Answers to reviewers:

Thank you for your comments and corrections. All the requested changes were accomplished and added to the manuscript. The comments of the reviewers are in italic .

Reviewer 1

The paragraph on FOLFIRINOX should make note of the significance and type of side effects, and that the treatment is really only viable in a certain patient population (stage IV).

The side effects of FOLFIRINOX and the viability of this regimen in a certain patient population were added to the manuscript.

Similarly, the GEM-elotinib paragraph needs to mention that the effect is modest and is really only beneficial to the subgroup of patients that develops a rash.

The specificities of this regimen were also added to the manuscript.

In particular, the section on miRNAs could be expanded to include, not only more detail about the miRNAs involved, but also specific citations about the contribution of miRNAs to radioresistance, in particular, through mechanisms related to Beclin-1.

The requested data was added as follow: Other microRNAs are in relation with radio-resistance of pancreatic cancer; in a recent study evaluating a radio-resistant pancreatic cell line, miRNA-216a was significantly down regulated, whereas the autophagy activity was controlled. Using bioinformatics analysis, it was concluded that forced expression of micro-RNA-216a enhances the radio-sensitivity of pancreatic cells by inhibiting beclin-1 mediated autophagy.

The authors mention that CDK2NA, TP53, and SMAD4 are regulated by miRNAs, but no pathways are mentioned.

The pathways were added as requested by the reviewer: For example, CDK2NA or p16 inhibits cycline -dependant kinase 1, 4 and 6 and also help to stabilize p53; CDKN2A itself is regulated by a microRNA, miR-10b [37]. TGFB (Transforming growth factor [beta]) is a potent tumor suppressor that signals via the SMAD pathway and intersects with the WNT beta-catenine signaling pathway; it regulates the cell cycle by inhibiting cyclin-dependant kinases, E2F and histone deacetylase during the G1 phase of the cell cycle. TGFB itself is regulated by different miRNA including mi-RNA 15/16 and mi-RNA 224.

Furthermore, the following paragraph concerning BRCA1 and BRCA2 would benefit from more detail about the "many ongoing trials" which are "studying this treatment options", in particular, citations of papers or ongoing trials.

Many details concerning those ongoing trials were added: NCT00515866 trial is evaluating the safety and tolerability of a PARP inhibitor in combination with gemcitabine in pancreatic cancer. Another randomized, phase II trial (NCT01585805) is testing the veliparib (anti-PARP) in combination with gemcitabine hydrochloride and cisplatine compared to gemcitabine hydrochloride and cisplatine alone in patients with pancreatic adenocarcinoma having a known BRCA/PALB2 mutation.

Anti-PARP drugs should be better defined, and the logic between moving a breast cancer drug into pancreatic cancer could be explained more clearly.

The definition and the relation was added as follow: Anti-PARP drugs cause multiple double strand breaks in the DNA and in tumors carrying one of these three mutations, these DNA breaks cannot be efficiently repaired, leading to the death of the cells.

What does Hyaluronidase target?

This explanation was added: Hyaluronidase acts by depleting pancreatic tumors of their high hyaluronan content in preclinical trials; hyaluronan being a glycosaminoglycan, one of the major components of extracellular matrix throughout the pancreatic tumor.

The genetics discussion could also include a paragraph about chromosomal instability (CIN) which is implicated in several pancreatic cell lines.

As an answer to the reviewer comment , this paragraph was added : As mentioned before, PC is caused by multiple genetic sequential changes. This subtype of cancer is associated also with chromosomal instability, since more than 90% of PC is aneuploidy: presence of losses and gains of large portions of chromosomes or whole chromosomes leading to abnormal karyotype. The accumulation of genetic instability mainly higher number of mutations of oncogenes and tumor suppressor genes is a part of the early event of the development of PC.

By enumerating the twelve signaling pathways that the authors mention in the introduction and the discussion, they could open up the review to discuss ongoing research that examines, for example, the programmed cell death pathway, oxidative stress, chronic inflammation, and the role of TLRs in cell proliferation and chemoresistance in PC: all topics which are of ongoing research in the field.

The pathways was added and requested ongoing researches were added:

The most commonly affected signaling pathways in pancreatic cancer are apoptosis , DNA damage repair , G1/S transition (CDKN2A/p16, CyclinD) , cell-cell adhesion , regulation of invasion , embryonic signaling (Notch pathway , Hedgehog pathway and Wnt pathway) and MAPK signaling (c-Jun N-terminal kinase , ERK and TGF-β signaling)[23] .

The biggest change that needs to made, is some mention and discussion of the fact that PC is so deadly due to the lack of early detection. In addition to targeted therapies being investigated, it would be good to discuss new diagnostic techniques for early detection, potential biomarkers, etc. The discussion needs to be expanded with some more significant conclusions about the state of PC, perhaps to emphasize the importance of ongoing clinical trials, the use of biomarkers to indicate the best treatment regiment, and ways to increase early detection.

In the discussion , the requested points were largely analyzed going from early detection of pancreatic cancer to biomarkers :

Detecting pancreatic cancer at an early stage is the most rationale and solid perspective in the future management of this disease. At present, serum CA-19-9 (carbohydrate antigen 19-9) is the only Food and Drug Administration-approved biomarker for PDA, and it has utility marker of disease recurrence and surveillance. There has been a recent explosion in the pancreatic cancer biomarker field with more than 2000 biomarker studies implicating thousands of informative genes as candidate biomarkers [53].

Many markers of early detection of PC are being evaluated in blood, pancreatic cyst fluid, pancreatic juice and stool based on the new advances in technology for whole genome, methylome, ribonucleome and proteasome interrogation. Many promising results are being reported for different markers of early detection of PC [54]. Circulating tumor cells are one of the promising markers in blood used to the early detection of pancreatic cancer;

the detection of a mutation, as KRAS for example, in the cells of pancreatic juice can help the early diagnosis of the PC [55,56]. Glycypan-1 circulating exosomes were detected in the serum of patients with pancreatic cancer with absolute specificity and sensitivity; this new diagnostic and screening marker may serve as a potential non-invasive tool to detect early stages of pancreatic cancer and consequently, to facilitate possible curative surgical therapy [57]. Recently, a new non-invasive urinary biomarker, based on a set of three urinary proteins (LYVE-1, REG1A, and TFF1) was identified, able to distinguish patients with early-stage PDAC from healthy individuals [58].

Answers to reviewer 2:

First authors should include with a short overview of known molecular mechanisms of PC, since without this analyses of targeted therapies lack necessary foundation. Reasons of difficulties to treat MPC are given scarcely and mostly at the end, while in this review it should be a focused area and needs to be given in more details as a separate section. Situation with the treatment of PMC should be reviewed vis-à-vis other similar types of cancer to better understand what is so unique (if any) in MPC.

A paragraph was added on the different mechanisms of resistant insisting on the different known molecular mechanisms:

Mechanisms of resistance of pancreatic cancer to chemotherapy and targeted therapies

PC was known to be resistant to chemotherapy before the era of FOLFIRINOX; many hypotheses tried to elucidate this chemo-resistance. Thus, alterations in key pathways involved in cell cycle control, namely apoptosis, were largely incriminated. NFKB, pro-inflammatory and anti-apoptotic factor, seemed to be a key link between inflammation and cancer chemo-resistance in pancreatic cancer. The genetic complexity and heterogeneity of PC is also a challenge in the treatment of this cancer, since more than 60 genetic alterations affecting more than twelve signaling pathways are involved in its pathogenesis. The most commonly affected signaling pathways in pancreatic cancer are apoptosis , DNA damage repair , G1/S transition (CDKN2A/p16, CyclinD) , cell-cell adhesion , regulation of

invasion , embryonic signaling (Notch pathway , Hedgehog pathway and Wnt pathway) and MAPK signaling (c-Jun N-terminal kinase , ERK and TGF-β signaling)[23] .

Actually, recent data revealed that tumor stroma is a major extrinsic mechanism of resistance to chemotherapy and targeted therapy of PC. The tumor stroma of PC is limiting the drug delivery to pancreatic tumor cells at therapeutically relevant concentrations [24].

Next to intrinsic resistance to targeted therapies due to the multitude of pathways incriminated in the pathogenesis of PC, extrinsic resistance seems to be as important in the mechanism of non-response to targeted therapies. This extrinsic resistance seems to be particular to PC, rendering it a hardly targetable malignancy.

Questions which a reader has reading this review are:

- *All mentioned targeted therapies were tested according to authors in combination with gemcitabine. Whether any attempts to use TT alone were made?*

The attempts to use TT alone were added also in our manuscript :

Many targeted therapies are tested as single agent in second line treatment of metastatic pancreatic cancer; selumetinib (anti-MEK) and everolimus (mTOR inhibitor) are two examples[21,22]. Combining two targeted therapies without chemotherapy is also being tested actually in new ongoing trial in a second line treatment of MPC with afatinib and selumetinib compared to capecitabine (NCT02450656) and sorafenib plus everolimus (NCT 00981162) .

Minor remarks:

- *Bortezomib is not epigenetic drug*

The correction was made.

- *CDKNA, TP53 and SMAD4 have many more important ways of regulation than miRNAs.*

A paragraph was added to explain the other ways:
For example, CDK2NA or p16 inhibits cycline -dependant kinase 1, 4 and 6 and also help to stabilize p53; CDKN2A itself is regulated by a microRNA, miR-10b [37]. TGFB (Transforming growth factor [beta]) is a potent tumor suppressor that signals via the SMAD pathway and intersects with the WNT beta-catenine signaling pathway; it regulates the cell cycle by inhibiting cyclin-dependant kinases, E2F and histone deacetylase during the G1 phase of the cell cycle.

- *In many instances authors mention ongoing trials without giving results or references to the trial, which is kind of useless without that.*

We tried to add all the references were possible and to give the preliminary results .