 284 placebo and gemcitabine) at 176 centers in 17 countries. Likewise, the clinical trail of nimotuzumab in Paragraph 4th did not examine the mutational status of EGFR in each PDAC patient. Similarly, the clinical trails in paragraph 6th did not examine the mutational status of EGFR in each PDAC patient. So the authors didn't mention the mutational status of EGFR/HER2 in each patients in those trails.

Comments: In page 9, section 3.5, 4th line, "15 stable responses" are mentioned. Although in determinate contexts stabilizations are considered and described as a component of the global clinical benefit, the concept "stable responses" is not correct and has to be changed for "stabilizations", "patients with stable disease" or similar.

Response: The authors thank the Reviewer for his kind suggestion. The authors revised "15 stable responses" to "15 patients with stable disease".

Comments: Summary Is correct, but lack one of the most relevant aspects indirectly exposed in the work. PDAC is a very complex entity, joining different molecular particularities and in a dynamic manner, not in a static one. As some guidelines already stated and can be concluded from de data shown here, is very important to spread the genetic and transcriptomic profiling of every PDAC in order to capture the vulnerabilities of the tumor as far as possible as the way to improve therapeutic results.

Response: The authors thank the Reviewer for his kind suggestion. Thank the Reviewer for not only pointing the problems, but also for showing the authors how to fix them. The authors revised the last paragraph of Summary to "PDAC is a very complex entity, joining different molecular particularities and in a dynamic manner, not in a static one. As some guidelines already stated and can be concluded from de data shown here, is very important to spread the genetic and transcriptomic profiling of every PDAC in order to capture the vulnerabilities of the tumor as far as possible as the way to improve therapeutic results. In conclusion, developing the targeted drug for pancreatic cancer has a long way to go. The complex interactions within targeted biological pathways, the pharmacokinetics of targeted drugs, predictive markers of the targeted drug benefit, and the combined application of targeted drugs still require extensive and in-depth studies.".

Reviewer #2:

Comments: The authors have carried out an extensive review of the literature taking into account numerous biological aspects of Pancreatic Cancer. They summarise current targeted therapies and clinical trials targeting dysregulated signaling pathways and components of the PDAC oncogenic process, analyse possible reasons for the lack of

positive results in clinical trials and ways to improve them, and also discuss emerging trends in targeted therapies as the most promising approach. The Manuscript is written correctly and the Literature Analysis appears vast and clearly illustrated. The subject is very topical given the poor disease prognosis and the increasing in incidence.

Response: Thank you for your high praise of this manuscript. In this revision, we found our shortcomings and made some revisions to the Title, Abstract, Introduction, abbreviations in the text, and the Summary section, so please review it.

The Title was revised to "Recent advances in Targeted Therapy for Pancreatic Adenocarcinoma".

The Abstract was revised to "Pancreatic adenocarcinoma (PDAC) is a fatal disease with a 5-year survival rate of 8% and a median survival of 6 months. In PDAC, several mutations in the genes are involved, with Kirsten rat sarcoma oncogene (KRAS)(90%), Cyclin dependent kinase inhibitor 2A(CDKN2A) (90%) and Tumor suppressor 53(TP53) (75%–90%) being the most common. Mothers against decapentaplegic homolog 4(SMAD4) represents 50%. In addition, the self-preserving cancer stem cells, dense tumor microenvironment (fibrous accounting for 90% of the tumor volume), and suppressive and relatively depleted immune niche of PDAC are also constitutive and relevant elements of PDAC. Molecular targeted therapy is widely utilized and effective in several solid tumors. In pancreatic adenocarcinoma, targeted therapy has been extensively evaluated, however, survival improvement of this aggressive disease using a targeted strategy has been minimal. There is currently only one FDA-approved targeted therapy for PDAC – erlotinib, but the absolute benefit of erlotinib in combination with gemcitabine is also minimal (two weeks). In this review, we summarize current targeted therapies and clinical trials targeting dysregulated signaling pathways and components of the PDAC oncogenic process, analyze possible reasons for the lack of positive results in clinical trials and suggest ways to improve them. We also discuss emerging trends in targeted therapies for PDAC: combining targeted inhibitors of multiple pathways. Method: The PubMed database and the NCDI clinical trial website (www.clinicaltrials.gov) were queried to identify completed and published (PubMed) and ongoing (clinicaltrials.gov) clinical trials (from 2003-2022) using the keywords: pancreatic cancer and targeted therapy. And the PubMed database were also queried to search for information about the pathogenesis and molecular pathways of pancreatic cancer using the keywords pancreatic cancer and molecular pathways.".

The keywords were revised to "Pancreatic carcinoma; Targeted therapy; Cancer stem cell; Monoclonal antibody; Epigenetic modifier.".

The last paragraph of Summary was revised to "PDAC is a very complex entity, joining different molecular particularities and in a dynamic manner, not in a static one. As some guidelines already stated and can be concluded from de data shown here, is very important to spread the genetic and transcriptomic profiling of every PDAC in order to capture the vulnerabilities of the tumor as far as possible as the way to improve therapeutic results. In conclusion, developing the targeted drug for pancreatic cancer has a long way to go. The complex interactions within targeted biological pathways, the pharmacokinetics of targeted drugs, predictive markers of the targeted drug benefit, and the combined application of targeted drugs still require extensive and in-depth studies.".