Editorial Office
World Journal of Gastroenterology

Jan 20, 2020

Manuscript **53284: "Gastrointestinal cancer stem cells as targets for innovative immunotherapy"** by Chivu-Economescu M. et al.

Dear Editor,

Thank you and the reviewers for the careful evaluation of the above referenced manuscript. We are very grateful for your comments and suggestions, which were valuable in improving the quality of our manuscript. We have considered them carefully and addressed them in full in the revised manuscript. All the changes made in the revised manuscript are highlighted and explained in an itemized, point-by-point response to the **Reviewers Comments** (see enclosed).

We hope that the revised manuscript is now acceptable for publication in World Journal of Gastroenterology.

We look forward to hearing from you.

Sincerely,
Mihaela Chivu-Economescu.

Response to Reviewers Comments (shown in italics):

Reviewer #02495872**:**

Comments:
This manuscript represents an interesting review of the current knowledge of the role of GI cancer stem cells in the progression and therapy of GI cancer. The potential of these stem cells to be developed into innovative immunotherapy is high and the whole topic is current and both scientifically and clinically relevant and important.

Response: Thank you for your comments and appreciations.

Reviewer #00503536**:**

Comments:
The manuscript written by Chivu-Economescu M et al. summarizes the current understanding on the immunotherapy against cancer stem cells, which could be an ideal target for anti-cancer therapy. The review describes comprehensive knowledge on the advances in the field of tumor immunotherapy. However, there is a concern that need to be addressed. Minor point, 1. Among various immunotherapy, combination immunotherapies of combining immune checkpoints therapy with DC-vaccines or CAR-T technology seem to be promising. The reports on the efficacy and limitation of the combination immunotherapy should be more described.

Response:
Thank you for the suggestion which was valuable in improving the quality of our manuscript. We have referred to literatures and papers and included information about efficacy and limitation of the combination immunotherapy.
We integrated the new data in new paragraphs at pages 15-16 highlighted in the text.

"Several clinical trials are now proposing interesting strategies for combining immune checkpoints therapy with DC-vaccines or CAR-T technology. Most of these clinical trials are either in the phase of patient recruitment or in phase I. There are several trials that are testing the combination between anti PD-1 compound (nivolumab) and gene-modified T-cells and dendritic cell vaccine targeting cancer-testis antigen (CTA). CTAs such as NY-ESO-1(New York esophageal squamous cell carcinoma 1) and MAGEA (melanoma-associated antigen A) are considered excellent candidates for cancer immunotherapy since a large majority of them have their espression limited to the embrionic stem cells, testes, ovaries and endometrium in normal tissue, and are re-

expressed in metastatic tumours[99]. MAGEA1-3, MAGEA9, LAGE1, and NY-ESO-1 were found to be highly expressed in hepatocellular carcinoma, oesophageal, gastric and colorectal carcinoma stem/progenitor cells and associated with poor survival, high risk of tumor recurrence[100, 101]. However, there is a small number of CTAs that are expressed on normal tissue as well. Although targeting CTA seems to be a promising strategy, carefull selection of CTA type for immunotherapy is manadatory. Serious neuronal adverse events followed by death were observed during clinical trials using anti-MAGE3 CAR-T cells in patients with solid cancers. Histopathological examination showed that normal neuronal cells also expressed MAGEA proteins that became targets of modified T cell therapy, thus being destroyed[102].

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Combined immune therapies may be very effective having the advantage of addressing both GCSC and TME simultaneously. Thus, they can target, for example, GCSC surface markers with monoclonal antibodies, DC-vaccines or CAR-T therapy, and at the same time, they can reactivate the immune system by blocking the negative signals induced by immunecheckpoints in effector immune cells. However, there are some limitations since most of the known solid tumor-associated antigens are expressed also in normal tissues, resulting in damaging off-target toxicity. Therefore, there is a continuu effort to identify tumor-specific antigens that can be addressed using immune therapies. Another limiting factor that can influence the clinical response is the level of inflammatory infiltration and the expression of immune checkpoints. Unlike liquid cancers, where immunotherapy has been a real success, in solid tumors, their efficiency has been diminished by the consistency, content and dynamics of TME that modulates the anti-tumor response through access and phenotype of immune cells.

To improve the efficiency and ensure the safety of the treatment it is imperative to carefully select the target antigens, assuring that they are highly immunogenic and expressed only in targeted the cell population."