Peer Review File
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Reviewer A

1) First, the authors need to indicate efficacy and safety and the animal study in the title.
Reply1: Thank you for your constructive suggestions. We have added safety and mice in title.
Changes in the text: Page 1, Line 2-3.

2) Second, the abstract needs further revisions. The background did not describe the clinical needs for this research focus and why mesothelin-targeted CAR T-cell is potentially effective and safe. The methods need to describe the number of rats and indicate the set of control groups, as well as specify the efficacy and safety outcomes. The results need to quantify the findings by reporting the outcome values and P values. The conclusion needs comments for the clinical implications and the unaddressed questions of this study.
Reply 2: Thank you for your constructive suggestions. We have rewritten this part according to the reviewer's suggestion.
Changes in the text: Page 2, Line 40-43; Line 49-55; Line62-64; Line 71-72.

3) Third, the introduction of the main text needs to explain why the animal study could inform the clinical management of human patients, review the limitations of available treatments for CRLM, and what the potential clinical contributions of this study are.
Reply 3: Thank you for your constructive suggestions. We have added the clinical meaning of CRLM orthotopic mouse model and patient-derived xenograft (PDX) mouse model, potential clinical contributions of this study.
Changes in the text: Page 4, Line 98; Line 115-116; Line 118-119.

4) Fourth, in the methodology, it would be helpful to use a flowchart to describe the research methodology of this study. Please also specify the efficacy and safety outcomes. In statistics, "unpaired t-test" is incorrect. Please consider SNK or other tests for post-hoc analyses. Please ensure $P<0.05$ is two-sided.
Reply 4: Thank you for your constructive suggestions. We have modified our text as advised.
Changes in the text: Page 8, Line 238-242; Line 245-248; Line 264-265.

5) Finally, please cite several related papers: 1. Klobuch S, Seijkens TTP, Haanen JBAG. The emerging role for CAR T cells in solid tumor oncology. Chin Clin Oncol 2023;12(2):19. doi: 10.21037/cco-22-125. 2. Luo Z, Bi X. Surgical treatment of colorectal cancer liver metastases: individualized comprehensive treatment makes a difference. Hepatobiliary Surg Nutr 2021;10(6):899-901. doi: 10.21037/hbsn-2021-23. 3. Ruffolo LI, Hernandez-Alejandro

R, Tomiyama K. Refining the surgical playbook for treating colorectal cancer liver metastases. Hepatobiliary Surg Nutr 2021;10(3):397-400. doi: 10.21037/hbsn-21-31.

Reply 5: Thank you for your constructive suggestions. As suggested by the reviewer, we have added more references to support this idea.

Changes in the text: Page 14, Line 473-478; Page 17, Line 550-551.

Reviewer B

The paper titled "Regional delivery of mesothelin-targeted CAR T-cell effectively targets colorectal cancer liver metastases" is interesting. Regional delivery of MSLN-targeted CAR T-cell therapy has encouraging results in the orthotopic CRLM NSG mice model and PDX model, and converts the tumor microenvironment from cold to hot. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) CAR T-cell therapy is an emerging option for cancer treatment, but its efficacy is limited, especially in solid tumors. This is partly because the CAR T cells become dysfunctional and exhausted in the tumor microenvironment. What are the key pathways responsible for impaired function of exhausted cells? It is suggested to add relevant contents.

Reply 1: Thank you for your constructive suggestions. As suggested by the reviewer, we have added key factors that influenced CAR T-cell dysfuntion in the text.

Changes in the text: Page 13, Line 415-416.

2) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply 2: Thank you for your constructive suggestions. We have rewritten this part according to the reviewer's suggestion.

Changes in the text: Page 2, Line 40-43.

3) Please provide a brief overview of MSLN as a therapeutic target and existing anti-MSLN antibody-based drugs and vaccines.

Reply 3: Thank you for your constructive suggestions. We have added the brief overview of anti-MSLN-related drugs and vaccines.

Changes in the text: Page 4, Line 104-106.

4) What role does the CAR T cell amplification program play in overcoming the immune metabolic barrier in tumor microenvironment? It is suggested to add relevant contents.

Reply 4：Thank you for your constructive suggestions. We have added the role of CAR T cell amplification in CRLM treatment.

Changes in the text: Page 13, Line 404-406.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “A narrative review of chimeric antigen receptor-T (CAR-T) cell therapy for lung cancer, PMID: 34268421”. It is recommended to quote the article.

Reply 5： Thank you for your constructive suggestions. As suggested by the reviewer, we have added the reference to support our text.

Changes in the text: Page 15, Line 503-504.

6) How to enhance the specificity and safety of CAR T cell therapy? What is the important information about the application of CAR T cells in solid tumors today and in the future? It is suggested to add relevant contents.

Reply 6: Thank you for your constructive suggestions. In the text, we have added the combination treatment with CAR T-cell therapy to enhance the antitumor effect, and added the artificial intelligence as the future direction

Changes in the text: Page 14, Line 450-453.