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Long-term prognostic significance of interleukin-17-producing T cells in patients with non-small cell lung cancer

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Abstract

The presence of interleukin (IL)-17-producing T cells has recently been reported in non-small cell lung cancer (NSCLC) patients. However, the long-term prognostic significance of these populations in NSCLC patients remains unknown. In the present study, we collected peripheral blood from 82 NSCLC patients and 22 normal healthy donors (NC). Percentages of IL-17-producing $CD4^+$T (Th17), $CD8^+$T (Tc17) and γδT cells (γδT17) were measured to determine their association with clinical outcomes and overall survival (OS) in NSCLC. All NSCLC patients were followed up until July 2018. Median follow-up time was 13.5 months (range 1-87 months). The 3- and 5-year survival rate was 27% and 19.6%, respectively. We found that Th17 cells and γδT17 cells were significantly increased, whereas Tc17 cells were markedly decreased in patients with NSCLC compared with those in NC. In addition, Th17 cells were significantly positively associated with T helper type 1 cells (Th1), whereas γδT17 cells were significantly negatively associated with $γδT^+$ interferon (IFN)-$γ^+$ cells. High percentages of peripheral Tc17 cells were significantly associated with favorable 5-year OS (P = .025), especially in patients with early TNM stage (P = .016). Furthermore, high percentages of peripheral Th17 cells were positively associated with favorable 5-year OS in patients with late TNM stage (P = .002). However, no significant association was observed between γδT17 cells and OS, regardless of the TNM stage. In conclusion, our findings suggest that enhanced Th17 and reduced Tc17 cells in the peripheral blood could be a significant predictor of a favorable prognosis for NSCLC patients.

Song and Ma contributed equally to this work.

KEYWORDS
γδT17 cell, IL-17, NSCLC, Tc17 cell, Th17 cell

1 | INTRODUCTION

Lung cancer is one of the most common human malignancies in the world and its mortality rate ranks first in cancer-related causes of death.[1] According to the histological features, lung cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), and NSCLC accounts for approximately 80% or more.[2] Although many novel treatment approaches have been developed for NSCLC, there are still no significant improvements in patient outcomes.[3] Therefore, finding prognostic biomarkers is important for choosing suitable therapeutic strategies for the individual patient to acquire maximum treatment benefits.

Interleukin-17 (IL-17) is a proinflammatory cytokine mainly secreted by Th17 cells, which are identified as a new subset of helper T cells that are different from T helper type 1 (Th1) and T helper type 2 (Th2) cells.[4,5] In addition to Th17 cells, several other cell types are described as sources for IL-17, including $CD8^+$ T cells,[6,7] γδ T cells[8,9] and innate lymphoid cells.[10] The presence of IL-17 and IL-17-expressing cells has recently been studied in several types of cancer, including NSCLC.[11-16] High serum IL-17 levels were detected in NSCLC patients and were significantly correlated with a late stage of NSCLC, overall survival (OS), and disease-free survival (DFS).[13-15] IL-17 in human NSCLC tissues was also significantly elevated and high IL-17 expression was significantly correlated with the clinical and pathological features of patients, such as TNM staging, OS and DFS.[11,12] In addition, the frequencies of IL-17-producing T cells ($IL\text{-}17^+CD4^+$ Th17, $IL\text{-}17^+CD8^+$ Tc17, and $IL\text{-}17A^+$ γδT17 cells) have been found to be dysregulated in NSCLS patients.[16]

Even though several studies have focused on the proportion of IL-17-producing T cells in human cancers,[16,17] the clinical significance of IL-17-expressing T cells, especially the long-term prognostic significance of these populations in NSCLC patients has not yet been well examined. In the current study, we investigated the frequencies of IL-17-producing $CD4^+$, $CD8^+$, and γδT cells, as well as their correlations with interferon (IFN)-γ-producing $CD4^+$, $CD8^+$, γδT cells, and regulatory T cells (Treg) in PBMC of NSCLC patients. Importantly, the clinical significance of IL-17-producing T cells in NSCLC patients with 5-year follow up was also evaluated.

2 | MATERIALS AND METHODS

2.1 | Patients and follow up

A total of 82 patients with newly diagnosed NSCLC at the Department of Oncology, The First Affiliated Hospital of Soochow University were included in the present study from May 2011 to December 2011. Twenty-two healthy volunteers were recruited as normal controls (NC). At the time of sample collection, none of the patients had received any anticancer therapy, corticosteroids, or other nonsteroidal anti-inflammatory drugs. Of all 82 patients, 16 (19.5%) patients were lost to follow up, and 66 patients were followed up until July 2018 or until death. Fifty-three patients (80.3%) died at the time of analysis. Mean follow-up time for survivors was 27.8 ± 19.7 months (median, 13.5 months, range 1-87 months). Follow-up information was obtained from hospital case records, or by telephone or mail, or from death certificates. Three-year and 5-year survival rates were 27% and 19.6%, respectively. OS was measured from the date of treatment until the date of death or last known follow up. The study protocol was approved by the ethics committee of The First Affiliated Hospital of Soochow University, and informed consent was obtained from all subjects. Characteristics of 82 cases and 22 NC are shown in Table 1.

2.2 | Cell preparation and flow cytometry

Peripheral blood mononuclear cells of heparinized peripheral blood from the study subjects were isolated by Ficoll density gradient centrifugation (HAO YANG, Tianjin, China). Flow cytometry analysis of IL-17-producing cells was carried out as previously described.[18] Briefly, 2×10^6 PBMC were stimulated with 50 ng/mL PMA and 500 ng/mL ionomycin in the presence of 10 μg/mL brefeldin A (BioLegend, San Diego, CA, USA) in 24-well plates for 4 hours. Cells were harvested, washed, and stained with FITC-antihuman CD3, or PerCP/Cy5.5-antihuman CD4, or PerCP/Cy5.5-antihuman CD8, or PerCP/Cy5.5-antihuman TCR-γδ (all from BioLegend) for 30 minutes on ice in the presence of FcR-block (BioLegend). After washing, cells were fixed with 4% paraformaldehyde and permeabilized with 1% saponin (Sigma Chemical Co., St Louis, MO, USA), and then stained with phycoerythrin (PE)-antihuman IL-17 and Allophycocyanin (APC)-antihuman IFN-γ (all from BioLegend) for 30 minutes on ice. Treg cell staining was carried out with a Human Regulatory T Cell Staining Kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. Treg cells were defined as $CD3^+CD4^+Foxp3^+$ cells. Data were acquired on a FACS Calibur (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using CellQuest Pro software (BD Biosciences).

2.3 | Statistical analysis

All data are expressed as means ± SEM and were analyzed using GraphPad Prism 5 software for Windows (GraphPad Software). Comparisons between groups were carried out by Student's *t*-test. Correlations between variables were determined by Pearson coefficient analysis. Survival curves were carried out by using the Kaplan-Meier method and tested using the log-rank test using SPSS 18.0 for Windows (SPSS Inc.). *P*-values <.05 were considered statistically significant.

3 | RESULTS

3.1 | Decreased CD8$^+$T and Th1 cells, increased γδT cells, Tc1 cells and Treg in the peripheral blood of NSCLC patients

We first examined T-cell subsets and cytokine profiles in PBMC of 82 patients with NSCLC, and 22 age- and gender-matched NC. We found similar levels of CD4$^+$T cells between NSCLC and NC (Figure 1A), whereas percentages of CD8$^+$T cells were significantly decreased (Figure 1B), and percentages of γδT cells were significantly increased (Figure 1C) in patients with NSCLC compared with NC. In addition, percentages of Th1 cells (CD4$^+$IFN-γ$^+$) were decreased (Figure 1D), whereas the percentage of Tc1 cells (CD8$^+$IFN-γ$^+$) and Treg cells (Figure 1E and G) were increased in NSCLC compared with NC. However, no difference in γδT$^+$IFN-γ$^+$ cells was observed between NSCLC and NC (Figure 1F). We further analyzed the percentages of the above cell populations according to different TNM stages of NSCLC patients. Nevertheless, no significant differences were observed between late-stage (III+IV) and early-stage (I+II) patients of four populations (Figure 1H-K), except that percentages of Treg cells in late-stage patients were slightly higher than those in early-stage patients (Figure 1K).

TABLE 1 Clinical characteristics of 82 patients with NSCLC and 22 normal controls

Clinical characteristics	NSCLC (n = 82)	Normal (n = 22)
Age, y		
<60	33 (40.2%)	13 (59.1%)
≥60	49 (59.8%)	9 (40.9%)
Gender		
Male	48 (58.5%)	12 (54.5%)
Female	34 (41.5%)	10 (45.5%)
Smoking status		
Smoker	39 (47.6%)	10 (45.5%)
Non-smoker	43 (52.4%)	12 (54.5%)
Histological type		
Adenocarcinoma	44 (53.7%)	
Squamous cancer	19 (23.2%)	
Other	19 (23.2%)	
TNM stage		
I	12 (14.6%)	
II	10 (12.2%)	
III	14 (17.1%)	
IV	46 (56.1%)	
Differentiation		
Moderate	19 (23.2%)	
Moderate-poor	4 (4.9%)	
Poor	31 (37.8%)	
Other	28 (34.1%)	
Lymph node metastasis		
No	19 (23.2%)	
Yes	63 (76.8%)	
Treatment		
Surgery alone	12 (14.6)	
Surgery + Chemotherapy	14 (17.1%)	
Chemotherapy (anti-VEGF agents)	24 (29.3%)	
Chemotherapy + Radiation	8 (9.8%)	
Chemotherapy + EGFR-TKI	5 (6.1%)	
Missing	19 (23.2%)	

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; VEGF-TKI, vascular endothelial growth factor-tyrosine kinase inhibitors.

3.2 | Decreased Tc17 cells, and increased Th17 cells and γδT17 cells in the peripheral blood of NSCLC patients

We then determined the frequencies of circulating Th17, Tc17, and γδT17 cells in patients with NSCLC. As shown in Figure 2, the percentages of Th17 cells (Figure 2A,B) and γδT17 cells (Figure 2G,H) were significantly increased, whereas the percentages of Tc17 cells (Figure 2D,E) were markedly decreased in patients with NSCLC in comparison with NC, which were consistent with the previous study.[16] In addition, the percentage of Th17 cells was higher in late-stage patients than in early-stage patients (Figure 2C), whereas there was no difference in the percentage of Tc17 cells regarding TNM stage (Figure 2F). γδT17 cells were slightly increased in late-stage patients compared to early-stage patients (P = .07, Figure 2I).

3.3 | Interferon-γ and IL-17 double-positive T cells in the peripheral blood of NSCLC patients

As cells producing both IFN-γ and IL-17 have been identified in many tumors and are responsible for antitumor effects in tumor immunity,[19-21] we then evaluated the frequencies of IFN-γ and IL-17 double-positive T cells in patients with NSCLC. As shown in Figure 3, the percentages of CD4$^+$IFN-γ$^+$IL-17$^+$ cells (P = .08, Figure 3A,B) and γδT$^+$IFN-γ$^+$IL-17$^+$ cells (Figure 3G,H) were increased, whereas the percentages of CD8$^+$IFN-γ$^+$IL-17$^+$ cells (Figure 3D,E) were markedly decreased in patients with NSCLC compared with NC. However, no differences between early- and late-stage patients were observed for these three cell populations (Figure 3C, F and I). CD4$^+$IL-17$^+$Foxp3$^+$ cells were found in human tumors, and could inhibit tumor-specific CD8$^+$T effector cells.[22,23] Herein, we found that this population was significantly increased in NSCLC patients (Figure S1A-C), suggesting that CD4$^+$IL-17$^+$Foxp3$^+$ cells may play a promoting role in the pathogenesis of NSCLC.

3.4 | Relationship between IL-17-producing T cells and their IFN-γ-producing partners in peripheral blood of NSCLC patients

To further explore the significance of IL-17-producing T cells in NSCLC, we analyzed the correlations between IL-17-producing T

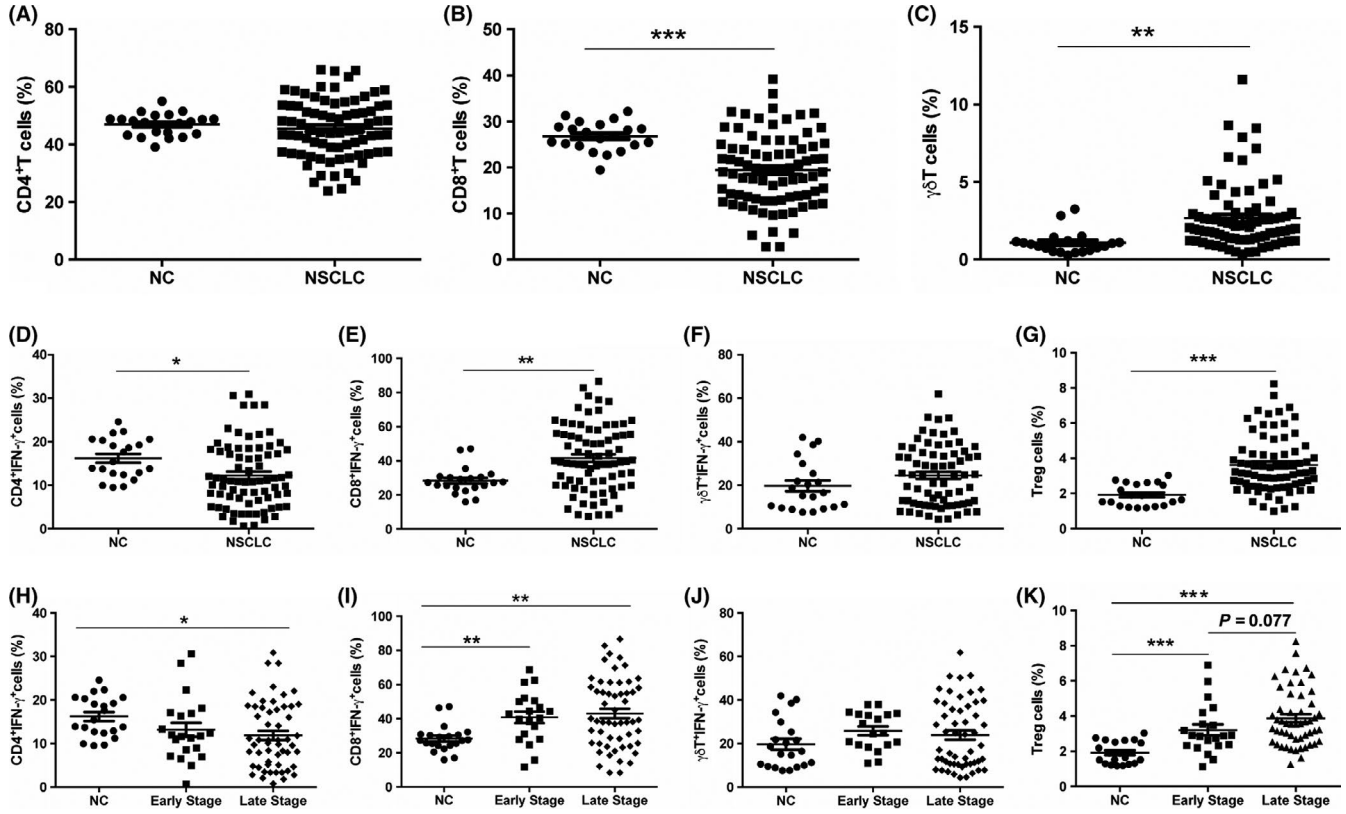

FIGURE 1 Decreased $CD8^+$T and T helper type 1 (Th1) cells, increased γδT cells, Tc1 cells and regulatory T cells (Treg) in the peripheral blood of patients with non-small cell lung cancer (NSCLC). PBMC from NSCLC patients and normal controls (NC) were isolated and evaluated by flow cytometry. Percentages of $CD4^+$T cells (A), $CD8^+$T cells (B), γδT cells (C), as well as Th1 cells ($CD4^+IFN\text{-}\gamma^+$) (D), Tc1 cells ($CD8^+IFN\text{-}\gamma^+$) (E), $\gamma\delta T^+IFN\text{-}\gamma^+$ cells (F), and Treg in NSCLC patients and NC are shown. (H-K) Frequencies of Th1, Tc1, $\gamma\delta T^+IFN\text{-}\gamma^+$ and Treg in NSCLC patients with tumors at different TNM stages (H-K) are shown. Bars represent means ± SD. $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$. IFN, interferon

cells and total $IL\text{-}17^+$ cells, IFN-γ-producing T cells and Treg cells. Data showed that the expression of $IL\text{-}17^+$ cells was significantly positively associated with the frequencies of Th17 cells (Figure 4A), but was not related to the frequency of Tc17 cells and γδT17 cells (Figure 4B,C), suggesting that the main source of intracellular IL-17 in the peripheral blood was Th17 cells in NSCLC patients. Furthermore, we found that Th17 cells were significantly positively associated with Th1 cells (Figure 4D), whereas γδT17 cells were significantly negatively related to $\gamma\delta T^+IFN\text{-}\gamma^+$ cells (Figure 4F). However, no correlation between Tc17 cells and Tc1 cells was observed in NSCLC patients (Figure 4E). Moreover, we also observed a significant positive correlation between Th17 cells and Treg (Figure 4G), but not for Tc17 cells and γδT17 cells (Figure 4H,I).

3.5 | Long-term prognostic significance of IL-17-producing T cells in NSCLC patients

We then evaluated the impact of peripheral IL-17-expressing T cells on OS for patients with NSCLC. Sixty-six NSCLC patients were divided into two groups based on their levels of IL-17-producing cells using cutoff values calculated by receiver operating characteristic (ROC) curve (Th17: 1.15%; Tc17: 0.3%; γδT17: 2.33%). Patients with a high percentage of Tc17 cells had a significantly higher 5-year OS rate compared to patients with a low percentage of Tc17 cells (Figure 5B, 23.81% vs 9.09%, HR = 1.865, 95% CI = −1.059 to 3.285, $P = .025$), whereas the 5-year OS rate of patients with high Th17 or γδT17 cells was comparable to that of patients with low Th17 or γδT17 cells (Figure 5A,C). In stratified analyses by TNM stage, we found that early-stage patients with a high percentage of Tc17 cells showed a significantly higher 5-year OS rate compared to patients with low Tc17 cells (Figure 5E). Remarkably, late-stage patients in the Th17-high group had significantly favorable OS compared with those in the Th17-low group (Figure 5G). However, we found no significant differences in OS of high or low Th17 cells and γδT17 cells in early-stage patients, and of high or low Tc17 cells or γδT17 cells in late-stage patients (Figure 5D, F, H and I). In addition to OS, we also evaluated the DFS. However, we did not find any significant correlations between these three cell populations and DFS (Figure S2), which may be due to the limited samples of NSCLC patients, Future studies will need to pay more attention to the impact of peripheral IL-17-expressing T cells on DFS for patients with NSCLC. Taken together, these results suggest that Tc17 cells may play a critical role in early-stage NSCLC patients, whereas Th17 cells are more favorable for late-stage NSCLC patients.

We also evaluated the prognostic significance of IFN-γ-producing cells, as well as IFN-γ- and IL-17 double-positive T cells in NSCLC patients. The data showed that patients with high percentages of $CD8^+IFN\text{-}\gamma^+$ cells or $\gamma\delta T^+IFN\text{-}\gamma^+$ cells had a significantly higher 5-year OS rate compared to patients with low percentages (Figure S3B,C), but this outcome was not observed in $CD4^+IFN\text{-}\gamma^+$ cells (Figure S3A). However, we found no significant differences in OS of high or low IFN-γ and IL-17-double-positive T cells (Figure S4A-C), as well as $CD4^+IL\text{-}17^+Foxp3^+$ cells in patients with NSCLC (Figure S1D).

4 | DISCUSSION

It has been reported that T lymphocytes and their associated cytokines are abnormal in patients with cancer and are significantly involved in tumor development and progression.[24] In the present study, we found that levels of $CD8^+$T cells and Th1 cells in peripheral blood of patients with NSCLC were significantly decreased, whereas Treg were increased, which were consistent with the previous findings.[25,26] Nevertheless, no significant differences were observed between these cell populations and TNM stage; the reason may be due to the limited number of patients in this study. Our findings suggest that the antitumor responses mediated by $CD8^+$T and Th1 cells may be suppressed in patients with NSCLC. Increased percentages of Treg may contribute to downregulation of the immune response against tumors.

Interleukin-17 and IL-17-producing cells have recently been detected in various types of human cancer and murine models. However, their roles in tumor development are still controversial. IL-17 is a proinflammatory cytokine that can promote tumor progression by inducing proangiogenic factors, such as vascular endothelial growth factor (VEGF), prostaglandin E1 and prostaglandin E2.[27-29] IL-17 can also induce chemokine expression and myeloid-derived suppressor cell (MDSC) infiltration into the tumor environment to suppress the antitumor immune response.[30] In contrast, IL-17 has been shown to inhibit the growth of hematopoietic tumors such as mastocytoma and plasmacytoma by enhancing CTL activity.[31] Endogenous IL-17 contributes to enhanced tumor growth and lung

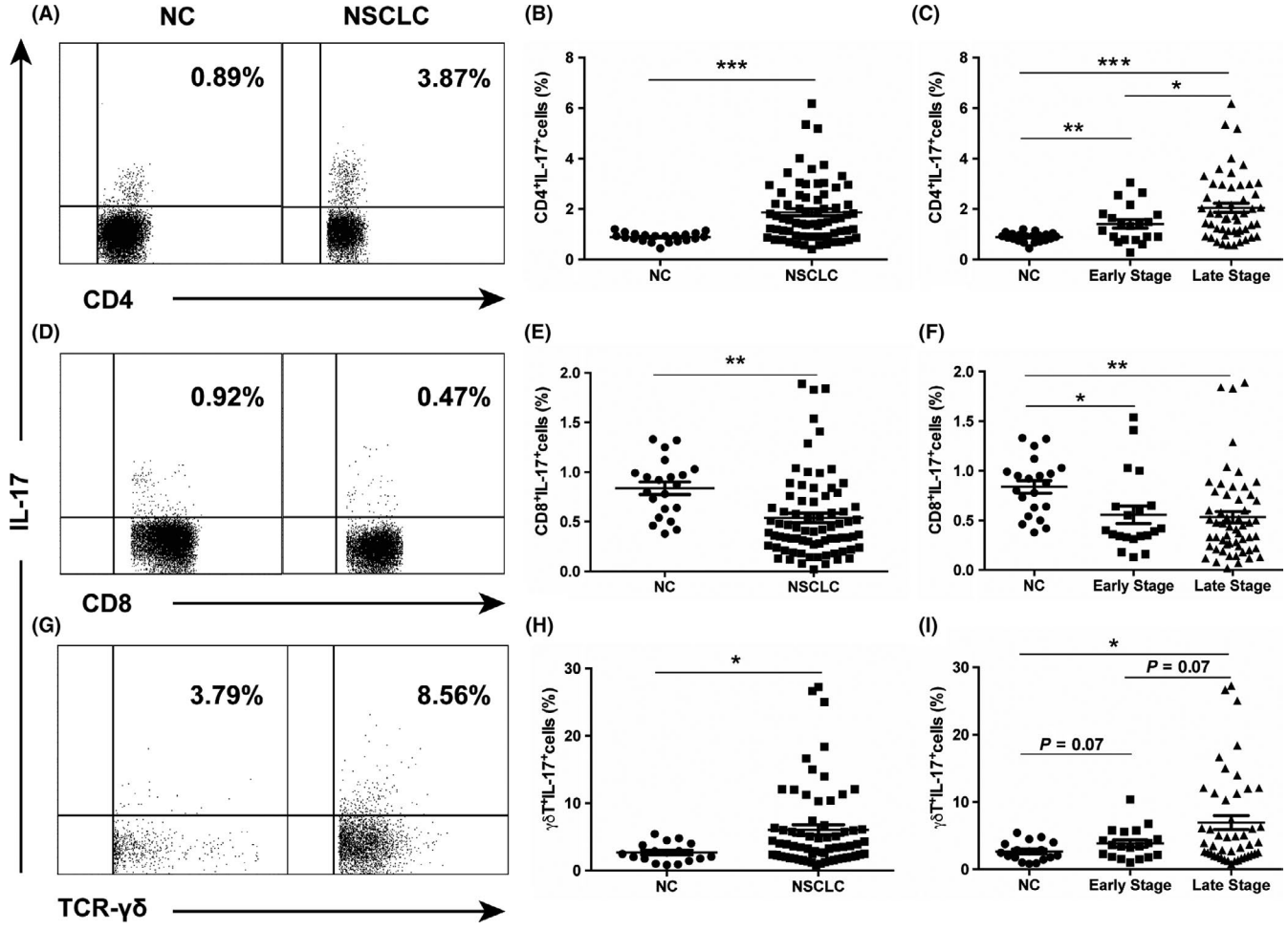

FIGURE 2 Decreased Tc17 cells, and increased Th17 cells and γδT17 cells in the peripheral blood of patients with non-small cell lung cancer (NSCLC). Populations of Th17 cells, Tc17 cells and γδT17 cells in the PBMC of patients with NSCLC and normal controls (NC) were evaluated by flow cytometry. Representative plots and the quantitative analysis of Th17 cells (A and B), Tc17 cells (D and E) and γδT17 cells (G and H) are shown. (C, F and I) Frequencies of Th17, Tc17, γδT17 cells in NSCLC patients with tumors at different TNM stages are shown. Bars represent means ± SD. *$P < .05$; **$P < .01$; ***$P < .001$. IL, interleukin; TCR, T-cell receptor

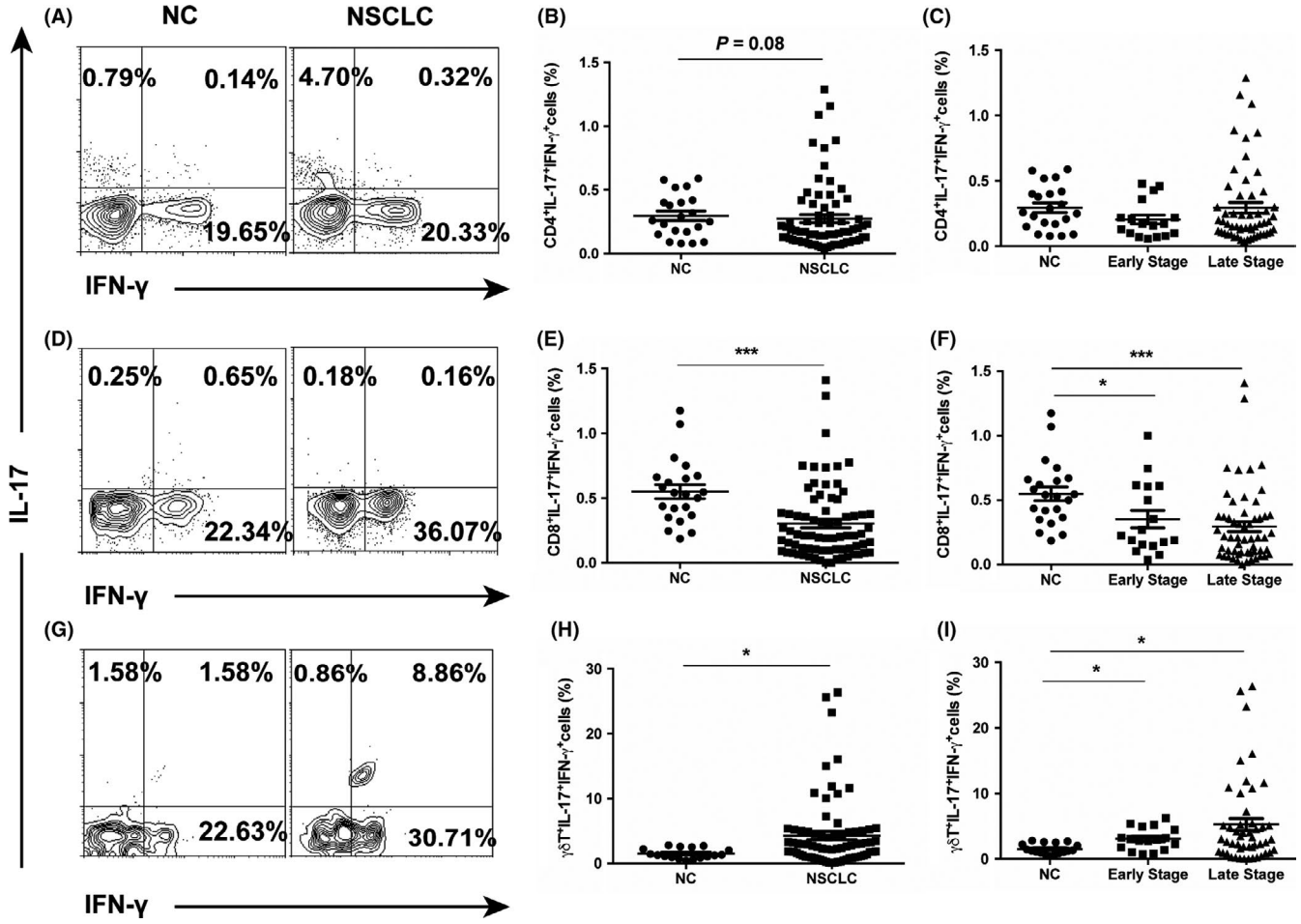

FIGURE 3 Interferon (IFN)-γ and interleukin (IL)-17 double-positive T cells in the peripheral blood of non-small cell lung cancer (NSCLC) patients. Population of $CD4^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells (A and B), $CD8^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells (D and E) and $\gamma\delta T^+IFN\text{-}\gamma^+IL\text{-}17^+$ cells (G and H) in the PBMC of patients with NSCLC and normal controls (NC) were evaluated by flow cytometry. Representative plots were gated on $CD4^+$T cells, $CD8^+$T cells, and γδT cells. (C, F and I) Frequencies of indicated cells in NSCLC patients with tumors at different TNM stages are shown. Bars represent means ± SD. $^*P < .05$; $^{***}P < .001$

metastasis in mice.[32] As in the controversial role of IL-17 cytokine in the tumor, IL-17-producing T cells also showed a paradoxical role in tumor immunity. Adoptive transfer of tumor-specific Th17 cells prevented tumor development.[33] Th17-polarized cells were found to be more effective than Th1 cells in eliminating large established tumors,[34] and showed a stem cell-like property in tumor immunity.[35] However, the infiltration of Th17 cells in the tumor site was associated with poor prognosis in hepatocellular carcinoma,[36] colorectal cancer,[37] pancreatic carcinoma,[38] as well as in thyroid tumor.[18] Th17 cells could convert to Th17/Treg hybrid cells in vivo and repress tumor-specific CD8+ T responses.[22,23] For IL-17-producing $CD8^+$T cells, adoptive transfer of tumor-specific Tc17 cells reduced large established tumors.[19] However, γδT17 cells were found to promote colorectal cancer progression through recruiting MDSC.[39] Therefore, IL-17-producing T cells may function as a double-edged sword in cancer.

γδT cells are the prototype of 'unconventional' T cells and represent a relatively small subset of T cells in peripheral blood. γδT cells can recognize and kill tumor cells in an MHC-unrestricted way.[40] They can also produce diverse cytokines (IFN-γ, tumor necrosis factor [TNF]-α, and IL-17) and chemokines (RANTES, IP-10, lymphotactin) in response to specific antigens.[40] A lower frequency of circulating γδT cells was observed in NSCLC patients than in healthy controls.[41] However, we found increased levels of γδT cells in peripheral blood of patients with NSCLC. In addition, γδT cells expressed comparable levels of IFN-γ but higher levels of IL-17 in NSCLC patients compared with NC. Strikingly, the majority of IL-17-producing γδT cells coexpressed IFN-γ (Figure 3G), suggesting that $IL\text{-}17^+IFN\text{-}\gamma^+\gamma\delta T$ cells are the main population in NSCLS patients. In addition, OS data indicate that γδT17 cells may play a protumor role in NSCLC, whereas $IFN\text{-}\gamma^+\gamma\delta T$ cells showed antitumor activity, and this was in accordance with previous studies.[16,30,39] Ma et al found that γδT17 cells could enhance the efficacy of anticancer chemotherapy through prompting the anticancer immune response.[42] However, we did not find a significant difference in 5-year OS of high and low γδT17 cells in patients who underwent chemotherapy (data not shown), suggesting that circulating γδT17 cells may not contribute to chemotherapy efficacy in NSCLC.

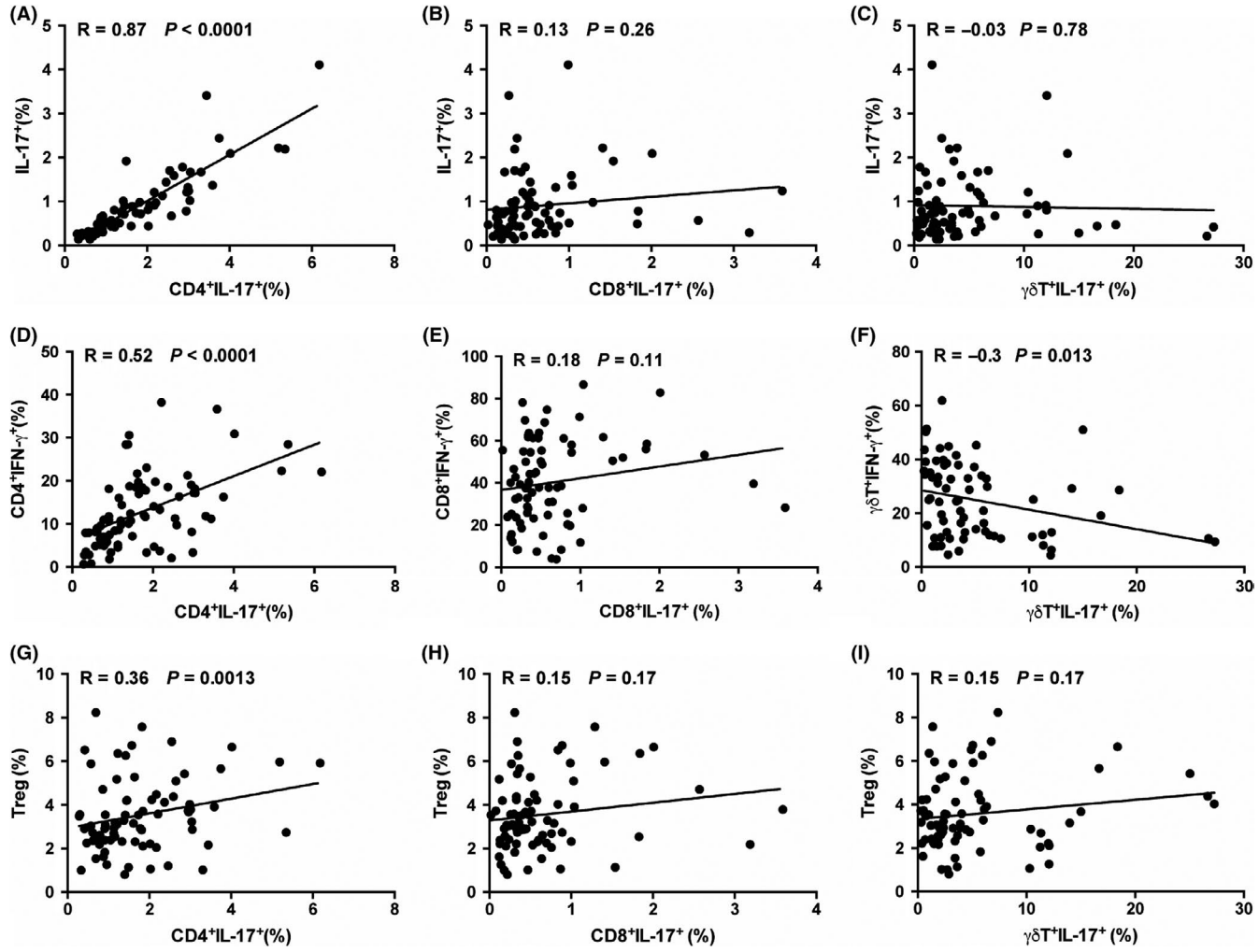

FIGURE 4 Relationship between interleukin (IL)-17-producing cells and their interferon (IFN)-γ-producing partners in peripheral blood of patients with non-small cell lung cancer (NSCLC). Correlations between the IL-17-producing CD4, CD8, γδT cells and IL17$^+$ cells (A-C), IFN-γ-producing T cells (D-E), γδT$^+$IFN-γ$^+$ cells (F) and regulatory T cells (Treg) (G-I) in patients with NSCLC variables were determined by Pearson coefficient analysis. Solid line, linear growth trend; r, correlation coefficient. *P*-values are shown

Interleukin-17 in human NSCLC tissues was significantly correlated with the clinical and pathological features of patients, such as TNM staging, OS and DFS.[11,12] In the current study, we found that high levels of peripheral Th17 and Tc17 cells were significantly associated with improved long-term OS in NSCLC, which was in accordance with previous studies in human esophageal squamous cell carcinoma (ESCC),[43] and in malignant pleural effusion.[44,45] However, in the present study, there were no significant correlations between IL-17-producing cells and DFS. Although the discrepancy might be as a result of the limited sample of NSCLC patients, it has been reported that higher intratumoral IL-17A$^+$ cells were associated with better OS in patients with gastric cancer who received adjuvant chemotherapy,[46] and γδT17 cells could enhance the efficacy of anticancer chemotherapy,[42] indicating that IL-17-producing cells in NSCLC patients may contribute to cancer therapy, resulting in the prolonged OS. In our case, we found that patients who underwent surgery had better OS compared to patients who received chemotherapy (Figure S5A-C). Patients who underwent surgery with low γδT17 cells or high Tc17 cells had the best 5-year OS rate (Figure S5B,C). No significant differences in OS of high and low IL-17-producing cells (Th17, Tc17 and γδT17 cells) in patients who received chemotherapy (Figure S5A-C) were seen, suggesting that circulating IL-17-producing cells may not contribute to chemotherapy efficacy in NSCLC, but may be related to surgical treatment. However, further studies are needed to evaluate the association of IL-17-producing cells with different treatment regimens.

We also found that high IFN-γ$^+$CD8$^+$T cells and IFN-γ$^+$γδT were correlated with favorable OS, indicating that Tc1 and Tc17 cells both play an antitumor role in NSCLC. However, Th1 cells and Th17 cells did not show a consistent function in NSCLC. The presence of IL-17/IFN-γ double-positive T cells has been identified in many tumors, and this population showed strong cytotoxicity against tumor cells.[19,47] In the present study, we concurrently evaluated the distribution and the prognostic significance of IL-17/IFN-γ double-positive T cells in the same NSCLC patients. Our data showed that patients with high IL-17$^+$IFN-γ$^+$CD4$^+$T cells

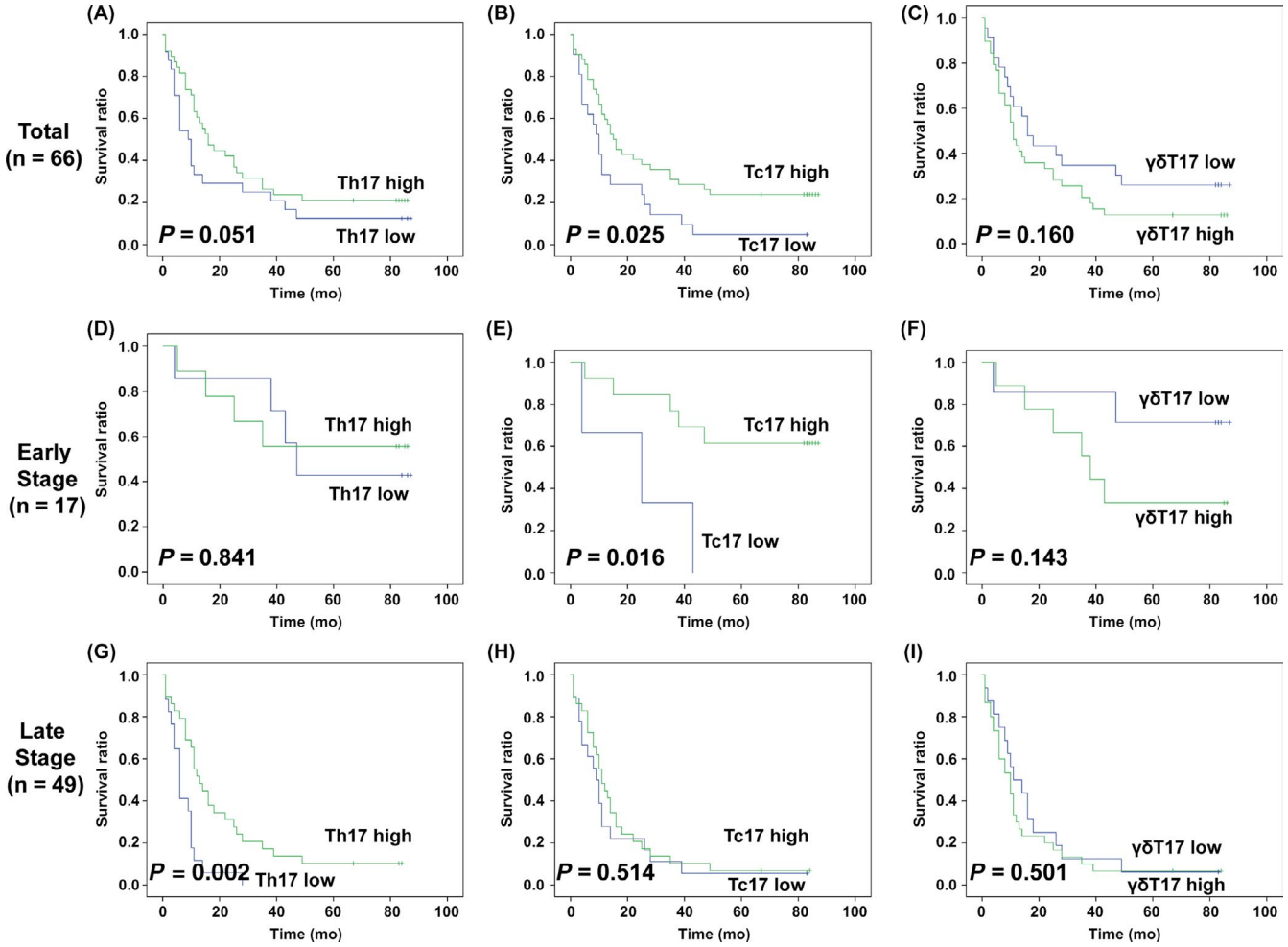

FIGURE 5 Kaplan-Meier analyses of the prognostic significance of interleukin (IL)-17-producing cells in non-small cell lung cancer (NSCLC) patients. Sixty-six NSCLC patients were divided into two groups based on their levels of IL-17-producing cells using cutoff values calculated by receiver operating characteristic curves (Th17: 1.15%; Tc17: 0.3%; γδT17: 2.33%). Five-year overall survival rates for NSCLC patients with a high and low level of (A) Th17, (B) Tc17, and (C) γδT17 cells are shown. (D-I) Five-year overall survival rates for NSCLC patients in stratified analyses by TNM stage. NSCLC patients with (D-F) early-stage (n = 17) or (G-I) late-stage (n = 49) cancer. Survival rates were determined using the Kaplan-Meier method and log-rank test. Vertical bars indicate censored cases

showed a slightly shorter OS (P = .097). This result suggests that IL-17/IFN-γ double-positive T cells may play a more multifaceted role in NSCLS. However, this needs further confirmation by other investigators. Further studies are needed to evaluate the role of peripheral IL-17-producing cells in tumor progress using a larger number of patient samples with long-term follow up of survival.

In conclusion, our study showed for the first time that the peripheral Th17 and Tc17 cells were associated with a favorable prognosis in patients with NSCLC. Percentages of Th17 and Tc17 cells in the peripheral blood could be significant predictors of a favorable prognosis for NSCLC patients.

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AUTHOR CONTRIBUTIONS

LHY and TM designed the study; TM provided patient samples and discussions; SL and MSB carried out the experiments; SL, CLP carried out the follow up. SL and MSB analyzed the data and wrote the manuscript. MLY helped with experiments and provided discussions. All authors have discussed and revised the manuscript.

DISCLOSURE

Authors declare no conflicts of interest for this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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