



Biological and clinical significance of circulating mucosal-associated invariant T cells in lung cancer

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Background: Lung cancer is among the most common malignant tumors worldwide. Circulating mucosal-associated invariant T (cMAIT) cells play an important role in cancer. This study investigated the biological and clinical significance of cMAIT cells in lung cancer.

Methods: Fasting peripheral blood mononuclear cells (PBMCs) were extracted from 30 newly diagnosed lung cancer patients and 30 healthy controls. The percentages of cMAIT among the CD3⁺T cells, their absolute values, and subpopulation distribution in both groups were compared by flow cytometry. The correlations of cMAIT with the neutrophil-to-lymphocyte ratio (NLR) and the expression of programmed cell death-ligand 1 (PD-L1) were analyzed. Enzyme-linked immunosorbent assay (ELISA) was used to detect plasma interleukin-6 (IL-6), interleukin-8 (IL-8), and interferon- γ (IFN- γ) levels in lung cancer patients and healthy controls. The percentage of MAIT cells in the tumor tissues and adjacent normal lung tissues was measured by flow cytometry.

Results: The percentages and absolute values of the cMAIT in lung cancer patients were lower than in healthy subjects ($P < 0.001$, $P < 0.01$, respectively). The CD8⁺CD4⁺ subgroup was dominant in both groups. There was no significant difference in percentages of the CD8⁺CD4⁺ subgroup between lung cancer patients and healthy subjects ($P = 0.63$), but the absolute values of CD8⁺CD4⁺ cells were lower in lung cancer patients ($P < 0.05$). The percentages and absolute values of cMAIT in lung cancer patients were negatively correlated with NLR ($r = -0.537$; $P < 0.01$ and $r = -0.423$; $P < 0.05$, respectively). The cMAIT cell percentage did not correlate with PD-L1 tumor expression ($r = -0.1740$; $P = 0.59$) and with the PD-L1 expression level ($P > 0.99$). No differences were found in the plasma IL-6, IL-8, and IFN- γ levels in lung cancer patients and healthy controls ($P = 0.63$, $P = 0.052$, $P = 0.13$, respectively). The percentage of mucosal-associated invariant T (MAIT) cells in lung cancer tissues was higher than in the adjacent normal lung tissues (1.44% vs. 1.29%, $P = 0.044$).

Conclusions: 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.

Keywords: Mucosal-associated invariant T cells (MAIT cells); lung cancer; prognosis

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Introduction

Mucosal-associated invariant T (MAIT) cells constitute a subset of innate-like T cells and were so named because they were initially discovered in the intestinal mucosa (1). MAIT cells express semi-invariant T cell receptors (TCRs), which consist of a constant alpha (α) chain paired with a semi-invariant beta (β) chain. TCRs vary among species. In humans, the α chain is Va7.2-Ja33, and the β chain is V β 13 and V β 2 (2,3). TCRs operate by recognizing microbial riboflavin (vitamin B2) ligands bound to the major histocompatibility complex class I-related protein (MR1) (4). In addition to the semi-invariant TCRs, cytokine receptors, chemokine receptors, and the transcription factor promyelocytic leukemia, zinc finger protein is present on the surface of MAIT cells and determines their functions (5). When stimulated by microbial antigens, MAIT cells can be recognized and activated by both MR1-dependent and MR1-independent mechanisms. Once activated, they can promptly generate certain cytokines, such as interferon γ (IFN- γ) and tumor necrosis factor α (TNF- α) and produce granzymes and perforin to mediate cytotoxic effects (6,7).

MAIT cells play important roles in infections, immunity, and malignant diseases (8-17). The data on MAIT cell alterations in cancer are inconsistent. For example, compared to healthy controls, the levels of circulating MAIT cells (cMAIT) are generally lower in mucosal malignancies, such as colorectal and gastric cancers, than in non-mucosal tumors, such as thyroid cancer, liver cancer, and breast cancer (18,19), but also in bladder cancer (20). Lower percentages of cMAIT in colorectal cancer patients

than in healthy controls may be caused by the migration of MAIT cells induced by chemokines. In turn, a negative correlation with the lymph node stage suggests that the levels of MAIT cells can reflect the tumor advancement (19). A study suggests 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). One study showed that MAIT cells play a protective role in a mouse colon cancer model and are involved in regulating the immune response to cancer (22).

The data on biological and clinical relevance of cMAIT in lung cancer are scarce and inconsistent. Some studies demonstrated significantly higher values of peripheral blood MAIT cells in lung cancer patients than in healthy controls, while others reported the opposite (22,23). There were also differences in the serum levels of some cytokines, e.g., interleukin-6 (IL-6), interleukin-8 (IL-8), and IFN- γ between lung cancer patients and healthy controls (19,23) and the numbers of MAIT cells in lung cancer and adjacent tissues (24,25).

Increased baseline neutrophil-to-lymphocyte ratio (NLR) was shown to carry adverse prognosis in several malignancies (26,27). In tumors of mucosal origin, the negative correlation between cMAIT levels and lymph node stage suggests their relationship with tumor advancement and prognosis (19). There is no data on the relationship between cMAIT and the NLR and on the prognostic value of this correlation in lung cancer. MAIT cell subpopulations have been found to predict the efficacy of immunotherapy, similarly to the expression level of programmed cell death-ligand 1 (PD-L1) in tumor tissue (24,28). The relationship between cMAIT and PD-L1 expression in tumor tissue, as well as their predictive value to immunotherapy efficacy, has not been investigated.

This study used flow cytometry to detect the number of MAIT cells and their subpopulations in the blood and lung cancer tissues and analyzed their correlation with clinical indicators, NLR, and tissue PD-L1 expression. We aimed to explore the alterations in cMAIT in lung cancer and their clinical significance. Additionally, we assayed by enzyme-linked immunosorbent assay (ELISA) plasma levels of IL-6, IL-8, and IFN- γ in lung cancer patients and healthy controls to better understand the role of cMAIT in lung cancer. We present this article in accordance with the STROBE reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-2025-178/rc>).

Highlight box

Key findings

- Lower percentage and absolute values of the circulating mucosal-associated invariant T (cMAIT) cells in lung cancer patients may be due to their migration into tumor tissues.

What is known and what is new?

- MAIT cells play an important role in malignant diseases.
- This study deepened the knowledge on biological and clinical significance of cMAIT in lung cancer.

What is the implication, and what should change now?

- In lung cancer patients, cMAIT may exert anti-tumor or pro-tumor effects through migration into tumor tissues.
- The role of cMAIT in lung cancer patients warrants further exploration.

Methods

Study subjects

The study group included 30 lung cancer patients admitted to The First Affiliated Hospital of Zhengzhou University between December 2022 and March 2023 and 30 healthy control subjects (age and gender-matched). Inclusion criteria included newly diagnosed lung cancer confirmed by histopathology, at least one measurable lesion on imaging, age between 18 and 80 years, a life expectancy >3 months, and the Eastern Cooperative Oncology Group score of 0–1. Exclusion criteria included immune deficiency, autoimmune disease or immunotherapy administration, a secondary lung cancer or other malignancies, recent history of fever and infection, bronchial asthma, a serious cardiovascular disease (including coronary heart disease, abnormal electrocardiogram results, and uncontrolled hypertension), severe lung function impairment, and serious liver and kidney function insufficiency. The clinical and demographic patient data, including age, sex, laboratory tests, imaging and pathology, were retrieved from the Jiahe medical records system. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Clinical Trial Ethics Committee of The First Affiliated Hospital of Zhengzhou University (No. 2025-KY-0302-002). All patients have signed informed consent for this study.

Experimental procedure

Principal reagent

The principal experimental reagents are detailed in supplementary materials (Table S1).

To prepare the complete medium, the following steps were performed:

- (I) The fetal bovine serum was removed from the -20°C refrigerator and allowed to thaw at room temperature.
- (II) The necessary materials [i.e., basic Roswell Park Memorial Institute-1640 (RPMI-1640) penicillin-streptomycin, a 500-mL sterile glass bottle, a pipette gun, and a pipette gun tip] were placed on a super-clean table under an ultraviolet lamp for disinfection for about 20–30 minutes.
- (III) 180 mL of the RPMI-1640 basic medium, 20 mL of fetal bovine serum, and 2 mL of $100\times$ penicillin-streptomycin double antibody were added to the 500-mL aseptic culture bottle,

and the mixture was thoroughly mixed by slow shaking.

- (IV) The prepared complete medium was divided into 50-mL centrifuge tubes, marked with the name and date, stored in a refrigerator at 4°C , and used within 2 weeks.

Peripheral blood mononuclear cell (PBMC) extraction

- (I) Fasting peripheral venous blood samples from newly diagnosed lung cancer patients and healthy controls were collected with 5 mL ethylene diamine tetraacetic acid.
- (II) The collected whole blood samples were centrifuged as follows: rise 5, fall 5, 25°C , and 2,500 r/minute for 10 minutes. After centrifugation, the whole blood was divided into the upper part (comprising light yellow plasma) and the lower part (comprising blood cells). The upper plasma was absorbed with a 2-mL pipette into a 1.5-mL Eppendorf (EP) tube, and placed in the refrigerator at -80°C for subsequent cytokine detection
- (III) The remaining blood was sucked and mixed with a pipette, then transferred to a 15-mL centrifuge tube, and an equal amount of phosphate buffered saline (PBS) was added to dilute the blood.
- (IV) Lymphocyte separation fluid was added to another 15-mL centrifuge tube. The ratio of the volume of lymphocyte separation fluid to blood volume before dilution was 2:1.
- (V) The diluted blood was drawn and mixed with a straw. The centrifuge tube containing the lymphocyte separation solution was tilted slightly, a blood sample was drawn, and the tip of the straw was pushed against the wall of the tube and slowly dropped into the surface of the separation solution, trying not to let the blood drip into the separation solution.
- (VI) Centrifugation was performed as follows: rise 5, fall 5, 25°C , and 2,500 r/minute for 25 minutes.
- (VII) After centrifugation, the fluid in the tube was clearly stratified from top to bottom into plasma, PBMCs, lymphocyte isolation fluid, and the red blood cell layer. The PBMCs in a cloud shape were collected with a straw, placed in another 15-mL centrifuge tube, and washed with 10 mL of $1\times$ PBS buffer.
- (VIII) Centrifugation was performed as follows: rise 9, fall 9, 25°C , and 2,000 r/minute for 5 minutes.
- (IX) The supernatant was discarded and left to precipitate. The precipitates are PBMC and other

components, such as plasma. Another 10 mL of 1×PBS buffer was added to the precipitate, and step 8 was repeated.

- (X) The sediment in the tube was PBMCs, and if the precipitate was visibly red, it was cleaved by adding a Red Blood Cell Lysis Buffer.
- (XI) Depending on the volume of sediment, 1–2 mL of 1×PBS buffer was added, and the PBMCs were evenly distributed in the buffer by blowing with a straw and then transferred to a 1.5-mL EP tube for the subsequent treatment. If freeze storage was necessary, 1 or 2 mL of cell freeze storage solution was added to PBMCs. The PBMCs mixed with the cell freeze solution were blown with a straw to mix well and then transferred to a 2 mL freeze tube. The freeze storage tube was placed in a gradient cooling box, which was then placed in the refrigerator at –80 °C. Resuscitation was required for use.

Structure treating

- (I) 5–10 mg of fresh lung cancer tissue and adjacent normal lung tissue were taken and stored in 10 mL of RPMI-1640 at 4 °C.
- (II) The tissue was washed twice with 10 mL of complete culture medium containing penicillin and streptomycin. The washing times were adjusted appropriately according to the tissue color until the tissue was gray-white, and no obvious red color was observable by the naked eye. The tissue was then cut into small pieces to destroy the tissue mechanically. Next, a small amount of RPMI-1640 was used to wash the scissors, and the flushing solution was collected in a sample tube to avoid any tissue attachment to the scissors and the loss of target cells.
- (III) The medium used to prepare the digestive tissue was added to the RPMI-1640 with 2% fetal bovine serum, 2 mg/mL type IV collagenase, and 0.05 mg/mL DNase I. The tube mouth was sealed with a sealing film. The tissue in the tube was digested in a water bath at 37 °C for 60 minutes and shaken well every 15–20 minutes.
- (IV) After mechanical and enzymatic digestion of the tissue, the single-cell suspension was obtained. A 70 µm sterile cell filter was moistened with RPMI-1640 in advance and filtered after moistening.

Preparation before flow cytometry

- (I) 300 µL of the prepared sample was placed in a 1.5-mL EP tube, and the remaining samples were kept for later use.

- (II) In each sample, 1 µL of Live-Dead Cell dye was added to mark the dead cells, and 1 µL of the following antibodies were then added: fluorescein isothiocyanate-cluster of differentiation 3 (FITC-CD3), phycoerythrin-TCR/γδ (PE-TCR/γδ), allophycocyanin-TCR V_α7.2 (APC-TCRV_α7.2), Brilliant Violet 605-cluster of differentiation 161 (BV605-CD161), phycoerythrin/cyanine 7-cluster of differentiation 4 (PE/Cy7-CD4), and allophycocyanin/cyanine 7-cluster of differentiation 8 (APC/Cy7-CD8). The tube wall was finger-tapped several times to mix the sample with the antibody.
- (III) After incubation at room temperature for 15 minutes in the dark, the target cells in the sample were fully bound to the labeled antibody.

ELISA

The plasma levels of IL-6, IL-8, and IFN-γ of lung cancer patients and healthy controls were measured by ELISA.

Preparations before operation:

- (I) Take the tested plasma out of the –80 °C refrigerator and fully thaw at room temperature before use.
- (II) Take the ELISA kit out of the 4 °C 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 °C 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 °C 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 °C for 1 hour.
- (VI) Washing: same as step 4.
- (VII) Add 100 µL horseradish peroxidase (HRP) labeled

Table 1 Characteristics of lung cancer patients and healthy controls

Characteristics	Lung cancer group (n=30)	Control group (n=30)
Age (years) (mean ± SD)	63.0±7.7	56.9±7.4
Gender, n (%)		
Male	21 (70.0)	19 (63.3)
Female	9 (30.0)	11 (36.7)
Pathological pattern, n (%)		
Squamous-cell carcinoma	6 (20.0)	–
Adenocarcinoma	17 (56.7)	–
Small cell lung cancer	7 (23.3)	–
Clinical stage, n (%)		
I	3 (10.0)	–
II	1 (3.33)	–
III	16 (53.3)	–
IV	10 (33.3)	–
Gene mutation type, n (%)		
<i>EGFR</i> 19 or 21 exon mutation	6 (20.0)	–
<i>ALK</i> gene fusion	1 (3.33)	–
<i>KRAS</i> mutation	2 (6.67)	–
PD-L1 expression, n (%)		
<1%	5 (16.67)	–
1–49%	4 (13.33)	–
>50%	3 (10.0)	–

EGFR, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; *KRAS*, kirsten rat sarcoma viral oncogene; PD-L1, programmed cell death-ligand 1; SD, standard deviation.

secondary antibody (1×) to each sample hole, cover with sealing plate film, and incubate at 37 °C 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 °C 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.

Data synthesis and statistical analysis

All experimental operations were performed independently by three researchers and the average values were taken as the result to eliminate potential bias. The absolute values of MAIT cells were calculated as follows: absolute value of MAIT cells = absolute value of lymphocytes × MAIT/lymphocytes (%).

SPSS version 25.0 (IBM company) and GraphPad Prism version 8.0.2 (GraphPad Software) were used for the statistical analysis. The data were expressed as the mean ± standard error of the mean (SEM). An independent or paired sample t test was used for comparisons between two groups, and a Pearson correlation analysis was used for the correlation analysis. A one-way analysis of variance was used to compare the means of multiple groups. A P value <0.05 indicated a statistically significant difference. The flow diagram was analyzed and processed using Cytexpert for Dxflex version 2.0 (Beckman Coulter, Inc.) and FlowJo version 10.6 (BD Biosciences).

Results

Patients

Study group included 30 lung cancer patients (six, seventeen and seven with squamous cell, adenocarcinoma and small cell carcinoma, respectively); 21 males and nine females, a mean age of 63 years. The control group included age and gender matched 30 healthy subjects (Table 1).

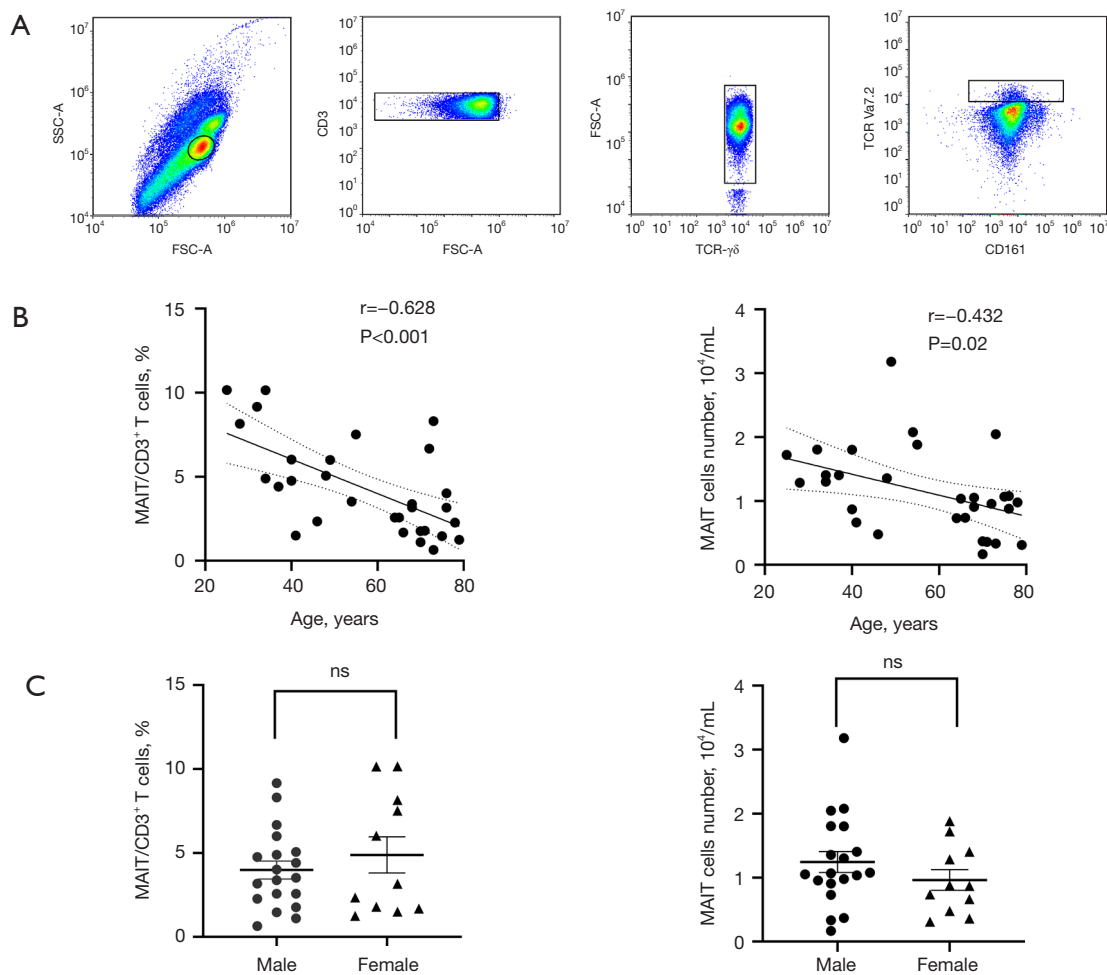


Figure 1 The gating strategy of cMAIT and the relationship between cMAIT and age and sex in healthy subjects. cMAIT, circulating mucosal-associated invariant T; FSC-A, forward scatter area; SSC-A, side scatter area; CD3, cluster of differentiation 3; TCR $\gamma\delta$, T cell receptor $\gamma\delta$; CD161, cluster of differentiation 161; TCR Va7.2, T cell receptor Va7.2; ns, not statistically significant.

cMAIT in healthy controls

cMAIT were defined as the T cells showing positive expression of CD3 and negative expression of TCR $\gamma\delta$, positive expression of TCR Va7.2, and strong expression of CD161, i.e., CD3⁺ TCR $\gamma\delta$ ⁻ TCR Va7.2⁺ CD161⁺⁺ T cells. The lymphocyte population was first identified out of the entire cell populations and the CD3⁺ T cell population was then identified out of the lymphocyte population (Figure 1A). Hence, the gating was gradual until the cell group CD3⁺ TCR $\gamma\delta$ ⁻ TCR Va7.2⁺ CD161⁺⁺ T cells (i.e., MAIT cells) were finally identified. In healthy controls, both the percentage and absolute values of cMAIT were negatively correlated with age (percentage: $r=-0.628$;

$P<0.001$, absolute value: $r=-0.432$; $P=0.02$) (Figure 1B) but not with gender (percentages: male 3.98%, female 4.88%; $P=0.41$, absolute values: $1.24\times 10^4/\text{mL}$ vs. $0.96\times 10^4/\text{mL}$, respectively; $P=0.27$) (Figure 1C).

The percentage and absolute values of cMAIT in lung cancer patients vs. healthy controls

The percentage of cMAIT among the CD3⁺T cells was significantly lower in lung cancer patients than in healthy controls (2.11% vs. 4.31%, $P<0.001$) (Figure 2A). The same was true for the absolute values of the cMAIT ($0.72\times 10^4/\text{mL}$ vs. $1.14\times 10^4/\text{mL}$ in lung cancer patients healthy controls, respectively; $P=0.008$) (Figure 2B).

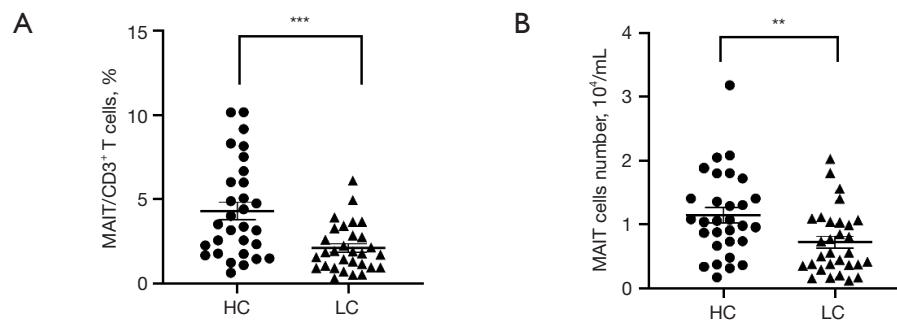


Figure 2 cMAIT percentages and absolute values in LC and HC groups (***, $P<0.001$; **, $P=0.008$). cMAIT, circulating mucosal-associated invariant T; LC, lung cancer group; HC, healthy controls.

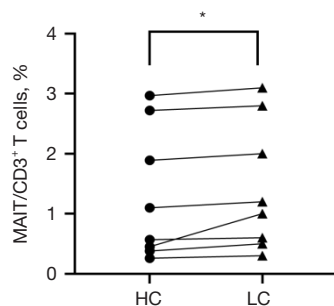


Figure 3 Proportion of MAIT cells/CD3⁺ T cells in tumor and adjacent tissues (*, $P=0.044$). MAIT, mucosal-associated invariant T; LC, lung cancer group; HC, healthy controls.

Comparison of the percentages of MAIT cells in tumor tissue and adjacent tissues

In this experiment, lung cancer tissues and corresponding adjacent tissues were collected and prepared into a single-cell suspension, which was combined with antibodies. The MAIT cells among the CD3⁺ T cells were identified by flow cytometry. The percentage of MAIT cells was higher in cancer tissues than in the adjacent tissues (1.44% *vs.* 1.29%, $P=0.044$) (Figure 3).

Analysis of the cMAIT cell subsets

According to the expression of CD4 and CD8 on the cell surface, MAIT cells were divided into the CD8⁺CD4⁻, CD8⁻CD4⁺, CD8⁺CD4⁺, and CD8⁻CD4⁻ subgroups. The cMAIT of lung cancer patients and healthy controls mainly comprised the CD8⁺CD4⁻ subsets (80.4% *vs.* 83.6%; $P=0.63$) (Figure 4A). However, the absolute values of

cMAIT were lower in lung cancer patients ($0.58 \times 10^4/\text{mL}$ *vs.* $0.92 \times 10^4/\text{mL}$, respectively; $P=0.01$) (Figure 4B).

Relationship between the percentage and absolute values of cMAIT and other variables in lung cancer patients

The percentage of cMAIT in lung cancer patients was negatively correlated with age ($r=-0.371$, $P=0.044$) and positively correlated with blood albumin level ($r=0.458$, $P=0.01$) (Table 2). The absolute values of cMAIT in lung cancer patients were negatively correlated with age ($r=-0.658$; $P<0.001$) and positively correlated with blood albumin level ($r=0.396$; $P=0.03$) (Table 3).

Relationship between the percentage of the cMAIT and tumor histology and stage in lung cancer patients

No significant correlation was found between the percentage of cMAIT and lung cancer histology ($P>0.99$ for all comparisons) and stage (2.77 *vs.* 2.20 for stages I–II and III–IV, respectively; $P=0.43$) (Figure 5).

Relationship between the percentage of cMAIT and tumor size (T), lymph node metastasis (N), and distant metastasis (M) in lung cancer patients

Lung cancer patients were divided according to the tumor diameter (<3 and ≥ 3 cm), lymph node metastasis (N0 and N+), and the presence of distant metastasis (M0 and M1). There was no significant correlation between the percentage of cMAIT and tumor size (2.87 *vs.* 2.10, $P=0.17$), lymph node metastasis (2.91 *vs.* 2.30, $P=0.39$), and distant metastasis (2.51 *vs.* 1.93; $P=0.29$) (Figure 6).

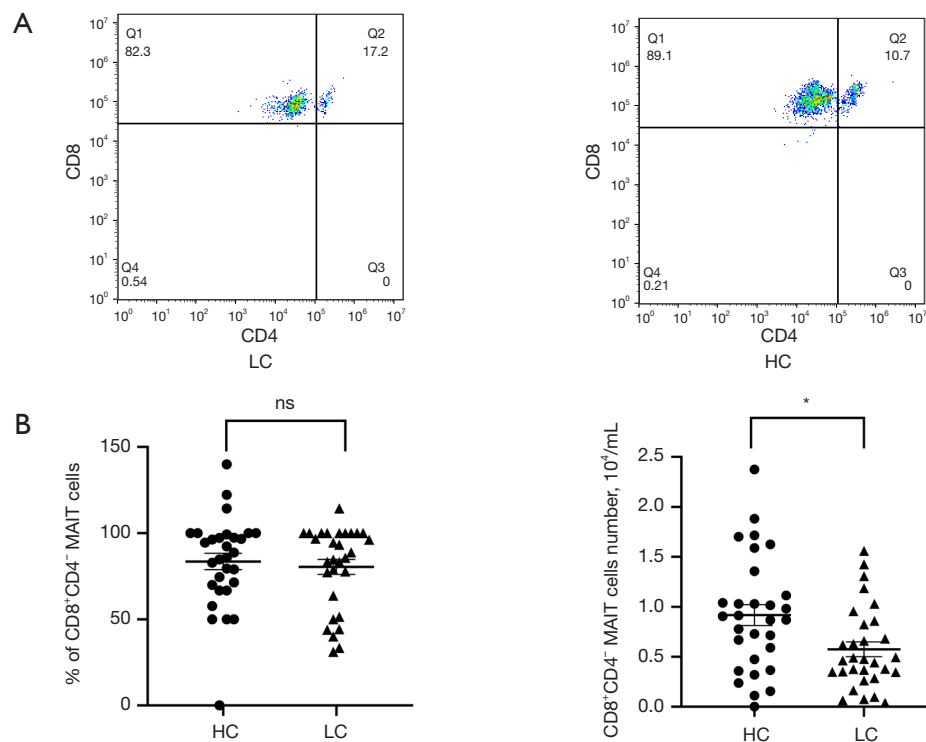


Figure 4 The distribution of MAIT cell subsets classified by CD4 and CD8 (A), and the percentages and absolute values of the CD8⁺CD4⁻ subsets (B). MAIT, mucosal-associated invariant T; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; LC, lung cancer group; HC, healthy controls (*, $P=0.01$); ns, not statistically significant.

IL-6, IL-8, and IFN- γ levels in lung cancer patients and healthy controls

There were no significant differences in the IL-6, IL-8, and IFN- γ levels between lung cancer and control groups (IL-6: 13.00 *vs.* 12.14 pg/mL, $P=0.63$; IL-8: 29.95 *vs.* 37.63 pg/mL, $P=0.052$; and IFN- γ : 18.78 *vs.* 13.99 pg/mL, $P=0.13$) (Figure 7).

Relationship between the number of cMAIT and baseline NLR in lung cancer patients

A negative correlation was found between the percentage of cMAIT and the baseline NLR in lung cancer patients ($r=-0.537$; $P=0.004$) (Figure 8A). The absolute values of cMAIT in lung cancer patients were negatively correlated with the baseline NLR ($r=-0.423$; $P=0.03$) (Figure 8B).

Relationship between cMAIT percentage and lung cancer PD-L1 expression

Lung cancer patients were divided into three groups

according to the level of PD-L1 in the tumor tissues: <1% (negative-expression), 1–49% (low-expression), and $\geq 50\%$ (high-expression). There was no significant correlation between the percentage of cMAIT and tumor PD-L1 expression ($P=0.59$) (Figure 9A) and the level of PD-L1 expression ($P>0.99$) (Figure 9B).

Discussion

This study analyzed the relationship between cMAIT values and other variables in lung cancer patients and healthy controls. Previous studies have demonstrated that the percentages of cMAIT in healthy subjects do not correlate with gender but decrease with age (29,30). Our study confirms these observations. The decreasing percentage of cMAIT with age may be related to the reduction of immune cells and the decline of immune functions.

In patients with infectious and autoimmune diseases, cMAIT participate and are consumed in anti-infection and autoimmunity processes, which decreases their number (31–34). In some malignancies, e.g., colorectal and gastric

Table 2 Relationship between the circulating MAIT cell percentages and other variables in lung cancer patients

Clinical variable	Correlation coefficient (γ s)	P value
Age	-0.371	0.044
Neutrophil absolute value	-0.019	0.92
Lymphocyte absolute value	-0.117	0.54
Monocyte absolute value	0.068	0.72
Hemoglobin	-0.034	0.86
Platelet count	0.117	0.54
Glutamic-pyruvic transaminase	-0.041	0.83
Glutamic oxalacetic transaminase	-0.307	0.10
Total protein	0.107	0.57
Albumin	0.458	0.01
Globulin	-0.205	0.28
Carbamide	0.069	0.72
Creatinine	0.326	0.08
Total bilirubin	-0.069	0.72
Total cholesterol	-0.031	0.88
Triglyceride	-0.253	0.21
High-density lipoprotein	-0.111	0.59
Low-density lipoprotein	0.064	0.75
CEA	0.036	0.85
CYFRA21-1	-0.290	0.13
NSE	0.120	0.54

CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; MAIT, mucosal-associated invariant T; NSE, neuron-specific enolase.

Table 3 Relationship between circulating MAIT cell absolute values and other variables in lung cancer patients

Clinical variable	Correlation coefficient (γ s)	P value
Age	-0.658	<0.001
Neutrophil absolute value	0.038	0.84
Lymphocyte absolute value	0.097	0.61
Monocyte absolute value	0.240	0.20
Hemoglobin	0.120	0.53
Platelet count	0.055	0.77
Glutamic-pyruvic transaminase	-0.113	0.55
Glutamic oxalacetic transaminase	-0.276	0.14
Total protein	0.121	0.53
Albumin	0.396	0.03
Globulin	-0.151	0.42
Carbamide	-0.229	0.22
Creatinine	-0.019	0.92
Total bilirubin	-0.283	0.13
Total cholesterol	0.099	0.63
Triglyceride	0.135	0.51
High-density lipoprotein	-0.214	0.30
Low-density lipoprotein	0.128	0.53
CEA	0.267	0.16
CYFRA21-1	-0.080	0.68
NSE	0.089	0.65

CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragment antigen 21-1; MAIT, mucosal-associated invariant T; NSE, neuron-specific enolase.

cancer, the values of cMAIT are lower than in healthy individuals, while the number of MAIT cells is higher in tumor than in healthy tissues. This phenomenon is presumed to be caused by cMAIT migrating to cancer tissues to play an immune role (31,32). The increased expression of chemokine receptors, such as C-C motif receptor 6 and C-X-C motif receptor 6 CCR6, on the surface of cMAIT, and the release of chemokines into peripheral blood by tumor tissues co-mediate the migration of cMAIT into tumor tissues (19).

Some studies showed significantly lower numbers of cMAIT in lung cancer patients than in healthy individuals, while others reported the opposite (19,23). A study has

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 the present study, the percentages and absolute values of cMAIT in lung cancer patients were lower than in healthy controls and the percentage of MAIT cells in lung cancer tissues was higher than that in the adjacent normal lung tissues. This suggests that a lower number of cMAIT in lung cancer patients may be caused by their migration into tumor tissues. The difference between this study and

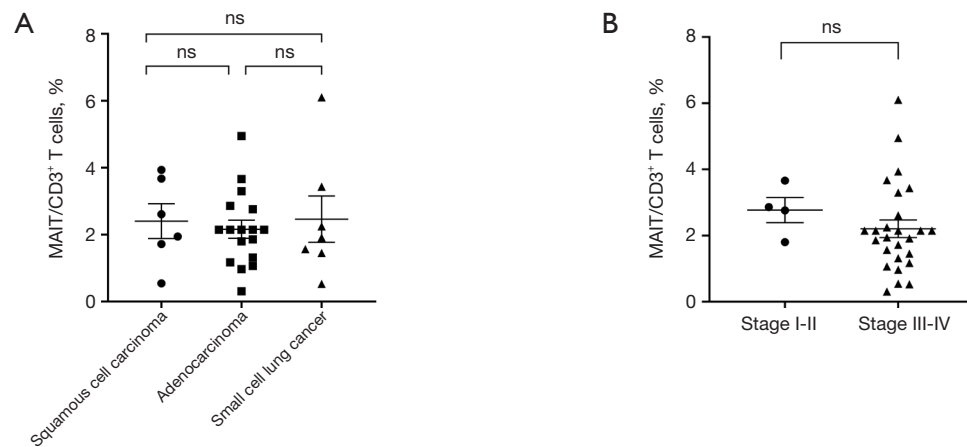


Figure 5 Relationship between percentages of the cMAIT and histology (A) and stage (B) of lung cancer. MAIT, mucosal-associated invariant T; ns, not statistically significant.

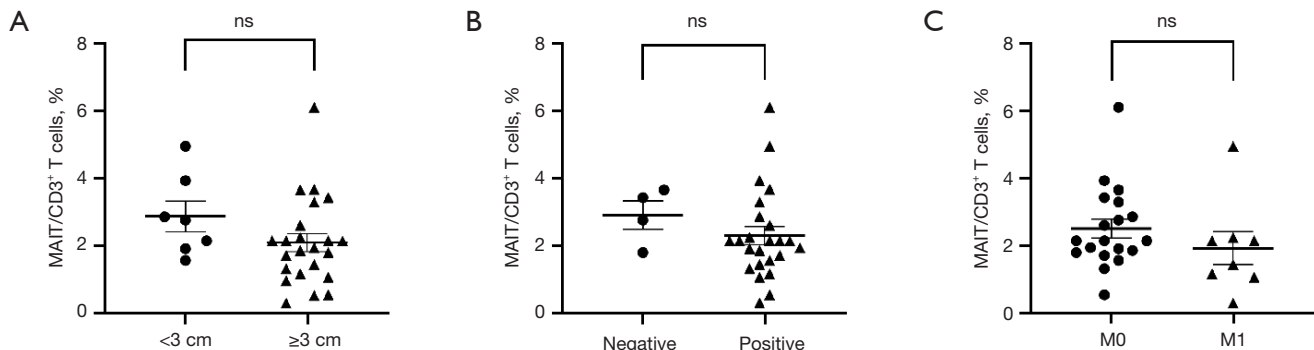


Figure 6 Relationship between the percentages of cMAIT and tumor size (A), lymph node metastasis (B), and distant metastasis (C) in lung cancer patients. MAIT, mucosal-associated invariant T; ns, not statistically significant.

the study, “Participation of Increased 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 (23).

Of the four phenotypes of MAIT cells (CD4⁺CD8⁻, CD4⁺CD8⁺, CD4⁻CD8⁺, and CD4⁻CD8⁻), CD8⁺ phenotypes accounted for the highest proportion in the peripheral blood of healthy participants (33,34). CD8⁺ cMAIT are the most common in gastric cancer patients, but the numbers of CD8⁺CD4⁻ cMAIT are lower than those in healthy controls (19). In lung cancer patients, the number of CD4⁺ and CD8⁺ cMAIT was found to be higher than in healthy controls, with no significant difference in the values of CD4⁻ and CD8⁻ cells between both groups (23).

This suggests that the increase of CD4⁺ and CD8⁺ cMAIT may be related to immune dysfunction in lung cancer patients. The role of CD8 and CD4 cells among MAIT cells is unclear. Our study showed the highest proportion of CD8⁺CD4⁻ subgroup among cMAIT in lung cancer patients. There was no significant difference in percentages of the CD8⁺CD4⁻ subgroup between lung cancer patients and healthy subjects, but the absolute values of CD8⁺CD4⁻ cells were lower in lung cancer patients. The specific role of this cell subgroup needs to be explored.

Several studies have analyzed the relationship between cMAIT and clinical variables in cancer patients. In tumors of mucosal origin, cMAIT were found to be negatively correlated with age, but the difference was insignificant (19). A negative correlation between cMAIT and lymph node

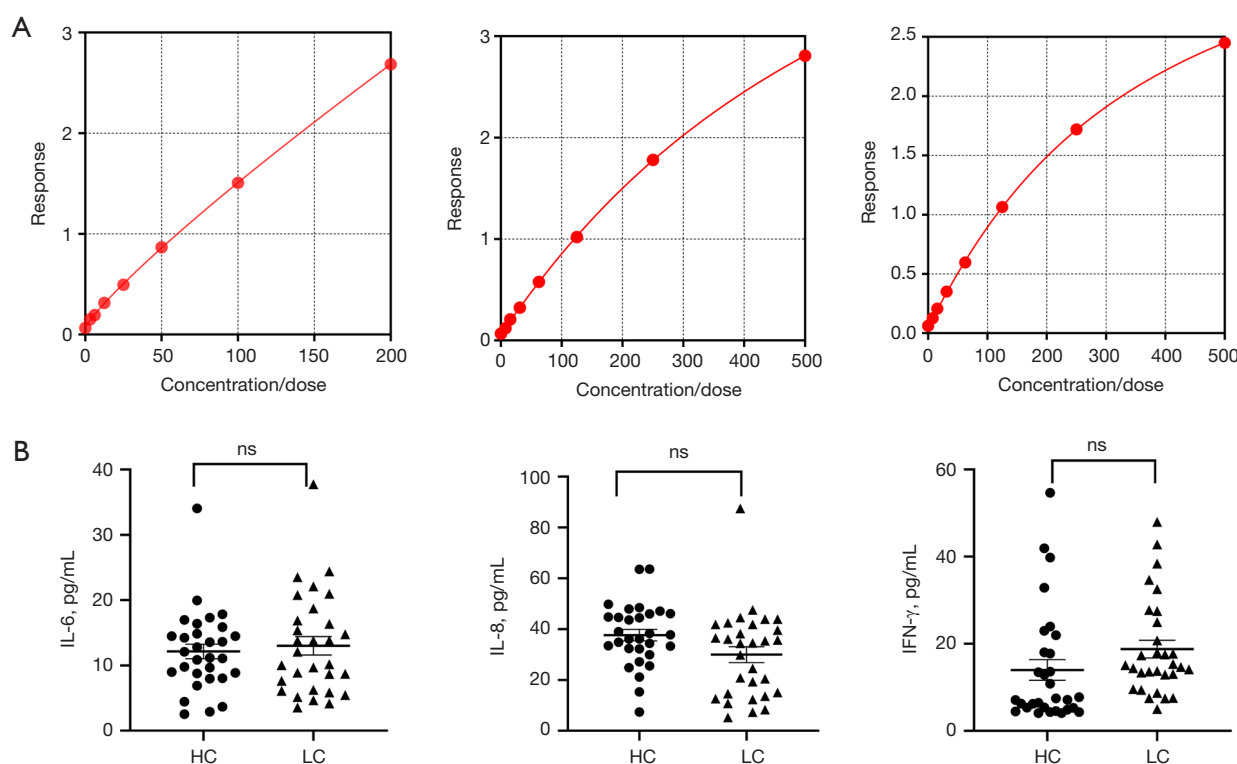


Figure 7 Standard curves of IL-6, IL-8, and IFN- γ (A) and comparisons of IL-6, IL-8, and IFN- γ (B) in lung cancer patients and healthy controls. HC, healthy controls; IL-6, interleukin-6; IL-8, interleukin-8; IFN- γ , interferon- γ ; LC, lung cancer; ns, not statistically significant.

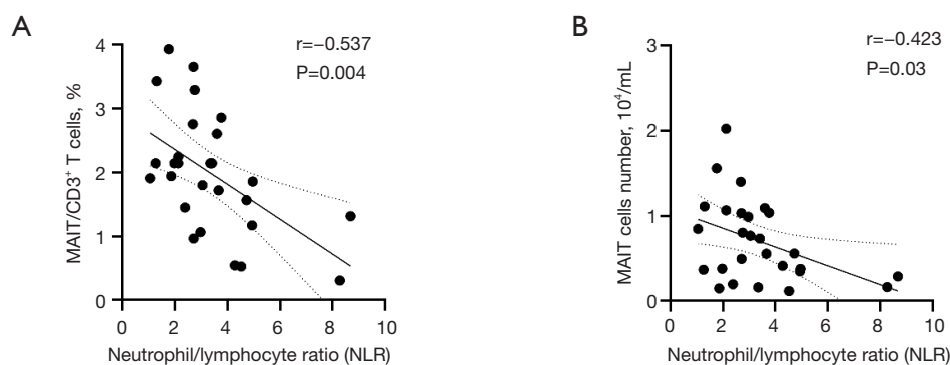


Figure 8 Relationship between the percentages (A) and absolute values (B) of cMAIT and baseline NLR in lung cancer patients. MAIT, mucosal-associated invariant T; NLR, neutrophil-to-lymphocyte ratio.

stage has been attributed to their relationship with tumor extension (19). Additionally, in colorectal cancer, higher MAIT cell infiltration in tumor tissues was correlated with worse survival (35). In gastric cancer, no significant correlation was found between MAIT cell numbers and tumor stage (29). In non-small cell lung cancer, a study found lower percentages of tumor-infiltrating MAIT

cells in patients with, compared to those without lymph node metastasis (25). The present study showed that the percentage and absolute values of cMAIT in lung cancer patients were negatively correlated with age, positively correlated with blood albumin levels, and not significantly correlated with tumor histology, tumor stage, and lung cancer serum markers [carcinoembryonic antigen (CEA);

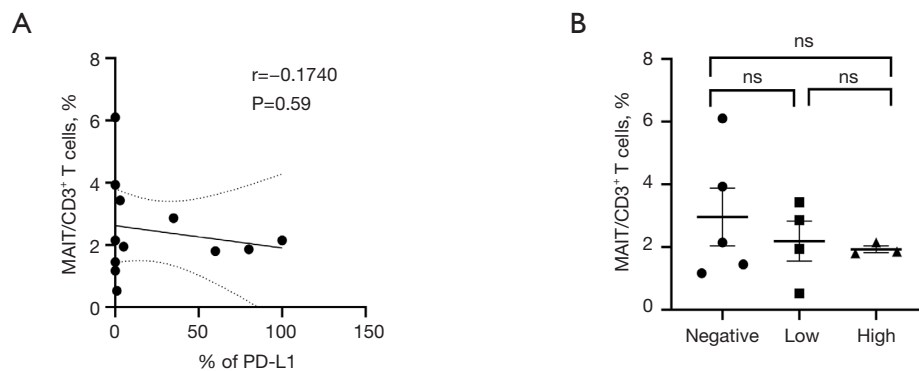


Figure 9 Relationship between the cMAIT percentages and tissue PD-L1 expression in lung cancer patients. cMAIT, circulating mucosal-associated invariant T; PD-L1, programmed cell death-ligand 1; ns, not statistically significant.

cytokeratin fragment 21-1 (CYFRA21-1); neuron-specific enolase (NSE)]. There is scarce knowledge on the correlation of cMAIT with blood albumin levels in cancer patients. The lack of correlation between cMAIT and lung cancer stage is consistent with the results of gastric cancer study (29).

Cytokines are active in many inflammatory processes and play a key role in the communication and connection of various cells. Cells often communicate with each other through cytokines. IL-8 can promote angiogenesis and the invasion and migration of tumor cells, and an increased IL-8 level is associated with tumor burden and poor prognosis (31,32). IL-6 not only promotes tumor proliferation but also plays an anti-tumor role. IFN- γ is an important anti-tumor factor that can act on natural killer cells and CD8 cytotoxic T cells. These cells can kill major histocompatibility complex-negative and positive tumor cells (36-38). The number of cMAIT and the plasma levels of IL-6, IL-8, and IFN- γ have been shown to be increased in lung cancer (23). Thus, MAIT cells may play a role in immunosuppression through the production or recruitment of IL-6, IL-8, and IFN- γ . cMAIT in lung cancer patients can produce IFN- γ , which in turn triggers an anti-tumor activity of lymphokine-activated killer cells. However, no significant difference was found in the plasma levels of IFN- γ , IL-17, and TNF- α in gastrointestinal cancers compared with healthy subjects (19). The present study showed no significant differences in plasma levels of IL-6, IL-8, and IFN- γ between lung cancer patients and healthy controls, which was inconsistent with the trend of decreasing cMAIT. cMAIT migrating into tissues can possibly still secrete these cytokines, but their role in lung cancer has not yet been determined. Thus, no conclusions can be drawn whether MAIT cells promote or

prevent tumor growth.

In tumors of mucous origin, the values of cMAIT are negatively correlated with the lymph node stage (19). In solid tumors, including non-small and small cell lung cancer, higher NLR is an adverse prognostic factor (26,39,40). We showed the negative correlation between percentages and absolute values of cMAIT and NLR, which indirectly suggests their prognostic value in lung cancer. However, due to a short follow-up time we have not directly explored the relationship between MAIT cells and survival outcomes.

High expression of anti-PD-L1 has been shown to correlate with the efficacy of anti-PD-1 and PD-L1 immunotherapy (28). The subsets of MAIT cells with high expression of depletion genes and T helper-17-related genes were demonstrated to correlate with anti-PD-1 therapy resistance, while MAIT cells expressing genes related to cytotoxic function indicated the effectiveness of immunotherapy (24). These results suggest that cMAIT may predict the efficacy of anti-PD-1 agents (24). 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. It demonstrated that ICB improves the activation and function of cMAIT in patients with non-small cell lung cancer (41). Our study demonstrated a negative correlation between cMAIT and tissue PD-L1 in lung cancer patients, but the difference was insignificant, likely due to an insufficient number of patients.

To summarize, our results suggest that cMAIT may migrate from peripheral blood to lung cancer tissues. We

also found that the percentages of cMAIT in lung cancer patients correlate with NLR and PD-L1 expression in tumor tissues, suggesting their relationship with immune efficacy.

This study has several limitations. First, the expression of cell proteins secreted by MAIT cells has not been assessed, so their function could not be fully clarified. Second, we have not analyzed chemokine receptors and thus have not provided further insight into the migration theory. Third, a short follow-up did not allow for a direct analysis of the relationship between MAIT cells and the prognosis. Hence, the function of MAIT cells warrants further exploration.

Conclusions

Weaker expression and lower absolute values of the cMAIT in lung cancer patients may be due to their migration into tissues. The possible prognostic value of MAIT cells warrants further studies. Elucidating the migration mechanism and revealing the interaction between MAIT cells and the tumor microenvironment may contribute to the development of lung cancer immunotherapy and provide new targets for this treatment.

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None.

Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Clinical Trial Ethics Committee of The First Affiliated Hospital of Zhengzhou University (No. 2025-KY-0302-002). All patients have signed informed consent for this study.

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