Author’s response to reviews

Title: Bortezomib prevents cytarabine resistance in MCL, which is characterized by downregulation of dCK and up-regulation of SPIB resulting in high NF-κB activity

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Dear Editor,

Please see our comments to reviewers comments below.

Specific comments (Reviewer 1, Shannon Buckley):

1) The manuscript title does not represent that key findings of the manuscript, and instead stresses down-regulation of dCK, which is already known. Editing the title would help the reader pinpoint the key findings in the manuscript.
Response: We appreciate the relevant feed-back and suggest that the new title is: “Bortezomib prevents cytarabine-resistance in MCL, which is characterized by down-regulation of dCK and up-regulation of SPIB resulting in high NF-κB activity”

2) Although the authors say in the text that dCK down-regulation was seen in three parallel experiments, the manuscript would benefit from seeing western blots for multiple resistant clones derived. Also, it is unclear the number of resistant clones analyzed and generated for both biological and technical replicates.

Response: A figure with western blots for multiple resistant clones has been added to the supplementary material (Fig S2) and a clarification regarding number of clones has been added to the figure text.

3) Does knockdown of NF-kB or SPIB in resistant cell lines alter cytarabine sensitivity?

Response: Knock-down of SPIB was assessed using multiple siRNA sequences. Although only a partially knock-down SPIB was achieved after technical optimization, assessment of proliferation shows a significant difference in proliferation between the resistant cells and the SPIB-knocked cells when cultivated in cytarabine-containing medium (see Fig 7).

Knock-down of NF-kB was not assessed in the current study, but the proliferative effect of NF-kB in MCL is well known. Further characterization of involved down-stream pathways is outside the scope of this study.
4) In Figure 11; the authors include findings from patient samples, however the significance of this data is unclear do to being diagnostic samples. The authors present that patients have high level dCK at diagnosis, however it is unclear if relapse or resistant patients have altered dCK expression. In patients that are resistant to cytarabine or in relapse, do they express lower levels of dCK?

Response: Unfortunately, relapse material is scarce as biopsies seldom are taken from relapsed patients. We have recently initiated the collection of such samples from an ongoing study of relapsed MCL, to be studied and presented in a follow-up study.

5) It is unclear whether the data represented in the gene expression analysis is technical or biologic replicates?

Response: As the figure text below the figure states, the data represented in Fig 3 are technical replicates.

6) A number of figures lack error bars, and it is unclear whether these experiments were repeated. ie; figures 1, 5, 6 and 8.

Response: the data in Fig 1 include error bars, and it is stated in the figure text what the bars represent and whether experiments were repeated. A clarification has been made in the figure texts for Figs 5, 6, 7 and 8, regarding how many times the experiments were repeated. Figures, line 776, 784, 790 and 799. A similar clarification has been added to Figure 10, line 817.