Reviewer’s report

Title: E-cadherin gene re-expression in chronic lymphocytic leukemia cells by HDAC inhibitors.

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Reviewer: A.K K Munirajan

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In their study, Jordaan et al., has analyzed the effect of HDACi on E-cadherin expression in CLL. They show that there is an increased expression of E-cadherin at mRNA as well as protein level after treating the CLL cells with HDACi which may be due to increased acetylation of histones followed by increased transcription of the E-cadherin gene. The re-expressed E-cadherin inhibits the wnt pathway and downregulates LEF and CCND1. They further show that the HDACi treatment preferentially allows the expression of the functional E-cadherin transcript over the aberrantly spliced transcript that lacks exon 11.

Major Compulsory Revisions

1. A major concern is the small number of samples used (n=10) and the difference in number of samples used for real time PCR analysis, western blotting, and ChIP. While one can agree that the initial use of 2 samples to choose the working concentration of MS-275 and for the preliminary check of H3 and H4 acetylation is adequate, why only 5 samples were used for E-cadherin protein expression by western blot? Similarly, the use of 2 samples for NMD inhibition and E-cadherin ChIP analysis, and the use of just 3 samples for HDAC real time PCR analysis is a question of further concern. Likewise a similar question can be posed for the choice of samples in figure 4 also.

The authors explain that they have checked the H3 and H4 acetylation in 2 samples, but the corresponding Fig.1B shows the increase in acetylation of H3 and H4 in only 1 sample. Further the sample name is missing in the same figure.

Also in fig. 4A, the two samples used are indicated as “CLL#” without the sample number.

In Fig 1A, if the authors could also show the expression level of E-cadherin in PBMC it will significantly establish the connection between E-cad silencing and re-expression in CLL samples.

In Fig.4A, under the title IP with # catenin Ab, the second panel did not show any decrease in # catenin when E-cadherin in re-expressed. Hence inclusion of additional samples may help in understanding the effect of E-cad on # catenin.

The conclusion drawn from Figure 1A as “The fold increase in the E-cadherin RNA expression at 1.0 #m concentration was between 60 and 150 fold” (page 8
last sentence and continued on page 9) is based only on 2 CLL samples and cannot be generalized for the 10 samples.

Further, the authors need to explain why they choose only LEF and CCND1 for studying the wnt pathway among the other genes involved.

In the results section, under the subtitle “E-cadherin expression with HDAC inhibitors”, second paragraph, the authors explain “E-cadherin expression was also tested by western blot analysis at the 0.1#m concentration of MS-275 and E-cadherin expression was observed in around 30% of primary CLL specimens...”. The 30% should be better described since nowhere they have mentioned whether they analysed the 10 samples or only 5 samples as in Fig.1C. If the data is not shown, then the same should be mentioned in the manuscript.

In page 11, the authors mention “As the NMD blocker emetine is toxic to cells the overall induction observed with HDACi is lower as compared to induction in cells that were treated with HDACi alone”. Based on this fact, how can emetine treatment be taken as a proper control for the expression of the aberrant transcript? (Since emetine will also cause a simultaneous decrease in the expression of the aberrant transcript).

Moreover, it would be of interest to the reader to know why emetine treatment was given only for the last 8 hrs.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests