Reviewer’s report

Title: Systemic treatment with CAR-engineered T cells against PSCA delays subcutaneous tumor growth and prolongs survival of mice

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Reviewer: Takemasa Tsuji

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In this study, authors tested the third generation chimeric antigen receptor (CAR) against prostate stem cell antigen (PSCA) that transduced the CD28 and OX-40 co-stimulatory signals in addition to the CD3zeta activation signal. PSCA-specific T cell reactivity was tested against a transfentant that overexpressed PSCA using in vitro and in vivo experimental models. Most experiments were performed and results were reported adequately. Recent clinical trials demonstrated favorable clinical outcome in hematologic cancer patients by adoptively transfer of CAR-expressing T cells. This study potentially provides a rationale to develop similar CAR-based immunotherapy in prostate cancer patients. The weakness of the study is that authors only demonstrated the reactivity using a model cancer cell line that artificially overexpress PSCA and could not demonstrate the expression and reactivity against cancer cells naturally expressing PSCA. Future study should address this issue.

Major compulsory revisions:

1. In Figures 2 and 3, only anti-PSCA-CAR-expressing T cells were used as effector T cells. Additional control effector T cells such as Mock-transduced T cells would be essential to exclude the possibility of non-specific stimulation by the PSCA-overexpressing target cell line.

Minor essential revisions:

1. In “Materials and Method”, CD107a staining was performed after long-term stimulation and did not use BFA or Monensin. Because CD107a/b expression is transient, the optimal detection of CD107a/b expression generally requires the presence of anti-CD107 antibody during culture in the presence of BFA or Monensin. Therefore, the results in Figure 3 A and B could significantly underestimate CD107 expression.

2. Figure 3C appears to show that cytotoxicity against TARP-expressing targets was higher than that against PSCA-expressing targets. Please confirm.

3. Authors described that they performed statistical analysis on survival, but the result was not shown.

4. There is no description about the number of independent experiments and consistency of the results in the legend of Figure 4. Because percentages of survival in Figure 4A-B, C, and D are not consistent and are confusing, it is important to indicate whether these data are from different experimental sets.
5. In “Conclusion”, authors described that expression level of CAR was not high. However, this conclusion was not supported by the experimental results.

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

**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.