Reviewer's report

Title: Wnt/beta-Catenin Pathway Regulates ABCB1 Transcription in Chronic Myeloid Leukemia.

Version: 3 Date: 6 December 2011

Reviewer: Michelle Perugini

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Major Compulsory Revisions

1. The authors should justify the use of the K562 and Lucena cells for the studies in Figure 6. In the raw (un-normalised) data, is ABCB1 mRNA expression in Lucena cells higher than that of the K562 controls? It would be necessary for this to be the case in order for this cell line to be an appropriate model for response and resistance phenotypes in CML. This data should be included in Figure 6 along with assessment of increased b-catenin activity in the Lucena cell line compared with K562 cells.

2. The authors should describe the differences in the patient cohorts between IM-responsive patients and IM-resistant patients. The IM-responsive cohort has no BC patients and a high proportion of CP patients, whereas the resistant group comprises nearly half BC-patients and only one CP patient and therefore is not representative of primary resistant CP CML. The authors need to justify these differences.

3. Methods, Bone marrow samples – more information should be included for disease endpoints ie. What is considered major molecular response and complete hematologic and cytogenetic responses, and how are they measured?

4. Methods, Statistical analysis – the authors have used a paired t-test for comparison of mRNA levels and cell viability between K562 and Lucena cells from different assays. An unpaired t-test would be more appropriate given the analysis is between two different cell lines, and the authors should use this test for their analysis. The authors should also describe the statistical tests used for comparing expression levels in the patient samples.

5. Results, b-catenin binds to the ABCB1 promoter at the TCF-binding site – Need to justify the inclusion and meaning of SMAD8 in Figure 2.

6. Authors should discuss the significance of the various TCF binding sites and their location in the promoter. Sites shown to be important in other cancers. Do any of the binding sites exhibit stronger binding to b-catenin than others, and why have the authors chosen 4 and 5 for the complete analysis, there is little discussion about the additional data in Supplementary Figure 1.

7. The authors need to show that the LiCl in their hands results in increased b-catenin stabilization and nuclear translocation, to ensure the concentration used is correct and that it is working as it should.
8. Results, The Wnt/b-catenin signaling pathway regulates ABCB1 expression (first para, last sentence) – The authors should provide an explanation as to why the increase in ABCB1 mRNA was more significant in K562 cells than the Lucena cells. Is this because ABCB1 expression is already higher in the Lucena cells than in the K562 cells (see comment 1)?

Minor Essential Revisions

1. Abstract (first paragraph, last sentence) – should read “b-catenin is… “.
2. Background (first sentence) – needs some references relating to the translocation that occurs in BCR-ABL in CML.
3. Background (second para, second sentence) – should read “Nevertheless, some patients do…”
4. Background (second para) – author name misspelled – should read Flahaut not Flauhaut.
5. Methods, Bone marrow samples – change “We selected six….” to “We selected 6….”
6. Methods, Electrophoretic Mobility Shift Assays – change “double-strained” to “double-stranded”
7. Methods, Chromatin Immunoprecipitation (ChIP) assays on Native Chromatin (second para) – change “unspecific binding” to “non-specific binding”.
8. Methods, Real-time Quantitative PCR (RT-qPCR) (second sentence) – change “RNA from cell lines was treat with….” to “RNA from cell lines was treated with…”
9. Methods, Real-time Quantitative PCR (RT-qPCR) (second to last sentence) – Cobertt misspelled, should be Corbett.
10. Discussion (first para, second sentence) – in the sentence “each of which has responds…” delete has.
11. Discussion (second para, third sentence) – statement requires referencing.
12. Discussion (third para, last sentence) – change “corroborates” to “corroborate”.
13. Discussion (fourth para, last sentence) – requires referencing.
14. Reference 5 is missing journal name and issue details.
15. Reference 48 is missing journal name and issue details.

Discretionary Revisions

No discretionary revisions required.

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

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.