Reviewer’s report

**Title:** Effects of cytarabine on activation of human T cells - cytarabine has concentration-dependent effects that are modulated both by valproic acid and all-trans retinoic acid

**Version:** 2  
**Date:** 5 January 2015

**Reviewer:** Sheryl Flanagan

**Reviewer’s report:**

This study aims to investigate the possible immunotoxicity of the combination of low dose cytarabine and valproic acid/all-trans retinoic acid in the treatment of acute myeloid leukemia (AML). Specifically, this group has examined the impact that these combined modalities have on the viability and proliferative capacity of peripheral blood T lymphocytes. Although standard treatment approaches for AML result in panleukopenia, and lymphocyte recovery after treatment induced deficits can predict superior relapse-free survival, little is known about the acute and long term effects of cytarabine + ATRA/valproic acid. This study represents an approach to identifying possible immunomodulatory effects of these standard treatment combinations.

I enjoyed the opportunity to read the authors’ manuscript and have detailed my suggested revisions/comments below:

**Major Revisions**

I found the approach scientifically sound, but when reading this manuscript I was left trying to put together the results in the context of what is already known in the field, and how this study’s findings help to further the field. The manuscript’s value could be increased by some rewriting. I have identified below portions within the manuscript where my concerns are highlighted and that could be reworked.

1) The authors identified the clinical question of what if any immunomodulatory effects cytarabine in combination with ATRA/valproic acid has on peripheral T lymphocytes and presumably how these effects may contribute to leukocyte recovery after treatment induced deficits. However, the authors’ conclusive findings as stated in the abstract “….immunomodulatory effects of low-dose cytarabine are strengthened by ATRA and valproic acid”, lacks specificity, i.e. what effects are strengthened? Are the effects immunosuppressive? What are the clinical implications? Similarly, the first paragraph of the discussion section ends with a sentence that indicates that results of the present study show that low-toxicity chemo will effect T cell system….but how? This is a declarative sentence that lacks detail, rendering it lacking in information.

2) related to point 1) above, a statement of the clinical problem or significance of the work is missing from the abstract. I was able to read between the lines and
surmise what the problem being addressed or what the critical questions were because I had first read the authors’ cover letter. A one sentence addition to highlight the problem being addressed or why the work is important or helps to inform the field would add relevance to the abstract and put the study into context of the field.

3) The Discussion section overall lacks discussion of how the results inform the field, or interpretation of the results. At times, the Discussion section reads more like Results. What inferences if any can be made from these results? Where do the authors see this work going? How does/could this new information impact this field? I was searching for a discussion of relevancy or significance. While the Conclusion section gives some hint of these points, this section could be expanded, or more conclusive/further interpretation statements could be made within the paragraphs of the Discussion section. Also, the Conclusion section is the first time we read of Treg cells and other background literature that may be related to this present study? This seems to come out of the blue. Including some of this literature in the Background section would help the reader to better put the present study results within the context of what is currently known in the field. Given the background and experience of the authors, an expansion of their interpretations of the data would provide valuable context to how the study results have furthered the field.

3) For the AML patients used in this study, were these patients receiving low dose cytarabine treatment? Chemo-naïve? If receiving treatment, for how long? The M and M section doesn’t give details. Also, I am assuming the effect of low dose cytarabine on AML cell viability/proliferative capacity wasn’t already known? If not, this should be stated. If the authors included the work with AML cells to highlight differences between T cells and AML cells, then what do the results mean? Line 371 of the Discussion section makes mention of the differential antiproliferative effects of cytarabine on the two cell types, but not what that may mean clinically.

4) Line 206: viability results of combination of araC and valproic acid are misstated, or do not match the data as presented in Figure 1. Text states, …..the only exception being cytarabine 0.35um that caused a minor reduction of T cell viability when tested alone but not when tested in combination with VA at 1000 um. This statement is true to the figure but what is missing is that the data in the figure indicate that 0.35 um araC in combination with 500 um valproic acid, DOES have a significant effect on viability.

5) Line 240, I think it should read, …..not in the presence of ATRA alone, otherwise the end of this sentence contradicts the first part of the sentence as it is presently written.

Minor Revisions:
Some grammatical errors/typographical errors need to be addressed. I have listed some below.
1) Line 66: This observation suggests…..
2) Line 72: Remove comma between low-toxicity AML-stabilizing chemotherapy.

Line 72: One alternative….this word choice suggests alternative to the low toxicity AML-stabilizing chemotherapy described in the prior statement although I believe the authors mean to describe in detail the low-toxicity AML-stabilizing chemotherapy. Would be less confusing if the authors said something like…..one such low-toxicity AML-stabilizing chemotherapy is…….

3) Line 96: drugs were thawed on the same day they were used in experiments…..

4) Line 155: should read either, “the effect of low dose cytarabine was…..

Or, the effect of low doses of cytarabine were…..

5) Line 205: awkward sentence….change to….with valproic acid at both 100 um and 500 um.

6) Line 250: combination before supernatant (doesn’t need to be pluralized)

7) Line 310: awkward sentence….the triple combination of…….is used to treat AML when…..(in what situations is this triple combination the preferred choice for treatment)

8) Line 311: VA ……has been investigated as an anticancer agent in several clinical studies for the treatment of AML (should be specific here in terms of what cancer),……..ATRA….and [eventually] …..I don’t believe essentially fits here. Perhaps, in addition to, or also with….

9) Line 357: …..and then have immunomodulatory effects

Discretionary Revisions

1) Some of the text is repetitive and perhaps doesn’t need to be included. i.e. the author states numerous times, in M and M, each results section, and figure legends, that the PBMCs were activated by…….this detail only needs to be included in the M and M, and perhaps figure legends (even there the reader can be directed to the M and M for details). Removing some of these repetitive sections would leave more room to discuss results.

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.

Declaration of competing interests:

I declare that I have no competing interests.