



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
<p>The authors clarify why they used the formula $2^{-\Delta Ct, \text{ case}} / 2^{-\Delta Ct, \text{ control}}$ 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.</p> <p>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.</p> <p>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.</p> <p>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.</p>	<p>Many thanks for your valuable comments and suggestions.</p> <p>We have sent the full paper used for the calculation of fold changes of gene expression here in with the reply.</p> <p>Your suggestion for performing the gene expression of samples in three independent experiments, as you bring a study for this subject entitled as "Know when your numbers are significant", would be led to a significant improvement in the quality of the paper, but as you studied in the paper, the author mentioned "if investigation evaluates a limited number of subjects, the results must confirmed with more independent tests, the sufficient number in this paper was considered to be thirty cases at least". In that study the cases were mice and we have 57 cases in our study. In fact if study involve adequate number of subjects, results are trustworthy because occurs in statistically acceptable number and this can reduce the effects of chance, thus although it is still worthy to do experiments in three independent format but it is still statically acceptable to do them in one triplicate experiment for each case alone.</p> <p>In other word, if gene expression of APC2 increase in 33/57 of patients, the overexpression is confirmed 33 time and when the P value of these data is less than 0.05 meaning that overexpression is not by chance(or the chance effect is less than 5%). We collect adequate number of samples based statistical formula and other studies in the field of gene expression analysis.</p> <p>Your suggestion to do the gene expression study along with western blot can also extremely enhance our data quality but as you know several of our cases were childhood patients less than 10 years and it is not possible to obtain sufficient sample volume to do Real-time and western blot analysis simultaneously because they have limited BM space for BM aspiration (which is a maneuver for obtaining patients sample from BM). Thus we could not perform western blot at the time of our analysis albeit we were eager to perform protein assay as well at that time.</p> <p>Although this is very informative and helpful to evaluate other related genes in our opinion as well, but this study was a student thesis program which is finished by this time, and the other related genes were not considered unfortunately at the time of project running.</p>