Reviewer’s report

Title: Synthetic Lethal Screen Identifies NF-kappaB as a Target for Combination Therapy with Topotecan for patients with Neuroblastoma

Version: 1 Date: 8 November 2011

Reviewer: Kelly Goldsmith

Reviewer’s report:

Major Compulsory Revisions

Given this paper is based on RNAi screen results, evidence for siRNA inhibition of target gene expression (by immunoblot) needs to be shown at least for the top nine hits that correlates with timing of siRNA-enhanced topotecan growth inhibition.

The methods mention that siRNA was added for 24 hrs followed by 72 hour incubation with topotecan, but exact time that measurement for enhanced effects was assessed is not written nor depicted in a figure or legend; Therefore it is not clear whether all siRNA’s were assessed for topotecan synergy at same time point or when maximal target gene knockdown occurred. This is critical to understanding functional relevance of enhancer gene knockdown in relation to potentiation of topotecan-induced cell growth arrest.

For Figure 2B, the Actin band at t=0 is much less intense than the other time points and gets darker with treatment time, suggesting unequal protein loading in lanes. Does the one actin blot represent protein loading for all blots tested, or just one? This would affect conclusions made about phospho-p65/relA that may be decreased in lane one due to decreased protein loading. Also, a faint line for phosphorylated IKB-alpha is evident at 6 and 24 hours post topotecan treatment. Phosphorylation of IKB-alpha precedes it’s degradation, yet in this experiment, phospho IKB-alpha is present at the same time and after total IKB protein is decreased to almost undetectable, begging the question of NFKB activation causing total IKB protein degradation through phosphorylation of IKB-alpha. Repeating the experiment with a stronger phospho IKB-alpha Ab and showing equal protein loading in all lanes for all blots used would support the conclusions that NFKB is activated.

For Figure 1C, evidence for target gene knockdown at protein level should be shown at the time where significant differences in siRNA treated cells are noted on the graph.

Although bortezomib enhances topotecan induced growth inhibition and apoptosis, the statement that this is specifically through NFKB inhibition by bortezomib needs to be shown experimentally in NB cell lines used in these studies before such conclusive statements can be made (last sentence;paragraph 1;page 13 and last sentence; paragraph 1;page 14). As
mentioned in the discussion, bortezomib is a proteasome inhibitor and can inhibit cancer growth by affecting multiple pathways. In other NB models, bortezomib potency does not inhibit NFKB at all but rather decreases pro-apoptotic proteins like Bcl-2 or Mcl-1 or increases anti-apoptotic Bcl-2 family members (Hagenbuchner, J., et. al., Jnl. Bio.Chem., 2010, Luo, P., et. al., Pharmazie 2010, and Naumann, I., et. al., Clin Can Res 2011).

Minor Essential Revisions

Table 1 should rank genes based on their “fold” change in cell growth (and p value if applicable) to denote the most significant enhancer genes.

The Methods state that “Combination Index was calculated using CompuSyn software” - A brief explanation of the CI calculation and how that determines/relates to synergy with reference to Ting-Chao Chou’s papers should be provided in the Methods section. Please denote if single agent IC50 determinations were also made using CompuSyn, or describe in Methods how IC50's were determined otherwise. Please explain the rationale for why only two very close concentrations of one of the two drugs are used to test for synergy. For example, the NSC676914 synergy concentrations chosen: 1uM and 1.5uM in SH-SY5Y and 1uM and 2.5 uM in NB-1691, while lower than the calculated IC50, they hardly span a wide range of concentrations. A statistically clearer explanation of synergy methods and results (beyond the fact that the points were away from the diagonal additive line) would help support conclusions for Figure 2A.

NSC676914 inhibits NFKB but does not inhibit tumor cell proliferation in other cancer cell lines (breast, etc.). The results mention single agent IC50 results for NSC676914. It would support NFKB as a true target in NB to show the IC50 results in a graph figure, supporting it has single agent activity at low uM concentrations in NB not treated with topotecan.

Figure 3 should include pictures from the control as well as bortezomib alone treatment arms and provide the time point in therapy that the picture was taken.

Discussion. References should be provided for comments stating Topotecan and Bortezomib dose limiting toxicities. Of note, in the Phase I Children’s Oncology Group study of Bortezomib in children with refractory solid tumors (ADVL0015), the dose limiting toxicity with Bortezomib was thrombocytopenia along with other hematologic grade 2-3 toxicities that were not dose limiting. Therefore, there are potential overlapping hematologic toxicities with bortezomib and topotecan that, while not precluding the combination, would need to be monitored (Blaney, SM, JCO 2004).

Discretionary revisions

Reference 16 does not refer to NSC676914 at all but to the millennium compound, MLN120B, which it seems has been compared to NSC676914 in
other literature, but is not the same compound.

There is no mention in Methods of the reagent NSC676914 or where it came from.

A more detailed description of the Metacore website/software assessment for pathway analysis of RNAi data should be provided or at least referenced.

There are more up to date references using topotecan in upfront NB therapy (JH Park, et al, JCO 2011).

**Level of interest:** An article of importance in its field

**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 I have no competing interests