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SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	Journal of Pharmaceutical Research International
Manuscript Number:	**Ms_JPRI_36743**
Title of the Manuscript:	**The expression patterns of APC2 and APC7 in newly diagnosed acute lymphoblastic leukemia**
Type of Article:	Original research paper

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	**Authors' response to final evaluator's comments**
The authors clarify why they used the formula $2^{-\Delta Ct,\ case}/2^{-\Delta Ct,\ control}$ instead of the $2^{-\Delta\Delta Ct}$ method, they added a reference to the manuscript that explains in which circumstances this formula can be used to calculate gene fold induction. Unfortunately I could not get access to the referenced paper as only the Abstract for this paper is available on the NCBI website, I trust that the authors used the aforementioned formula on the basis of the referenced paper. I am still extremely concerned regarding the statistical analysis performed by the authors and I feel that my comments were not appropriately addressed. The authors mention doing the experiments in triplicates, but as I mentioned in my previous comments – the data presented should be the result of at least three independent experiments to have any statistical value/significance. This again was not addressed or clarified by the authors, which leads me to believe that the results presented are the result of a single experiment done in triplicate. If this is the case the authors cannot calculate P values based on a single experiment. The P values should be calculated from the averages of at least three independent experiments, meaning that the qPCR experiments should have been performed at least three times (independently) and each time in triplicates. A great paper that explains the difference between triplicates and independent experiments for biologists is the following: "Know when your numbers are significant" by David L. Vaux, NATURE, VOL 492, 13 DECEMBER 2012. In this article David L.Vaux explains that triplicates merely show the accuracy of the operator/researcher and in no way represent reproducibility of the results. To show reproducibility the experiments need to be done at least three times with similar results, the P values should be calculated from the average of these experiments and not from the triplicates used in each experiment. The Figure legends in the revised manuscript were improved and there was editing of the English language throughout the text, although there are still some minor issues. Finally, none of the recommendations for further experiments were taken into consideration by the authors and no additional experiments were proposed or done by the authors to strengthen the scientific value of the research work. Although the experiments suggesting knocking down or knockout genes in ALL cell lines might be out of the technical capability of the authors, I feel that protein analysis is a fairly easy and standard technique that could be performed. In fact, if the authors were able to extract RNA from the ALL samples, they should not have any problems extracting protein and doing western blot analysis for the target gene/proteins. Alternatively, they could have done a more exhaustive analysis by qPCR of other genes related to APC2 and APC7, or proposed other studies.	

Reviewer Details:

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