Reviewer’s report

Title: Small Interfering RNA Targeting Mcl-1 Enhances Proteasome Inhibitor-Induced Apoptosis in Solid Malignant Tumors

Version: 1 Date: 18 August 2011

Reviewer: Gian Luigi Russo

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The present ms addresses the functional relations (if any) between proteasome inhibitor (PI) induced apoptosis and Mcl-1 expression in several malignant cell lines. The Authors concluded that lowering Mcl-1 expression by siRNA could present a novel strategy to potentiate the anticancer activity of several proteasome inhibitors. My first impression has been to read a paper confirmatory of previous findings. Regarding the suggested combined treatment of PIs plus Mcl-1 siRNA, the Authors seem to ignore several key issues which must be experimentally addressed before reaching the conclusion reported in the text.

Discretionary revisions:
1- In my view, the ms contains an evident contradiction which must be explained or, at least, discussed. PIs are considered anticancer molecules able to induce apoptosis. However, as reported here and by others, they also increase stability and, consequently, expression of Mcl-1 which is a pro-survival factor and it is expected to counteract the pro-apoptotic effect of the inhibitors. How these two events can coexist in the cell? Is it possible that Mcl-1 increase following PI treatment is an epiphenomenon without a functional meaning?

2-One of the key experiment is presented in Fig. 5B where the relative cell viability in the combined treatment (MG132+Mcl-1 siRNA) is slightly better than MG132 + control siRNA. However in considering potential clinical application, issues regarding costs and toxicity of a combined treatment must be taken into account. The relatively limited benefit resulting from the analysis of Fig. 5B does not justify the positive message transmitted by the authors throughout the text on the efficacy of the combined treatment.

Major essential revisions:

1-The Authors stated in the abstract and introduction that “The current study was designed to analyze the levels of several anti-apoptotic members of Bcl-2 family in different human cancer cell lines after they were treated with proteasome inhibitors…”. However, they didn’t mention any data on other Bcl-2 family members. Why ? No effect following PI treatment ? Please clarify this point.

2-About sensitivity to bortezomib, the Authors stated that “These results demonstrate that the cells’ susceptibility to bortezomib was not obviously associated with the amount of Mcl-1 accumulation”. To reach this conclusion a
correlation index must be calculated combining data from Tab. 1 and Fig. 4.

3-Regarding Fig. 5A, the Authors stated “Nevertheless, treatment with MG132 resulted in obvious Mcl-1 accumulation in cells pretreated with Mcl-1 siRNA, although the level of this accumulation was dramatically lower than in cells pretreated by control siRNA” To say the true, analysis by eyes suggest that Mcl-1 level in the data point MG132+Mcl-1 siRNA is not so “dramatically lower” as described. How many immunoblottings have been performed regarding data in Fig. 5A? Probably more than one. It is mandatory to add a graph showing densitometric analysis plus/minus SD.

4-Fig. 6 present several problems. Probably a copy/paste mistake happened regarding Fig. 6B which is identical to Fig. 5B. This is impossible since in Fig. 5 and 6 cell viability has been calculated using two different assays. Lack of the real Fig. 6B makes difficult to judge data reported in Fig. 6A. Percentages in the different squares of Fig. 6A indicating positivity to propidium and annexin-V are missing. Control data point in Fig. 6A doesn't help Authors’ conclusions since it shows a clear signal (top-left) probably attributable to propidium staining.

5-The rationale behind the selection of the cell lines used in the present study must be clarified.

Minor revisions:
1-In Fig. 3B and in Table 1 SD is not indicated
2-Several controls are missing. As an example, MG132 mono-treatment in Fig. 5B and Fig. 6.
3-How do the Authors explain that Mcl-1 siRNA alone in Fig. 5B does not decrease cell viability considering the anti-apoptotic nature of Mcl-1?

Level of interest: An article of limited interest

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