ANSWERING REVIEWERS

Dear Editor,

We appreciate the very valuable feedback provided by the four reviewers and we believe that their suggestions have allowed us to improve the overall quality of our manuscript. Below, we address each of their comments individually.

Reviewer 1 (3408355)

The paper entitled "The CpG island methylator phenotype in adenocarcinomas from the digestive tract: methods, conclusions, and controversies" focused on the role of a specific phenotype in the development of multiple pancreatic and gastrointestinal adenocarcinomas. The study was well conducted and presented.

We thank the reviewer for these kind words and appreciate the reviewer's positive evaluation of our study.

The following were some minor concerns:

1. The subtitles could be numerated, which could help the readers understand better.

Thanks for the suggestion. We have no objection to adding the kind of numbering that the reviewer proposes, as long as this is approved by the journal formatting editor (none of the sample papers provided in the formatting guidelines of the journal website appear to include this kind of numbering, which makes us wonder whether or not it is ok to add it).

2. Pancreatic cancer and gastrointestinal cancer could be described, respectively.

An overview of previous work on gastrointestinal cancer – including colorectal, gastric and esophageal adenocarcinomas – is already included in our manuscript (see pages 5-8).
We use the commonly accepted terminology "pan-cancer" to refer to analyses that are done across multiple types of cancer (see, for example, http://www.nature.com/tcga/), but pancreatic cancer is not a subject of study in this manuscript. .

3. This paper could be revised following the text style of a review, and the methods could be removed or shortened.

Following the reviewer suggestion, we added a table where we provide a summary of previous work on gastrointestinal CIMP (Table 1 in the new version of the manuscript), in the style of classic review papers. We have also shortened some paragraphs and rearranged several parts of our manuscript in order to improve the flow of the text and make it read more like a review. Finally, we have also tried to keep our methods section as concise as possible, while ensuring that the article remains self-contained and all the necessary details are provided to ensure reproducibility.

Reviewer 2 (3475586)

A well written and thorough review by an experienced group. Congratulations for this excellent work.

We thank the reviewer for his/her positive evaluation of our work and these very encouraging comments.

Reviewer 3 (181208)

This is a valuable commentary on CIMP in GI cancers summarizing part of the literature and authors' own studies.

We thank the reviewer for acknowledging the value and the scientific interest of our work.

Some specific comments for the authors to consider:

1. In page 3, 2nd paragraph, it is mentioned that hypermethylation results in the formation of repressive chromatin. In the 2nd paragraph of p.4 is noted, though, that repressive modifications are already present when DNA methylation is effectuated. These seem to be contradictory.

We believe that the two statements are not contradictory, although we understand the confusion. What we meant to explain is that DNA methylation tends to occur at gene promoters that already contained repressive histone marks, so that DNA methylation replaces the previous heterochromatinization that was mediated by histone methylation, and therefore provides a more permanent layer of transcriptional repression. In order to make this clearer, we have reworded the phrase in the 2nd paragraph of page 3 as follows:

"Hypermethylation of CpG dinucleotides within these regions results in the establishment or reinforcement of repressive chromatin and the steric occlusion of transcription factor binding"

Authors should provide an overall model possibly accompanied with a figure of the processes and key players such as DNMTs and polycomb repressors. This model may discuss also how disparate upstream molecular lesions in different cancers may result to similar down-stream methylation events (or vice versa) and could be integrated with the BRAF discussion in p. 11. 2.

The kind of detailed model that the reviewer describes is already explained in detail in the reference by Cedar and Bergman, 2009 (PMID: 19308066) that is cited in the 2nd paragraph of p4. The kind of figure that the reviewer suggests seems to correspond well with Figure 5 of that paper. We appreciate the reviewer's suggestion of adding a similar kind of figure and additional details here, but - since other reviewers have already expressed their concerns about our current manuscript being quite lengthy – we believe that it is sufficient to refer the interested readers to the Cedar and Bergman paper in Nature Reviews.

2. In page 5, the panels used for the evaluation of methylation could be of interest for the reader, possibly in a table form.

We agree in the interest of these panels, and this is why we provide a reference for each one of them, so that interested readers can easily access more details about each individual study. However, since some of these panels include a large number of genes, possibly in the several tens or hundreds, we do not believe that including a table with full details for each one of these previous studies would constitute an effective use of space within the context of our manuscript.

3. Related to point 1, in p. 15 and table 5, one would like to make some categorization and pathway integration of the differentially mutated genes to fit into the model.

Following the reviewer's suggestion, we have added a column to Table 6 where we indicate potentially relevant pathways that are known to be associated with each of the mutated genes that we report.

4. In p.16 in the discussion on confounding factors, authors should discuss how significant they believe that the limited number of samples and tumor heterogeneity is for the validity of their results. A further discussion of alternatives to computation modeling for future sampling (e.g. tumor microdissection) could be appropriate at this point.

A detailed discussion of the effect of tumor heterogeneity upon the validity of our results is already provided in page 16, under section titled "Assessing the impact of tumor heterogeneity on CIMP classification". The results that we report in that section show that our classification remains consistent and robust when the analysis is restricted to high-purity samples, and that tumor heterogeneity does not have a negative effect upon our true-positive rate.

A detailed mathematical analysis of power vs. sample size that rigorously assesses the number of samples required for robust stratification is beyond the scope of the current manuscript. However, in line with the reviewer's comment, we believe that it is important to stress the need for a sufficiently large set of non-cancer controls. Because of this, we have rephrased the corresponding paragraph in p.16 as follows:

"*For example, our classification algorithm relies on the assumption of having a sufficiently large and sufficiently heterogeneous set of controls for each individual tumor type in order to guard against potentially confounding variables such as age, gender, race or anatomic location. Since only two non-tumor control samples were available for STAD, we may have encountered false positives in the probe selection process for this cancer type (Sanchez-Vega et al. 2015).*"

Following the reviewer's recommendation, we have also added the following sentence to the end of the same paragraph:

"*As an alternative, future studies may benefit from improved sample collection requirements (e.g., tumor micro dissection) that lead to enhanced tumor purity and lower stromal contamination.*"

Reviewer 4 (77543)

In this commentary, Sanchez-Vega et al. presented an overview of previous studies on the molecular profiles of GI tumors based on CIMP status, and their own research using TCGA data. This manuscript is well written and described in detail.

We thank the reviewer for his/her positive remarks about our work.

However, it is not easy to follow the flow.

Following the reviewer's suggestion, we have done a detailed proofreading of the text and we have shortened and rearranged several paragraphs with respect to our original submission in order to improve the flow of the text and to streamline the narrative.

Overall, this manuscript seems to be diffused. The authors tried to cover too many subjects.

As described in the journal's website, the purpose and scope of Frontier articles is not to focus on a single, specific question and try to answer it in full detail, but to review recent developments and discuss ongoing and future directions of research in a topic of interest. We believe that our manuscript fits well within this description.

A table containing information for previous studies will be extremely helpful for audience.

Following the reviewer's suggestion, we have added a table that summarizes some milestone findings for CIMP in gastrointestinal cancers from previous studies. This is provided as Table 1 in the new version of our manuscript.

The conclusion of this manuscript is not clearly described.

Since this is a review/perspective article, we did not intend to reach any single, strong, final "conclusion" from the present work. If anything, the main "take home message" from our study is that stratifying patients with gastrointestinal cancers based on CIMP status helps to reduce cohort heterogeneity at the molecular level, which in turns offers advantages in terms of (a) improving biomarker discovery and disease understanding and (b) producing more uniform responses in clinical trials. We believe that this is clearly explained in the "Conclusions and perspectives" section of the manuscript.