Reviewer’s report

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

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Reviewer: Frank Thevenod

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This study describes the role of Wnt signaling in ABCB1 overexpression in a CML cancer cell line (K562). A multidrug resistant variant overexpresses ABCB1, which is in accordance with bone marrow cells from multidrug resistant CML patients. EMSAs and ChiP assays confirm binding of beta-catenin to the ABCB1 promoter region in vitro and in vivo, which is more pronounced in multidrug resistant cells. LiCl treatment to stabilize beta-catenin enhances ABCB1 expression in the parent cell line, but not in the multidrug resistant cell line. Moreover, Wnt1 siRNA abolishes ABCB1 expression in multidrug resistant cells, but not in the parent cell line. The authors conclude that Wnt/beta-catenin signaling drives ABCB1 overexpression in multidrug resistant CML cells and could account at least in part for the multidrug resistance phenotype in CML.

General:
This is an important and ambitious issue, which however requires better data to convincingly demonstrate Wnt/beta-catenin dependent overexpression of ABCB1 in CML cells. A large body of data is of confirmatory character (Figures 1, 2, 4).

Major Compulsory Revisions:
1. Figure 1: Protein expression is required, e.g. by FACS analysis with ABCB1 antibodies.
2. Figure 2: Competition by Opt and beta-catenin is variable and not convincing for both TCF4 and TCF5 probes. Why was Smad8 tested?
3. Figure 3: The left panel probably the results of quantitative RT-PCR, the right panel the results of a qualitative RT-PCR. The right panel is not convincing (very weak signal in bound and unbound experiment. Why was a qualitative RT-PCR not shown for ABCB1?
4. Figure 4: The Figure is redundant and could be described in the text.
5. Figure 5: These data are not clear. The “ctrl” is normalized to 1 in both parent and multidrug resistant cells. I assume that the ABCB1 levels are higher in control “Lucena” compared to control “K562”. The interpretation (saturation of Wnt signaling) would be convincing only if all the data are normalized to the “K562” control only.
6. Figure 6: It is astonishing that siRNA against Wnt1 is so effective in multidrug resistant cells, but has no effect in parent cells considering that at least 19 Wnt
ligands have been identified. Why did the authors exclusively focus on Wnt1? Are the other Wnt ligands irrelevant? A more credible strategy would be to knockdown a more down-stream target, e.g. beta-catenin or TCF4/5.

7. To demonstrate increased activity of Wnt signaling a TCF luciferase reporter gene assay is mandatory.

**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.