Reviewer's report

Title: Immunotherapy using slow-cycling tumor cells prolonged the overall survival of mice bearing colon carcinoma

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Reviewer: Yukai He

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It is known that, after selection by many rounds of chemo or radiation therapy and after going through the process of immunoediting in tumor bearing host where the tumor cells have been sculptured by host immune system, re-emerging tumor cells will be different from original tumor cells. In this manuscript, the authors tested a novel idea that slow-cycling tumor cells may provide more relevant Ag to protect tumor relapse and generate more clinically relevant responses to control tumor growth. The idea is excellent and deserves a careful and well designed investigation, which could generate significant impact on tumor immunotherapy. Thus, the reviewer believes that this is a very interesting study that will provide values to this field. However, several issues can be clarified to strengthen the manuscript. In addition, this manuscript requires some professional editing in order to be published in this Journal.

Major issues:
1. Will the slow-dividing tumor cells change their character with time? In another word, if you keep culturing the slow-cycling cells, will they become fast-dividing or vice versa?
2. The viability of each population should be examined. The Dil high population may be more viable. Staining of apoptosis related protein may be helpful.
3. The high tumor intake after inoculation of the Dil+ cells indicates that they may be better escape the immune system, which contradict the findings that drug-treated CT26 tumor cells (which is also slow dividing) have high MHC II and CD86 (which means they should be more immunogenic).
4. The authors did not touch the issue of Ag repertoire change between slow dividing and fast dividing cells. But could that be one of the reason why drug treated tumor cells be more immunogenic? It is understandable that extensive array assay may be needed to identify the difference of Ag repertoire. Thus, some explanation in the discussion should be added to address this question.
5. The data showed that the drug treated CT26 cells have higher MHC II and CD86 (table 3). The authors believe that this will attribute to the induction of higher immune responses. If so, this may be significantly different from what has been observed in vivo. The relapsed tumors after chemo or radiation therapy often escape the immune system, indicating that in vivo cancer chemo treatment rather select the tumor variant that may be invisible to the immune system. The authors should provide some explanation.
Minor issue: There are many grammar and spelling related minor problems that need to be fixed. For example:

a. Dil-labeling should be “Dil-labeled” (Fig 1 legend on page 29)
b. Finally, not finaly on page17.

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.