Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-2025-178

Reviewer A

The paper titled “Biological and clinical significance of circulating MAIT cells in lung cancer” is interesting. Lower percentage and absolute values of cMAIT in lung cancer patients may be due to their migration into tissues. The number of cMAIT in lung cancer patients may potentially be considered as a prognostic indicator. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the immune characteristics of MAIT cells in lung cancer? What is the correlation between its prognosis and response rate to ICIs treatment? Suggest adding relevant content.

Reply 1：I'm sorry, but these issues haven't been reported much in the literature.

2) In the results of 'Relationship between the number of cMAIT and baseline NLR in lung cancer patients', “The absolute values of cMAIT in lung cancer patients were negatively correlated with the baseline NLR (r=-0.423; P=0.03) (Figure 8A). "This result should correspond to Figure 8B. Please carefully check and make corrections.

Reply 2 ： We have modified our text as advised (see Page 12, line 383).

Changes in the text：The absolute values of cMAIT in lung cancer patients were negatively correlated with the baseline NLR (r=-0.423; P=0.03) (Figure 8B).

3) MAIT cells accumulate in tumor tissue but exhibit a depleted phenotype. Does ICB of PD-1 or PD-L1 antibodies affect the function of circulating MAIT cells from lung cancer patients? Suggest adding relevant content.

Reply 3 ： We have modified our text as advised (see Page 15, line 486-492).

Changes in the text：A study of non-small cell lung cancer showed that Immune checkpoint blockade (ICB) of PD-1 or PD-L1 antibodies increased proliferation and co-expression of the activating markers HLA-DR and CD38 on MAIT cells in most patients after the first treatment cycle. The production of cytokines (especially TNF and IL-2) and the versatility of MAIT cells were also increased. This study demonstrated that immune checkpoint blockade improves the activation and function of cMAIT in patients with non-small cell lung cancer.

4) What is the difference between this study and published study [Participation of Increased Circulating MAIT Cells in Lung Cancer: a Pilot Study, J Cancer, PMID: 35371315]? What is the innovation? These should be described in the discussion.

Reply 4 ： We have modified our text as advised (see Page 13, line 418-422).

Changes in the text：The difference between this study and the study, “Increased participation of circulating MAIT cells in lung Cancer: a pilot study”, is that the innovation is that it demonstrates that cMAIT can migrate to the tissue to function by showing that lung cancer patients have fewer cMAIT and tumor tissues have more MAIT cells.

5) The description of “ELISA” method is too simplistic. Suggest providing a detailed description.

Reply 5 ： We have modified our text as advised (see Page 8, line 253-290).

Changes in the text：

Preparations before operation:

(I) Take the tested plasma out of the -80 ℃ refrigerator and fully thaw at room temperature before use.

(II) Take the ELISA kit out of the 4 ℃ refrigerator and place it at room temperature for 20-30 min.

(III) Open the enzyme marker and the connected computer, open the SoftMax Pro6.4.2 software, and set the temperature to 37 ℃ for preheating.

Operation process:

(I) Take out the required slats according to the number of samples to be tested.

(II) Set blank holes, standard holes and sample holes to be measured on the slats, and add samples according to the instructions.

(III) Cover each sample hole with sealing plate film and incubate at 37 ℃ for 2 hours.

(IV) Washing:Gently uncover the sealing plate film, pour out the liquid in the hole, and pat it on the absorbent paper to dry it.Wash each sample hole with washing liquid, pour out the liquid in the hole after washing,pat it on the absorbent paper to dry it, repeat washing 4 times.

(V) Add 100 μL antibody (1×) to each sample hole, cover with sealing plate film, and incubate at 37 ℃ for 1 hour.

(VI) Washing: same as step 4.

(VII) Add 100 μL HRP labeled secondary antibody (1×) to each sample hole, cover with sealing plate film, and incubate at 37 ℃ for 40 min.

(VIII) Washing: same as step 4.

(IX) Color development: Add 100 μL of Tetramethylbenzidine (TMB) color development solution to each sample hole, and incubate at 37 ℃ for 15-20 minutes in the dark state for color development.

(X) Termination reaction: Add 100 μL termination solution to each sample adding hole, and the liquid in the hole can be observed to change from blue to yellow.

(XI) Measurement: Put the strip to be measured into the enzyme label instrument within 5

minutes after adding the termination solution, set the wavelength to 450 nm, and measure the Optical density (OD) of each hole.

(XII) Drawing of the standard curve: the OD value of the standard product is subtracted from the OD value of the blank hole, and the average value of the two multiple holes is the final value. With the standard concentration of each gradient as the horizontal coordinate and OD value as the vertical coordinate, the standard curve can be obtained by using ELISA Calc software for four-parameter fitting.

(XIII) Calculation of sample concentration: The OD value of the sample is substituted into the standard curve to obtain its fitting concentration, and the actual concentration of the sample can be obtained by multiplying its dilution.

6) Suggest adding the mechanism of airway MAIT cell function, their impact on lung immunity, and the potential for targeting lung MAIT cells in the therapeutic context to the discussion.

Reply 6: I'm sorry, but these issues haven't been reported much in the literature.

Reviewer B

1. Figure 1B

Please indicate the unit of age.

R: Thanks for your comment. We have added the unit in Figure 1B, the figure in the main manuscript was replaced, and the revised original figure was provided in the attachment.

2. Figure 4

Please explain the "*" in figure legend.

R: "*" in figure legend represents P<0.05, which should be marked as an exact value according to magazine requirements. It has been revised in the manuscript.

3. Figure 1C, 4B, 5, 6, 7B, 9B

Please confirm if any P value are missing. e.g.,

R: This special symbol means that the comparison is not statistically significant. We have added the meaning of special symbols in the manuscript, please review.

4. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise.

Some studies *suggest that the ability of MAIT cells to secrete IFN-γ in colorectal cancer tissues is significantly lower than that in the adjacent tissues, possibly due to the influence of the tumor microenvironment* ***(21).***

Other ***studies*** *have found increased levels of chemokines in the peripheral blood of lung cancer patients and higher numbers of MAIT cells in lung cancer tissues compared with adjacent tissues, but no significant differences in cell proliferation and apoptosis between these tissues* ***(24)****, suggesting that cMAIT migrate to tumor tissues to play a tumor promoting roles.*

In non-small cell lung cancer, ***some studies*** *found lower percentages of tumor-infiltrating MAIT cells in patients with, compared to those without lymph node metastasi****s*** ***(25).***

The lack of correlation between cMAIT and lung cancer stage is consistent with the results of gastric cancer ***studies (29).***

R: Thanks for your comment. We have revised it in the manuscript, please review.

5. **Please list this study "*Increased participation of circulating MAIT cells in lung Cancer: a pilot study*" in the reference list, and cite it in this sentence.**

The difference between this study and the study, "Increased participation of circulating MAIT cells in lung Cancer: a pilot study", is that the innovation is that it demonstrates that cMAIT can migrate to the tissue to function by showing that lung cancer patients have fewer cMAIT and tumor tissues have more MAIT cells.

R: Thanks for your comment. We have revised it in the manuscript, please review.