Review of: "Development of 3D breast cancer models with human T cells expressing engineered MAIT cell receptors"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The manuscript entitled “Development of 3D breast cancer models with human T cells expressing engineered MAIT cell receptors” authored by Dey. et al. introduced different 3D breast tumor models with increasing complexity to study the efficiency of engineered T cells in destroying metastatic breast cancer cell line: MDA-MB-231. These 3D model platforms may be an important tool for studying the fundamental interactions between the engineered immune cells and tumors in a biomimetic 3D environment and are anticipated to evolve into an essential immune therapy model. Overall, the obtained data sheds light on the importance of different tumor models in studying underlying immune-cancer interactions.

The premise of the paper is interesting and important. The methods are used appropriately and the data supports the conclusion.

However, some minor concerns need to be extensively addressed (in a detailed step-by-step response) before considering this claim.

1- The title may be modified to be more specific so that it would reflect the subject of the paper.

2- In the introduction, define MR 1 and 5-ARU (authors defined them in the materials and method section, but that should be done in their first appearance in the text).

3- In the materials and methods section:
   • Indicate the company of all reagents including the media and IL-2.
   • Indicate the city for beads (Invitrogen, …), EDTA, lipofectamine, Ethidium bromide, thrombin, cell tracker violet, collagen, and Qbeads immunoassay…. to keep the same format for all reagents.
   • Interleukin- 2 (IL-2) should be defined on page 5 (last line) instead of page 6.
   • The authors should include the number of independent experiments performed (“n”) for each data set (not only in the figure legend).

4- In the results section:
   • It would be interesting to study direct cell-cell communication between HFFs and MDA cells (gap junctions for example).
   • Given the fact that MDA-MD-231 cells are metastatic cells, authors may study the expression of epithelial to mesenchymal transition markers and show if the is a shift from mesenchymal to epithelial state under different experimental condition and in the different models.
- Why did the authors choose day 3 as their only time point?
- It is important to compare the observations with non-invasive breast cancer cells like MCF-7 cells and normal-like breast cancer cell lines such as MCF-10A to check the cytotoxicity on normal counterparts so that the data would be conclusive.
- Fig 2, 3, 7, 9, and 11: Enhance resolution (Increase the font size and remove borders of shapes used)
- Fig 4: It would be more informative if the authors took images at higher magnification and used a dye to stain nuclei to access gross cell morphology.

In panel (B), at day 3 it is expected to get super confluent cells since MDA-MB-231 cells duplicate every 35 hours approximately. This is not reflected in day 3 of control cells, there is only an increase in the background signal. Adding data obtained from ethidium bromide staining may be helpful in this case.