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The Simultaneous Increased Expressions of IL-37, Treg and Th2 Correlate with Poor Clinical Outcomes in Non-small Cell Lung Cancer Patients

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ABSTRACT

Interleukin-37 (IL-37) has been shown to regulate immune responses in various disease conditions. Furthermore, $CD4^+CD25^{hi}$ regulatory T cells (Tregs) and T helper 2 (Th2) cells, which inhibit anti-tumor immunity, are implicated in tumor development and progression. In this study, we aimed to identify whether the combination of serum IL-37 levels and peripheral blood T lymphocyte subsets can provide biomarkers to predict clinical outcomes in patients with non-small cell lung cancer (NSCLC).

We obtained longitudinal blood samples from NSCLC patients. Levels of IL-37 were determined by enzyme-linked immunosorbent assay (ELISA) and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Frequencies of peripheral Tregs and Th2 cells were detected by flow cytometry (FCM).

We found that serum IL-37 levels and frequencies of circulating Tregs and Th2 cells were significantly elevated in NSCLC patients compared to healthy controls. Elevated IL-37 levels were positively correlated with increased Tregs and Th2 counts. Although elevated levels of IL-37, Tregs, or Th2 cells individually failed to significantly predict overall survival (OS), their simultaneous elevation was strongly associated with poorer clinical outcomes.

To conclude, the combination of high serum IL-37 with increased circulating Treg and Th2 cells constitutes a novel "high-risk" immune signature that predicts poor clinical outcomes in NSCLC patients, outperforming any single marker alone.

Keywords: Helper T cell; Interleukin-37; Non-small cell lung cancer; Regulatory T cell

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INTRODUCTION

Lung cancer is one of the malignant tumors with the highest mortality rate. There is an urgent need to find new diagnostic and predictive biomarkers for early

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detection and assessment of treatment outcome.[1,2] In recent years, increasing evidence has confirmed that tumor formation and development are closely related to the immune system. Immune cells (especially T lymphocytes) and various cytokines play a key role in tumorigenesis and antitumor immune response.[3,4] Interleukin 37 (IL-37) is a recently identified member of the IL-1 superfamily and functions as an inhibitor of intrinsic inflammatory and immune responses. [5] The anti-inflammatory effects of IL-37 have been extensively studied over the past decades, but the biological role of IL-37 in tumor development is still poorly understood. Data from prior studies suggested the potential of IL-37 in novel therapeutic strategies.[6,7] Moreover, exogenous IL-37 was shown to induce apoptosis in human lung adenocarcinoma A549 cells, suggesting a direct inhibitory effect on non-small cell lung cancer (NSCLC) tumor cells. Elevated serum IL-37 levels have been associated with poor clinical outcomes in melanoma conversely.[2,8] While limited or inconsistent effects have been reported in breast cancer,[9] these findings suggesting context-dependent functions of IL-37 in malignancy.

Accumulating evidence indicates a relationship between IL-37 and T lymphocytes. In vitro studies have demonstrated that IL-37 directly promotes $CD4^+$ T-cell activation and proliferation, particularly within the regulatory T cell (Treg) subset.[10] Tregs represent a specialized subpopulation of T cells that suppress excessive or aberrant immune responses to maintain immune tolerance and homeostasis.[11] In the tumor microenvironment increased Tregs infiltration can inhibit antitumor immunity and facilitate tumor progression.[12] Furthermore, high Treg abundance has been associated with poor clinical outcomes in various malignancies.[13]

Moreover, Tregs can regulate helper T-cell (Th) responses, particularly T helper 2(Th2)differentiation, by modifying transcription factor expression.[14] In previous studies, Th1 cells have been shown to be involved in proinflammatory response, whereas Th2 cells secrete cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13, which suppress Th1-mediated immunity and promote anti-inflammatory humoral response and immunosuppression.[3] Previous studies have shown that Th2 associated cytokines predominate in the peripheral blood of patients with NSCLC and may contribute to tumor progression.[14]

While previous studies have examined IL-37, Tregs, or Th2 cells individually as potential prognostic markers in NSCLC, several critical knowledge gaps remain. First, IL-37 has been reported to exhibit both anti-tumor and pro-tumor effects depending on context, with its prognostic value in NSCLC remaining controversial.[6,15] Second, elevated Treg infiltration has been consistently associated with poor patient survival outcomes, but studies using peripheral blood Tregs have shown variable results.[16-19] Third, Th2 polarization is recognized as immunosuppressive in NSCLC, yet its independent prognostic utility is limited.[20] Importantly, no study has investigated whether the simultaneous elevation of all three markers creates a distinct “high-risk” immune signature with superior prognostic accuracy compared with any single marker alone. Our study addresses this gap by evaluating the combined IL-37/Treg/Th2 profile as a novel multi-parameter biomarker for NSCLC prognosis. Therefore, this study investigated the association between levels of serum IL-37 and peripheral blood $CD4^+CD25^{hi}$ Tregs and Th2 cells in patients with NSCLC. Furthermore, we identified that $IL\text{-}37^{hi}/CD4^+$ $CD25^{hi}$ $Tregs^{hi}/Th2^{hi}$ as a novel prognostic immune checkpoint in lung cancer patients.

MATERIALS AND METHODS

Patients and Study Design

The study protocol was approved by the Ethics Review Board of Qingdao Central Hospital. Between January 2022 and November 2024, 60 patients with NSCLC and 33 age-matched healthy controls were recruited. All participants provided written informed consent. There was no significant age difference between groups. Tumor staging followed the Union for International Cancer Control (UICC) pathological Tumor, Node, and Metastasis (pTNM) classification. None of the patients had received anticancer therapy.

Patients were eligible for enrolment if they were confirmed to have NSCLC with no prior anticancer treatment and an absence of other pulmonary diseases (e.g., bronchiectasis or asthma). Exclusion criteria consisted of congestive heart failure; severe pulmonary infection or sepsis; and major cardiac, renal, or central nervous system dysfunction. Additionally, patients were excluded if they presented with autoimmune diseases, required long-term immunomodulatory therapy, or utilized steroids as antiemetics.

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Table 1. Demographic and clinical characteristics of the subjects

Characteristics	NSCLC	HCs
Median age (range, years)	65(38-84)	62(34-74)
Gender, *n* (%)		
Male	40 (66.7)	21(63.6)
Female	20 (33.3)	12(36.4)
Histology, *n* (%)		
Adenocarcinoma	36 (60.0)	NA
Squamous cell carcinoma	19 (31.7)	NA
Others	5 (8.3)	NA
Stage at diagnosis, *n* (%)		
I	8(13.3)	NA
II	13(21.7)	NA
III	12 (20.0)	NA
IV	27(45.0)	NA

NA: not available; HCs: healthy controls; NSCLC: non-small cell lung cancer

Blood Experiments

Peripheral blood (10 mL) was collected before treatment. Anti-coagulated whole blood (2.5 mL) was analyzed by flow cytometry within 4 hours. Plasma was stored at –80°C for enzyme-linked immunosorbent assay (ELISA). Plasma IL-37 and IL-4 concentrations were measured using a commercial ELISA kit (Abcam, Cambridge, UK). All assays were performed in triplicate. The intra-assay coefficient of variation (CV) for serum IL-37 and IL-4 concentrations measured by ELISA were≤2.10%.

peripheral blood mononuclear cells (PBMCs) were isolated after lysis of red blood cells, samples were stained using the following antibodies from Beckman Coulter (FL, US): CD3 (UCHT1), CD4 (13B8.2), CD8 (clone SFCI21Thy2D3), CD196 (B68132), CD25(B1.49.9) and CD183 (1C6/CXCR3) from BD Bioscience (NJ, USA). The $CD3^+$T cell subsets included $CD3^+CD4^+$ helper T (Th) subsets and $CD3^+CD8^+$ suppressor T (Ts) subsets; the $CD4^+$ Th cell subsets included $CD196^-$ $CD183^+$ Th1 subsets, $CD196^-CD183^-$ Th2 subsets, and $CD196^+$ $CD183^-$ Th17 subsets. Surface staining was further prepared as described previously. Flow-count fluorospheres kit (Beckman Coulter, FL, USA) was used for the absolute count of lymphocyte subsets. The cells were analyzed on FC500 flow cytometry. Data were analyzed using Flow Jo software (Tree Star).

RNA was extracted from PBMCs (Qiagen RNeasy Mini Kit，DE) and reverse-transcribed using MMLV reverse transcriptase (Promega，US). Quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems, CA, US) on an ABI 7500 Fast system.

For relative quantification of *IL37* mRNA expression, *GAPDH* was used as the endogenous reference gene to normalize for variations in RNA input. The relative expression levels were calculated using the comparative $2^{-\Delta\Delta Ct}$ method. To ensure data reliability, all qRT-PCR reactions were performed in triplicate, yielding an intra-assay coefficient of variation (CV) of≤5.08%. Primer sequences:

IL37

F: 5′ -GCA TTC ATG ACC AGG ATC AC-3′ ;

R: 5′ -CAA AGA AGA TCT CTG GGC GTA-3′

GAPDH

F: 5′ -TGC ACC ACC AAC TGC TTA GC-3′ ;

R: 5′-GGC ATG GAC TGT GGT CAT GAG-3′

Statistical Analysis

Data are presented as mean ± SD and analyzed using GraphPad Prism 9.0. Student's *t*-test was used for two-group comparisons and one-way ANOVA for multi-group analyses. Overall survival (OS) was defined as the interval from diagnosis to disease-related death or last follow-up. Correlations were assessed using Pearson's method. A *p* value of less than 0.05 was considered statistically significant.

ROC curves were generated using GraphPad Prism statistical software to evaluate the diagnostic efficacy of each marker for NSCLC. The AUC and its 95%

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confidence interval were calculated. The optimal diagnostic cut-off value was determined by the maximum Youden index.

Data were analyzed using R (v4.5.2) and RStudio (v2025.09.2). Univariate and multivariate Cox regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for disease progression. Forest plots were generated using the forestplot and broom packages. All tests were two-sided, with statistical significance defined as $p<0.05$.

The analyzed variables included IL-37/Treg/Th2 levels (IL-37>118.3 pg/mL, Treg >102.5 cells/μL, and Th2 >312.0 cells/mL vs. all other patients), NSCLC stage (stage III–IV vs Stage I–II), age (>60 years vs.≤60 years), and histology (non-adenocarcinoma vs. adenocarcinoma). The vertical reference line at HR=1.0 indicates no association with disease progression.

RESULTS

The clinical characteristics of the subjects are presented in Table 1. No significant differences in age or sex distribution were observed between patients with NSCLC and healthy controls. Among patients with NSCLC, adenocarcinoma was the most common histological subtype, followed by squamous cell carcinoma.

IL-37 levels, assessed by ELISA in serum and qRT-PCR, were significantly higher in patients with NSCLC than in healthy controls (Figure 1A-B). Consistent with this finding, the frequencies of circulating Tregs, Th2 cells, and $CD8^+$ T cells were also significantly increased in the NSCLC group. In contrast, the frequencies of $CD3^+$, $CD4^+$, and Th1 cells were significantly reduced. No significant difference was observed in Th17 cell frequencies between the two groups (Figure 1C-E). Furthermore, the levels of these immune parameters demonstrated a significant positive correlation with disease stage. Compared to healthy controls, patients with early-stage disease (stage I/II) already exhibited significantly elevated levels of serum IL-37, as well as higher frequencies of circulating $CD4^+CD25^{hi}$ regulatory T cells (Tregs) and $CD4^+CD196^-CD183^-$ Th2 cells. Moreover, patients with advanced-stage disease (stage III/IV) showed even more pronounced elevations, with levels of serum IL-37, Tregs, and Th2 cells being significantly higher than those in both early-stage patients (stage I/II) and healthy controls (Fig. 1F, G). No significant associations were observed between these immune parameters and other clinical variables, including age, sex, histological subtype, or serum carcinoembryonic antigen (CEA) levels (Table 2).

We performed receiver operating characteristic (ROC) analysis to assess the diagnostic performance of serum IL-37 levels, Treg cell counts, and Th2 cell counts as screening markers for NSCLC. As shown in Fig. 1H, all three indicators effectively distinguished NSCLC patients from healthy controls, with area under the curve (AUC) values of 0.963, 0.906, and 0.851, respectively; cut-off values of 84.89 pg/mL, 55.50 cells/μL, and 357.00 cells/μL, respectively; 95% confidence intervals (CI) of 0.9280–0.9972, 0.8372–0.9749, and 0.7703–0.9319, respectively; sensitivities of 93.94%, 90.91%, and 84.85%, respectively; and specificities of 90.00%, 83.33%, and 74.47%, respectively (all $p<0.01$).

Furthermore, these three immune parameters also accurately discriminated early-stage (stage I/II) from advanced-stage (stage III/IV) NSCLC patients (Fig. 1I), with AUC values of 0.913, 0.713, and 0.740, respectively; cut-off values of 118.3 pg/mL, 102.5 cells/μL, and 312.0 cells/μL, respectively; 95% CIs of 0.8409–0.9858, 0.5769–0.8480, and 0.5836–0.8963, respectively; sensitivities of 84.62%, 64.10%, and 96.77%, respectively; and specificities of 95.24%, 76.19%, and 50%, respectively (all $p<0.01$).

Collectively, these findings demonstrate that serum IL-37 levels, Treg cell counts, and Th2 cell counts each exhibit considerable diagnostic value for NSCLC.

Correlation analysis demonstrated that serum IL-37 levels were positively correlated with the absolute counts of circulating Tregs and Th2 cells (Figure 2E, G), but not with $CD3^+$, $CD4^+$, $CD8^+$T cells, Th1, or Th17 cells in patients with NSCLC (Figure 2A-D, F). Notably, serum IL-37 levels showed stronger correlations with absolute counts of Tregs and Th2 cells compared to percentage, suggesting that absolute count of T cells-based correlation analysis is potentially more suitable for predicting patients' clinical outcomes than percentage-based analysis. Similar results were observed between IL-4 levels and Th2 cells (Figure 2H).

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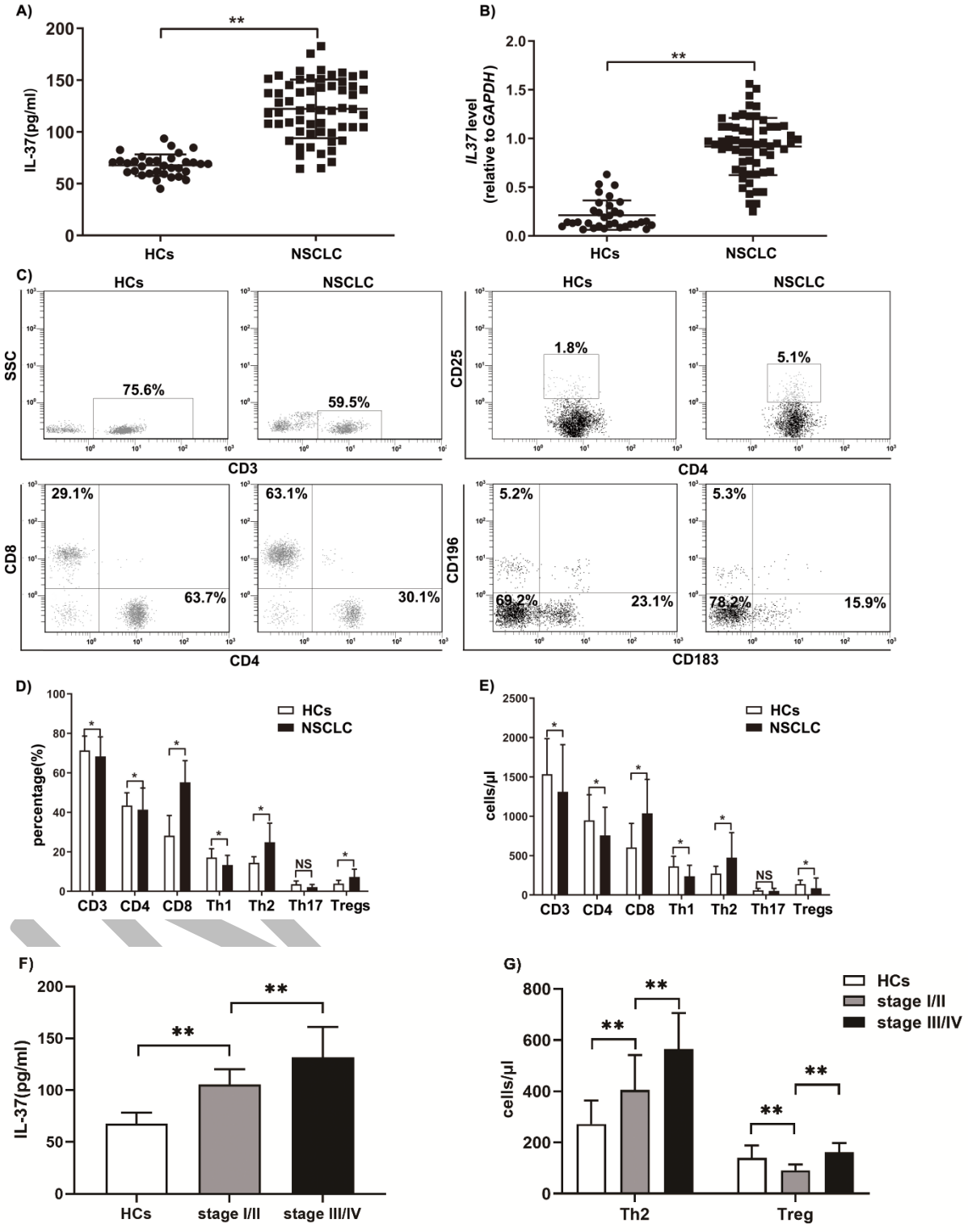

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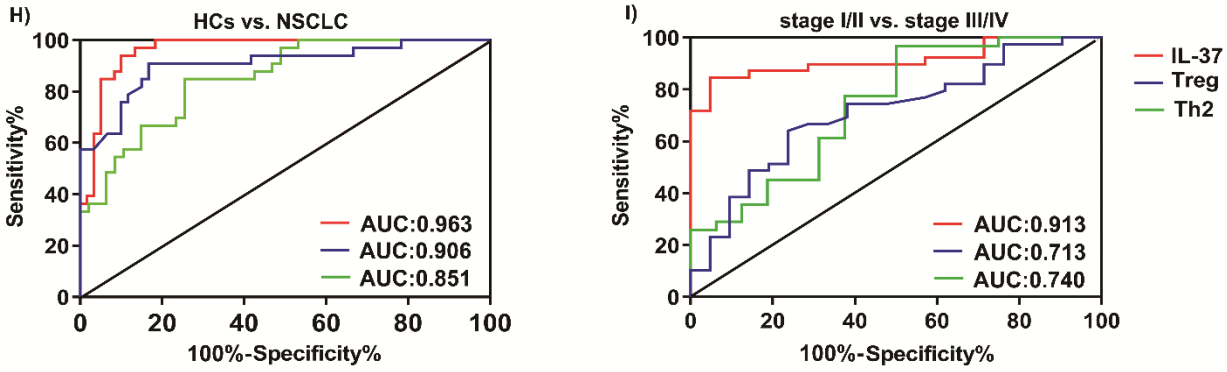

Figure. 1 Levels serum interleukin-37 (IL-37) and T lymphocyte subsets in non-small cell lung cancer (NSCLC) patients and healthy controls (HCs). (A) Serum IL-37 levels in NSCLC patients and HCs. (B) Expression levels of *IL37* mRNA in NSCLC patients and HCs. *IL37* gene expression was determined based on the relative levels to *GAPDH* mRNA. Data are expressed as mean ± SD. (C) Representative flow cytometry results. The $CD3^+$ T cell subsets included $CD3^+CD4^+$ helper T (Th) subsets and $CD3^+CD8^+$ suppressor T (Ts) subsets, The $CD4^+$ Th cell subsets included $CD196^-CD183^+$ Th1 subsets, $CD196^-CD183^-$ Th2 subsets, and $CD196^+CD183^-$ Th17 subsets. (D) Total lymphocytes of T cell subsets (%); (E) Absolute count of T cell subset (cells/μL). Data are expressed as mean±SD. (F) Serum IL-37 levels in healthy controls, patients of stage I/II/ and III/IV. (G) Frequencies of $CD4^+CD25^{hi}$ regulatory T cells (Tregs) and Th2 cells in healthy controls, patients of stage I/II and III/IV. Each dot represents one case. (H) The ROC curves of serum IL-37 levels, frequencies of Tregs and Th2 cells demonstrated effective discrimination between NSCLC patients and healthy controls. (I) The ROC curves of serum IL-37 levels, frequencies of Tregs and Th2 cells demonstrated effective discrimination between NSCLC patients of stage I/II and III/IV. Data are expressed as mean±SD. **p<0.01 (NSCLC patients, n=60; HCs, n=33; stage I/II, n=21; stage III/IV, n=39).

Table. 2 Levels of IL-37, Tregs and Th2 cells in NSCLC patients with different demographic and clinical characteristics

characteristics	n (%)	IL-37 (pg/mL)	*p*	Treg cells (cells/μL)	*p*	Th2 cells (cells/μL)	*p*
Age (years)							
>60	41 (68.3)	129.21±32.28	0.1223	152±58	0.1219	530±100	0.5612
<60	19 (31.7)	116.95±15.54		128±48		570±78	
Sex, *n* (%)							
male	40 (66.7)	131.19±43.16	0.1443	133±60	0.2058	490±92	0.4149
female	20 (33.3)	114.97±29.76		146±46		470±86	
Histology, *n* (%)							
Adenocarcinoma	36 (60.0)	125.62±38.47		141±55		480±93	
Squamous cell carcinoma	19 (31.7)	121.41±25.93	0.1079	155±64	0.5470	410±96	0.1562
others	5 (8.3)	118.19±16.83		124±43		450±88	
Serum CEA level (IU/ml)							
>4.11	33(55)	135.90±33.43	0.4742	131±53	0.3587	510±82	0.0613
<4.11	27(45)	128.87±42.18		143±46		470±79	

NSCLC: non-small cell lung cancer; CEA: carcinoembryonic antigen; IL-37: interleukin-37; Treg: regulatory T cell; Th2: T helper 2

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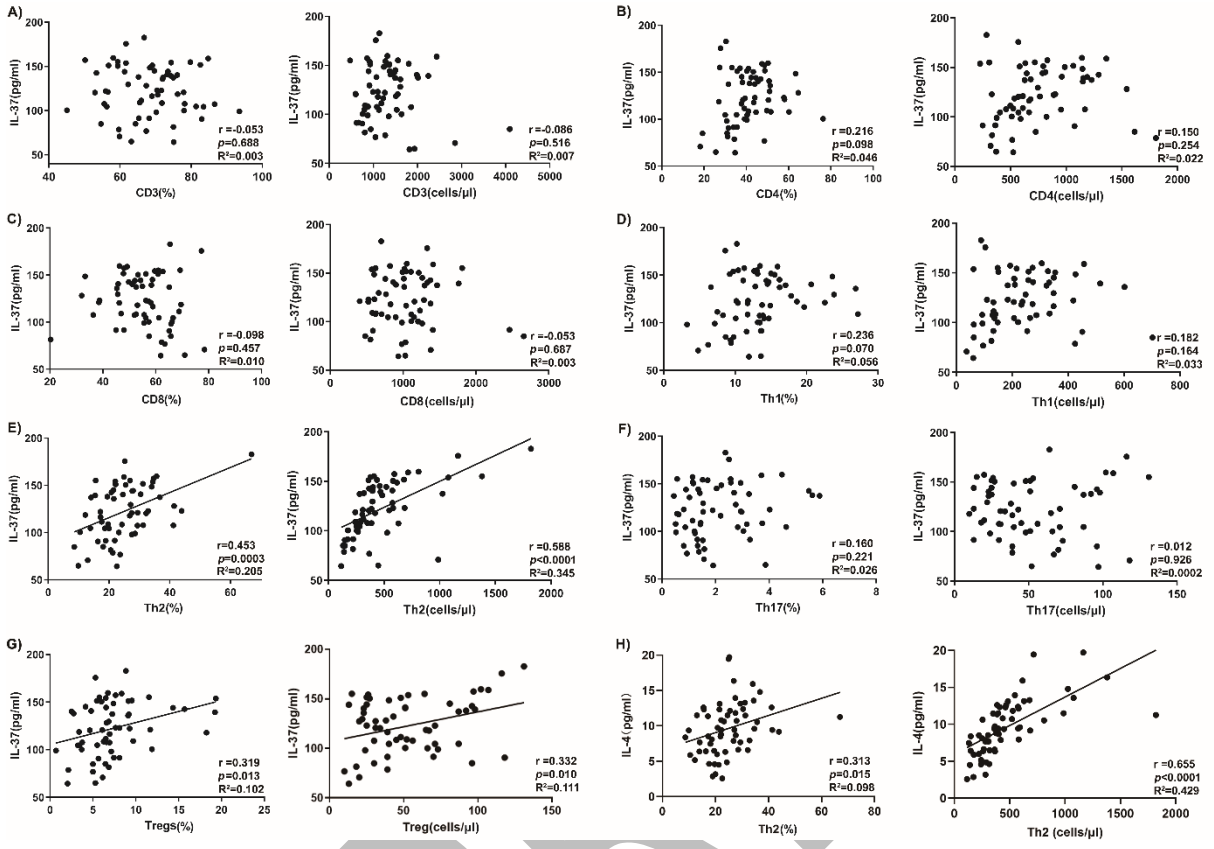

Figure 2. The correlation of interleukin-37 (IL-37) and T cell subsets in non-small cell lung cancer (NSCLC) patients (*n*=60). (A) Correlation between IL-37 and CD3$^+$ T cells. (B) Correlation between IL-37 and CD4$^+$ T cells. (C) Correlation between IL-37 and CD8$^+$ T cells. (D) Correlation between IL-37 and Th1 cells. (E) Correlation between IL-37 and T helper 2 (Th2) cells. (F) Correlation between IL-37 and T helper 17 (Th17) cells. (G) Correlation between IL-37 and regulatory T cells (Tregs). (H) Correlation between IL-4 and Th2 cells. Correlations were analyzed by Pearson or Spearman rank correlation test. Each data point represents an individual subject.

Based on the cutoff values of serum IL-37, Tregs and Th2 cells provided by the ROC curve in the above context, corresponding 118.3 pg/mL, 102.5 cells /μL and 312 cells /μL were determined as cut-off values to divide patients into groups with high (n=22) and low (n=17) levels. The median follow-up time was 18 months (range, 1-36 months). For the overall survival (OS) rates, there was no significant difference between patients with higher level of IL-37 and those with lower level (Figure 3A). Similar results were observed in Tregs or Th2 cells groups as well as the combination of IL-37 and Tregs or Th2 cells groups (Figure 3B-E). However, Log-rank test showed that patients with the combination of high-level IL-37 and increased Treg and Th2 cells had significantly poorer overall survival (Figure 3F). In the univariate Cox regression analysis, elevated IL-37/Treg/Th2 levels were significantly associated with an increased risk of disease progression (HR=1.79, 95% CI: 1.04–3.08, *p*=0.035). However, after adjustment for clinical stage, age, and histology in the multivariate model, this association was attenuated and no longer statistically significant (HR=1.68, 95% CI: 0.85–3.34, *p*=0.135). Other clinicopathological variables, including NSCLC stage, age, and histology, did not demonstrate statistically significant associations with disease progression in either univariate or multivariate analyses (Figure 3G).These findings suggest that high IL-37/Treg/Th2 levels may be associated with disease progression at the univariate level; however, the loss of statistical significance in the multivariate model indicates that this effect may be influenced by potential confounding factors or limited sample size.

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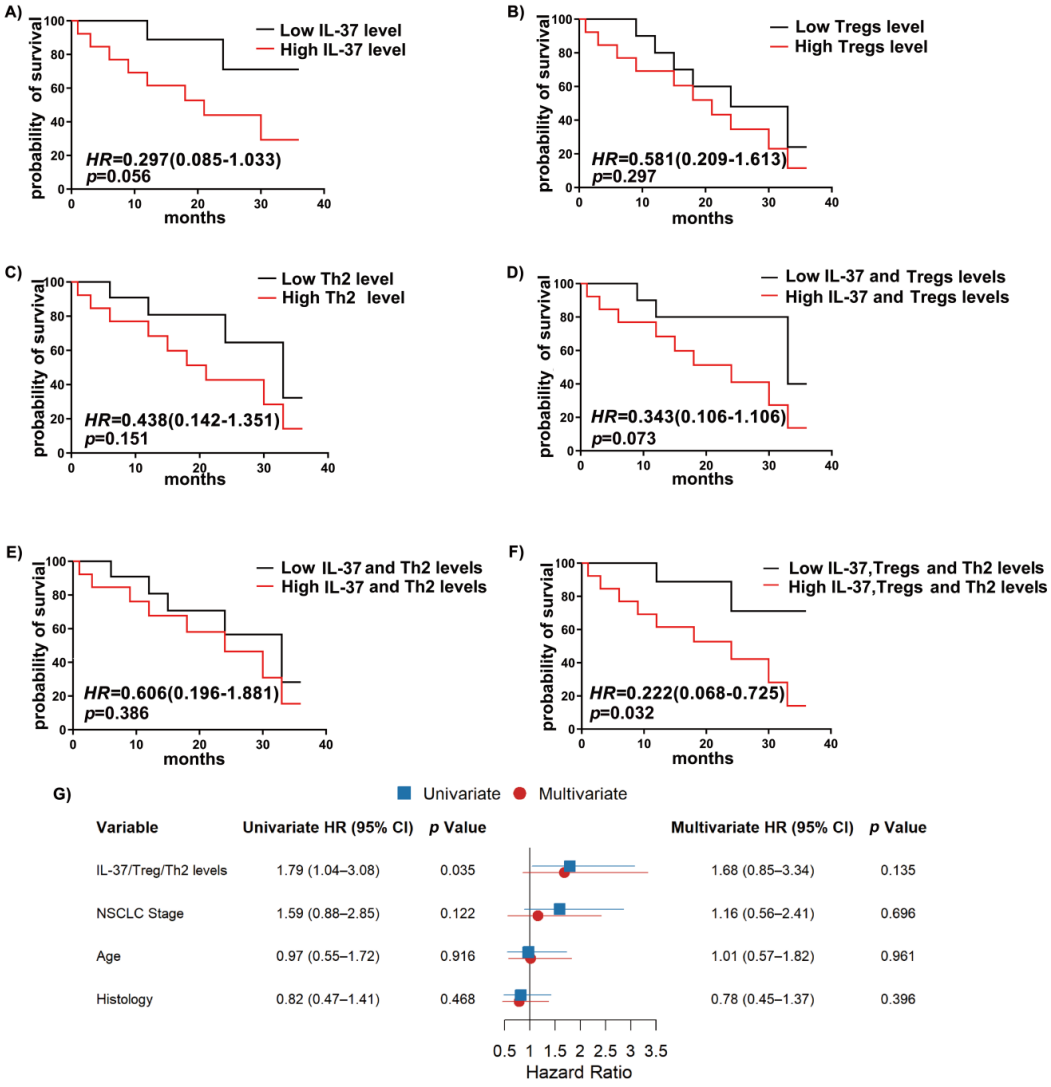

Figure 3. Kaplan Meier survival curves for the correlation between serum interleukin (IL)-37 levels, frequencies of $CD4^{+}CD25^{hi}$ Tregs and helper T (Th)2 cells and overall survival. (A) Overall survival of non-small cell lung cancer (NSCLC) patients with high and low IL-37 serum levels. (B) Overall survival of NSCLC patients with high and low frequencies of $CD4^{+}CD25^{hi}$ regulatory T cells (Tregs). (C) Overall survival of NSCLC patients with high and low frequencies of Th2 cells. (D) Overall survival of NSCLC patients with high and low combination of IL-37 and Tregs. (E) Overall survival of NSCLC patients with high and low combination of IL-37 and Th2 cells. (F) Overall survival of NSCLC patients with high and low combination of IL-37, Tregs and Th2 cells. A value of $p<0.05$ was considered to be statistically significant. (G) Forest plot of univariate and multivariate Cox regression analyses for disease progression in NSCLC patients. The forest plot displays HR and 95% CI derived from univariate (blue squares) and multivariate (red circles) Cox proportional hazards models. The analyzed variables include IL-37/Treg/Th2 levels (IL-37>118.3 pg/mL, Treg>102.5 cells/μL, and Th2>312.0 cells/μL vs. all other patients), NSCLC stage (Stage III–IV vs. Stage I–II), age (>60 years vs.≤60 years), and histology (non-adenocarcinoma vs. adenocarcinoma). The vertical reference line at HR=1.0 indicates no association with disease progression.

DISCUSSION

In this study, we demonstrated that patients with NSCLC exhibited significantly elevated serum IL-37 levels and increased frequencies of circulating Tregs and Th2 cells compared with healthy controls. Importantly, the concurrent elevation of these immunosuppressive factors was associated with

advanced disease stage and poorer overall survival.

While the individual roles of IL-37, regulatory T cells (Tregs), and Th2 cells in non-small cell lung cancer (NSCLC) have been documented, their combined prognostic utility remains unexplored. A key finding of our study is that the prognostic significance of IL-37 appears to be context-dependent on the concurrent status of adaptive immune cells. In our cohort, individual elevations of IL-37, Tregs, or Th2 cells did not consistently predict survival outcomes with high precision. However, patients exhibiting simultaneous high levels of all three markers formed a distinct 'high-risk' group with significantly reduced overall survival. This synergistic effect suggests that IL-37 drives tumor progression primarily drives tumor progression when it coincides with a broader immunosuppressive landscape (i.e., Treg/Th2 enrichment)—a nuance that single-marker analyses fail to capture.

Furthermore, the apparent conflict between our findings (high IL-37 associated with poor prognosis) and previous reports of IL-37 as a tumor suppressor can be reconciled through a stage-dependent conceptual framework. In early tumorigenesis, IL-37 likely functions as a tumor suppressor by inhibiting chronic inflammation. However, in advanced NSCLC—the population investigated in our study—the context shifts. Here, the tumor appears to exploit IL-37's potent anti-inflammatory capacity to evade immune surveillance. By suppressing cytotoxic Th1/Th17 responses and fostering an environment rich in Tregs and Th2 cells, IL-37 facilitates an adaptive immunosuppressive niche that supports tumor progression.

To explain the observed correlations, we propose a biological mechanism where IL-37 acts as an upstream regulator of the tolerogenic microenvironment. First, IL-37 signaling through its receptor SIGIRR inhibits innate inflammatory drivers (e.g., IL-1β, TNF-α), potentially inducing tolerogenic dendritic cells that secrete TGF-β, a potent driver of Treg expansion. Second, by suppressing Th1 and Th17 responses, IL-37 alters the cytokine milieu to favor Th2 polarization. This dual action—dampening effective anti-tumor immunity while promoting regulatory and humoral-shifted populations—creates a self-reinforcing cycle of immune escape. This framework aligns with our findings that high IL-37 levels coincide with elevated Treg and Th2 counts in patients with poor prognosis.

In recent years, tumor immunology has become a new hotspot in tumor biology research, revealing that immune cells and cytokines play important roles in tumor immunity.

Accumulating evidence also suggests that IL-37 acts as a natural immunosuppressant and is associated with tumor immunity.[21] In addition, T cells play an important role in tumorigenesis. However, only limited studies have explored the correlation between serum IL-37 levels and T cell subsets, and the feasibility of using IL-37 levels and specific T lymphocyte populations as biomarkers for human lung cancer.

Here, our data show that NSCLC patients have higher levels of serum IL-37, Tregs and Th2 cells than healthy controls. We also found that the serum levels of IL-37, Tregs, and Th2 cells were higher in stage III-IV patients than in stage I-II patients. IL-37 is expressed by regulatory T cells and is associated with their immunosuppressive phenotype. Tregs are known to suppress effective antitumor immune responses and can influence helper T-cell polarization, which is often associated with a Th2-dominated immunity. Th2-associated cytokines further inhibit Th1-mediated cytotoxic responses, thereby facilitating immune escape.

Huo et al. found that IL-37 levels in patients with EOC were higher than in healthy controls, and high serum IL-37 levels were significantly associated with FIGO stage.[2] In addition, high IL-37 mRNA expression in specific lymphocyte populations may play a potential immunosuppressive role in melanoma tumorigenesis.[22] In this study, serum IL-37 levels were positively correlated with Tregs/Th2 cells in peripheral blood. IL-37 can be expressed by human Tregs.[23] Tregs are characterized by their immunosuppressive activity, which involves modulating CD4+ T cell differentiation and suppressing $CD4^+$ T cells.[24] The positive correlation observed in our study is consistent with reports that IL-37 may be linked to a Treg-mediated shift towards a Th2 profile. Activated Tregs can mediate the balance between Th1 and Th2. IL-37 can also induce cell drift towards Th2 by enhancing the effect of Tregs.[25] It is well known that Tregs play an important role in suppressing T cell responses and cytotoxic lymphocyte effects, and Tregs have been confirmed to be essential in tumor persistence/progression.[26] Here, our data show an association where elevated IL-37 levels co-occur with an increased in immunosuppression mediated by Tregs and Th2 cells.

In our study, NSCLC patients with higher levels of serum IL-37, Tregs and Th2 cells had poorer OS. In most tumors, the elevation of Treg and Th2 cells was

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associated with poor clinical outcomes. It is worth noting that in our study, elevated IL-37 levels are correlated with an immunosuppressive state that may be less conducive to antitumor immune responses. Similar to our results, Huo et al. reported that high serum IL-37 level was a poor predictive biomarker for overall survival and shorter progression-free survival in EOC patients.[2] However, contrary to our findings, HCC patients with high IL-37 protein levels had better OS and DFS.[27] Moreover, several studies show IL-37 enhanced antitumor effects by inhibiting multiple signaling pathways.[27-29] Different cancer types, complex tumor microenvironments, and infiltrating immune cells within tumors may be an important reason for this difference. IL-37 has intracellular and extracellular functions in the tumor microenvironment. Notably, IL-37 has also been defined as a specific cytokine that can be induced by many other cytokines or produced by a variety of cells, such as Treg.[10,30] And tumor cells can exploit these immunosuppressive cytokines and cells, creating a highly suppressive environment to evade the immune system. Furthermore, these differences may be due to samples from different resources. In our study, we chose blood samples, and most of other studies used tumor tissue and other samples in vitro or in vivo. The association between higher serum levels of IL-37, Tregs/Th2 and poorer OS is consistent with the established role of these factors in promoting an immunosuppressive environment, which may facilitate immune escape in NSCLC and warrants further investigation and clarification in future studies in future studies.

Notably, our analysis revealed no significant correlation between the IL-37/Treg/Th2 immune profile and serum CEA levels, a conventional tumor marker. This dissociation implies that while CEA primarily reflects tumor mass and proliferation, the simultaneous elevation of IL-37, Tregs, and Th2 cells captures distinct aspects of the host's immunosuppressive landscape. Consequently, this immune signature may offer complementary prognostic value to traditional biomarkers. Integrating both tumor-derived markers (like CEA) and host immune parameters could potentially improve the risk stratification accuracy.

Furthermore, our findings have important implications for immunotherapy responsiveness. The simultaneous elevation of IL-37, Tregs, and Th2 cells defines a profoundly immunosuppressive microenvironment that may confer primary resistance to immune checkpoint inhibitors (ICIs). Specifically, high Treg infiltration is known to suppress CD8+ cytotoxic T-cell activity, while Th2 polarization diverts the immune response away from the tumor-destructive Th1 pathway. IL-37 may further reinforce this tolerogenic state. Consequently, patients harboring this high-risk immune signature might exhibit suboptimal responses to PD-1/PD-L1 monotherapy. We propose that pre-treatment assessment of this profile could identify candidates for combination regimens, such as ICIs plus Treg-depleting agents or Th2-pathway inhibitors.

From a practical standpoint, integrating the IL-37/Treg/Th2 profile into routine clinical workflows is feasible given that serum ELISA and standard flow cytometry are widely available. This signature could serve as a non-invasive decision-support tool at diagnosis in three key ways: First, it enables risk stratification by identifying “high-risk” patients who may benefit from more intensive surveillance. Second, it informs therapeutic selection; since this profile indicates a strongly immunosuppressive microenvironment, such patients might be prioritized for combination regimens. Third, it supports risk-adapted management, ensuring that early-stage patients harboring this high-risk signature receive adequate adjuvant treatment.

Several limitations should be acknowledged. This study was conducted at a single center with a relatively limited sample size. Second, the median follow-up of 18 months is relatively short for a comprehensive assessment of long-term overall survival in NSCLC. While this duration proved adequate to detect significant early mortality differences in our cohort, the prognostic utility of the signature over extended periods warrants validation in future studies with longer follow-up. Third, a significant limitation is the reliance on peripheral blood immune parameters rather than direct analysis of tumor-infiltrating lymphocytes (TILs). Additionally, we acknowledge that intracellular FoxP3 staining is the gold standard for Treg identification. However, we defined circulating Tregs as $CD4^{+}CD25^{hi}$ based on its established validity as a surrogate marker in peripheral blood, with strong concordance with $FoxP3^{+}$ populations.[31-33] Additionally, surface marker staining is more feasible for routine clinical use than intracellular FoxP3 staining, aligning with our goal of developing a translatable biomarker profile. Future studies incorporating FoxP3 staining are warranted to further validate our findings. The tumor microenvironment (TME) represents a distinct immunological compartment, and circulating measures

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may not perfectly mirror the local immune landscape. A primary limitation of this study is the reliance on peripheral blood immune parameters rather than direct analysis of tumor-infiltrating lymphocytes. While circulating levels may not fully mirror the complex spatial architecture of the tumor microenvironment, they provide a crucial assessment of the systemic immune status. In advanced NSCLC, tumor-induced immune suppression frequently extends beyond the local site, making peripheral markers valuable indicators of the host's overall immune competence and metastatic potential. Our data showing significant correlations between these peripheral markers and disease stage support their clinical relevance. Nevertheless, we acknowledge that peripheral counts do not directly measure intratumoral functional activity, and future studies utilizing paired tissue and blood samples are essential to confirm these findings at the tumor site.

In conclusion, our study demonstrates a significant correlation between elevated serum IL-37 levels and increased circulating Treg and Th2 cells, which together are associated with poor clinical outcomes in NSCLC patients. The simultaneous elevation of these three markers forms a novel prognostic immune signature. More importantly, this profile may offer complementary value to traditional biomarkers and could inform risk stratification and therapeutic decisions. However, due to conflicting evidence in the literature and the limitations of this study, more research involving larger patient cohorts and paired tissue analyses is needed to confirm the role of the IL-37/Treg/Th2 axis in NSCLC.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee Medical College of Qingdao University (Ethical Code Number: IEC-AF-081-03.1).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

Due to privacy concerns, the data are not publicly available but can be obtained from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

No AI assistance involved.

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