Reviewer’s report

Title: Targeting of CD34+CD38- cells using Gemtuzumab Ozogamicin (Mylotarg) in combination with Tipifarnib (Zarnestra) in Acute Myeloid Leukaemia

Version: 1 Date: 30 June 2012

Reviewer: Jörg Cammenga

Reviewer’s report:

Jawad et al. investigate the effects of gemtuzumab ozogamycin (myelotarg) and Tipifarnib on bulk and CD34+/CD38- cells in an in vitro culture system using serum-free conditions and the presence of fibronectin and cytokines. The paper is well written and touches upon a clinically relevant subject.

Major compulsory revisions:

Table 1:
It would be helpful for the reader if the two AML patient samples that did not show a defined leukaemic phenotype from the table making it easier to follow the discussion.

The addition of two columns to Table 1 showing whether the sum of the individual toxicities was >100% or the effects of GO and Tip resulted in an additive or supra-additive (synergistic?) effect would make things easier for the reader to appreciate and evaluate the data. I am no pharmacologists but it seems that the term supra-additive is not well defined.

The fact that the authors have to exclude samples and which the sum of the individual treatment exceeds 100% shows that measuring % cell loss is probably a problematic way of investigating additive and synergistic effects. The reviewer is totally aware of the fact that using sorted primary AML CD34+/CD38- patient samples makes it impossible to establish IC 50 doses for each drug and investigate synergistic effects of the two drugs using this method.

Looking over the individual columns with the response of the bulk cells and the LSPC to the drugs alone or in combination and reading the material & methods I cannot really understand when an effect is called supra-additive and I do not know whether the Wilcoxon signed rank test is the appropriate test.

Figure 2:

The authors state: GO alone induced chk2 phosphorylation in primary cell culture in bulk cells and in the CD34+CD38-.... (Figure 2b, c). Figure 2b includes only double treated cells according to the Figure legend. If space allows it would be helpful to include the single treatments on the different populations in Figure 2b.

Why is % phosphoCHK2 RFI vs untreated and %increase in phosphorchk2 used for the Y-axis in Figure 2a and Figure 2c respectively?
Figure 4:
Unfortunately, I do not understand what the experiments in Figure 4 really add to the message of the paper because the authors change (for technical reasons) the experimental set-up completely. Instead of using GO and Zarnestra they now use Daunorubicin treatment for two hours with or without pretreatment with a TOR inhibitor to enrich for dormancy. It is also difficult to put these data in a clinical context because daunorubicin (in combination with cytarabin) has been used for the treatment of AML for several decades which has probably very little effect on leukemia initiating (stem) cells.

Minor essential revisions:

Figure 5:
The p-values should be included in Figure 5c to make it easier to understand.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

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