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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 89393

Title: VX-509 attenuates the stemness characteristics of colorectal cancer stem-like cells by regulating the epithelial-mesenchymal transition through Nodal/Smad2/3 signaling

Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00225346
Position: Peer Reviewer
Academic degree: MD, PhD
Professional title: Director, Head, Professor
Reviewer's Country/Territory: Italy
Author's Country/Territory: China

Manuscript submission date: 2023-10-30

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-11-22 10:44

Reviewer performed review: 2023-11-24 14:13

Review time: 2 Days and 3 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation

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Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

This study provides interesting observation on a matter of significant biological and clinical relevance. However, the manuscript presents relevant problems in its present form. In the Introduction section: (Lines 98-99 onwards): "As the traditional RA treatment, is the inhibitory effect of VX-509 on CRC stemness related to JAK3 or other pathways?" This sentence is totally misleading, and unplugged from the rest of the introduction section. In this regard, there is no introduction of the interplay and relevance between TGF-Beta/Nodal, Smad, and JAK3 within the context of colorectal tumorigenesis. The Authors should specify that other studies pointed at the issue of the relevance of JAK3 in colorectal cancer and CSCs, including the fact that other JAK3 inhibitors have been already shown to be effective in colorectal cancer treatment in in vivo animal model and in inducing apoptosis and colorectal cancer putative CSC cell cycle arrest in vitro. Among these studies: Novel Potent EGFR-JAK3 Dual-Target Inhibitor that Overcomes KRAS Mutation Resistance in Colorectal Cancer. Wu T, Yu J, Wang C, Jin Y, Zheng X, Chen L, Ma X, Sun X .Anticancer Agents Med Chem. 2023;23(4):440-449. doi: 10.2174/1871520622666220609112816.PMID: 35692150 Role of

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cytokines and Jak3/Stat3 signaling in the 1,2-dimethylhydrazine dihydrochloride-induced rat model of colon carcinogenesis: early target in the anticancer strategy. Saini MK, Vaish V, Sanyal SN.Eur J Cancer Prev. 2013 May;22(3):215-28. doi: 10.1097/CEJ.0b013e3283584932 Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. Lin Q, Lai R, Chirieac LR, Li C, Thomazy VA, Grammatikakis I, Rassidakis GZ, Zhang W, Fujio Y, Kunisada K, Hamilton SR, Amin HM.Am J Pathol. 2005 Oct;167(4):969-80. doi: 10.1016/S0002-9440(10)61187-X. Design, synthesis, biological activity evaluation of 3-(4-phenyl-1H-imidazol-2-yl)-1H-pyrazole derivatives as potent JAK 2/3 and aurora A/B kinases multi-targeted inhibitors. Zheng YG, Wang JA, Meng L, Pei X, Zhang L, An L, Li CL, Miao YL.Eur J Med Chem. 2021 Jan 1;209:112934. doi: 10.1016/j.ejmech.2020.112934. There is also prior evidence that JAK3 inhibition can stop proliferation and malignancy from CSCs: https://pubmed.ncbi.nlm.nih.gov/?term=JAK3%20inhibitor%20and%20cancer%20stem%20cells In the introduction section, a major part is lacking, that is explaining how the present study poses itself within the context of the already existing, above mentioned, literature, ad what are the main features of originality in the present study itself. Minor points in the Introduction section are: (lines 60-61): “It is predicted that 3.2 million new CRC cases and 1.6 million deaths worldwide by 2040”. Please modify: …that 3.2 million new CRC cases and 1.6 million deaths will occur worldwide… (line 63): “that is a long time for CRC patients to face”. Change into: …”there is still a long time…. (lines 70-71): “which endows CSCs with the potential of cell proliferation and differentiation. Please change into: which endows CSCs with the potential of aberrant cell proliferation and differentiation… (Lines 74-75): “CSCs……provides”. Change into: CSCs….. provide (lines 89-90): “A recent study demonstrated that Nodal specifically expressed in…”.

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Change into: ……Nodal is specifically expressed… (Line 96): "A phase II clinical trial of VX-509 for the treatment demonstrated that…". Change into: A phase II clinical trial of VX-509 for the treatment of rheumatoid arthritis (RA demonstrated that…. (line 141): "Washing for three times,…." Change into: after washing for three times… (Line 145): "106 cells suspended….". Change into:…cells were suspended… In the Methods Section, the Authors should specify the rationale for Aldefuor assay. ALDEFLUOR™ is a non-immunological fluorescent reagent system that has supported over 1000 publications for the detection of aldehyde dehydrogenase-bright (ALDHbr) cells in over 80 different tissues. High expression of ALDH has been reported for normal and cancer stem and progenitor cells of various lineages. The Authors must specify why such an assay would be affordable enough to identify CSCs, when the kit itself reports that ALDH is also expressed in normal cells. (Line 214): "For vivo animal treatment…" Change into: …For in vivo…. (line 259): "cDNA was reversibly transcribed…" Change into:…reverse-transcribed… (line 268): "Statistic"…Change into: Statistics (line 274): "Result"…Change into: Results In the Results section: (lines 330-331…): "EMT transformation drives cancer cells to dedifferentiate into CSCs cells by improving cell plasticity [22], we proposed that whether VX-509 exerts its CSCs stemness inhibitory effect by involving EMT process?". Please, rephrase as: Since EMT transformation drives cancer cells to dedifferentiate into CSCs by improving cell plasticity [22], we decided to investigate whether the inhibitory effect elicited by VX-509 on CSC may have involved changes in EMT patterning. (lines 333-335…): "Consistent with our hypothesis, VX-509 treatment repressed the expression of EMT related proteins (E-cadherin, N-cadherin and Vimentin) compared with mock group (Figure3H-I)". Please, integrate as it follows: A well-known hallmark of EMT is the upregulation of N-cadherin followed by the downregulation of E-cadherin. Consistent with our hypothesis, VX-509 treatment remarkably changed the expression of EMT related proteins, by eliciting an

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up-regulation of E-cadherin, while inhibiting the expression of N-cadherin, as well as vimentin expression, as compared with the mock group (Figure3H-I). In the Results section (lines 372-374), the Authors report "After treatment with VX- 509, gene expression levels were determined using qRT-PCR and the results showed that Nodal were increased in in HCT116 CSCs and HT29 CSCs compared to levels in their control cells (Figure 5C)". There are NO Data showing such an increase. Panel 5C shows a decrease in the gene expression of Nodal following cell exposure to VX- 509. In Figure 5, panel E reports the densitometric analyses of Nodal and pSmad2/3 in HCT116 and HT29 CSCs treated in the absence or presence of different concentrations of VX-509. However, the legend of the same figure (line 779) reports that panel (E) is "The densitometry analysis of Oct4, Nanog, SOX2, normalized against GAPDH". The Western blot and the densitometric analyses of these proteins are not shown in the figure! These data must be presented in additional panels. (lines 420-421): In the Discussion Section. "Somatic cells can pluripotent stem cells through transient ectopic overexpression of transcription factors such as…". A verb is missing: can transform into? In the Discussion section (lines 524-526): "VX-509 treatment inhibited EMT-related phenotypic proteins, suggesting that its ability to reverse the activation of induced by Nodal stimulation and inhibit dedifferentiation processes in CCSCs". Please rephrase. Please, also rephrase the last part of the Conclusion section: "Moreover, VX-509 regulated the transcription and protein expression of Nodal and inhibited the phosphorylation of downstream protein Smad2/3 to prevent the EMT process of CCSCs and inhibit continuous self-renewal of CCSCs and consequently reduced the generation of CCSCs, offering a potential clinical use of CRC". Change in: …. and consequently reduce the generation of CCSCs, offering potential clinical perspectives….

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 89393

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Provenance and peer review: Unsolicited manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00225346
Position: Peer Reviewer
Academic degree: MD, PhD
Professional title: Director, Head, Professor
Reviewer's Country/Territory: Italy
Author's Country/Territory: China

Manuscript submission date: 2023-10-30
Reviewer chosen by: Jing-Jie Wang
Reviewer accepted review: 2023-12-20 23:30
Reviewer performed review: 2023-12-27 00:32
Review time: 6 Days and 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The Authors have remarkably improved the quality of their manuscript that can now be accepted in its present form. Only minor typographical changes should be made, as it has been shown by track changes in the attached manuscript file. Such changes may be easily performed by the Editorial Team.