Reviewer's report

**Title:** Targeting and killing of glioblastoma with activated T cells armed with bispecific antibodies

**Version:** 2  **Date:** 7 December 2012

**Reviewer:** Jun Wei

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**Major Compulsory Revisions**

1. Bi-specific antibody (BiAb) conjugated T cell therapeutic efficacy against glioblastoma should be validated in xenograft immunodeficient mice model and included in this study.

2. The data of EGFR and HER2 expression by FACS on glioma cell lines and ex vivo glioma cells need to be presented in the manuscript in order to make a clear view for the correlation of sensitivity of glioma cells to the BiAbs with EGFR and HER2 expression.

3. Validation of MTT assay in Fig.1 is problematic, because absorbance measurement of residual viable cells consists of not only glioma cells, but also includes armed ATCs conjugated to the glioma cells. Inconsistency between HER2Bi/MTT and HER2Bi/51Cr at higher BiAb concentration in Fig.1 supports it.

4. In Fig.4 histogram, majority of CD133+ cells and CD133- cells still overlap, and such CD133 purity is not acceptable and suitable for further study.

5. TCR downstream signaling activity of BiAb-ATCs needs to be investigated for mechanistic study of BiAb mediated ATC cytotoxicity.

**Minor Essential Revisions**

1. The highest E:T ratio (12.5:1) is used in Fig.3, and this may saturate killing activity for each BiAb armed ATCs. Improved killing might be achieved when titrating down E:T for simultaneous use of two BiAbs.

2. It would be interesting to test BiAb armed ATCs killing on fresh ex vivo glioma stem cells (GSCs).

3. It is not clear that ATCs are from healthy donor PBMCs or glioma patients. If they are generated from healthy donor, the possible allogeneic responses or GVHD side effects need to be discussed.

**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.