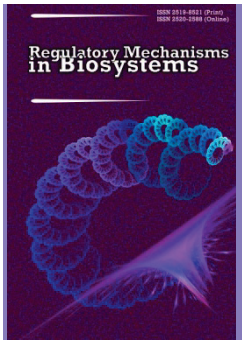

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Prognostic role of STAT6 in patients with non-small cell lung cancer

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Lung cancer is the leading cause of death from cancer in Ukraine and worldwide. The impact on the tumor microenvironment is the most promising direction for lung cancer therapy. Tumor-associated macrophages of type M2 have the most powerful immunosuppressive properties. A new potential way of influencing M2 macrophages is the signaling protein and activator of transcription 6 (STAT6). The aim of our study was to evaluate the role of STAT6 in the formation of the immunosuppressive microenvironment and the prognosis of patients with radically treated non-small cell lung cancer (NSCLC). We performed an immunohistochemical examination of the tumor tissue of 42 NSCLC patients with antibodies to CD8+, forkhead box P3 (FOXP3+), CD163+, and STAT6. The impact on survival was assessed by Cox regression analysis. The median follow-up period for the studied cohort was 57.9 ± 4.2 months. 50.0% of patients with NSCLC have high expression of STAT6. We established that STAT6 correlates with the histology of NSCLC and the gender of patients. High expression of STAT6 is significantly more determined in squamous cell carcinomas than in adenocarcinomas. In patients with squamous cell carcinomas, metastasis to regional lymph nodes is associated with an immune exclusion phenotype and is mediated by a high infiltration of tumor stroma M2 macrophages. An inflammatory immune phenotype with low CD163+, FOXP3+, and STAT6 expression is most typical for adenocarcinoma. Low STAT6 and an inflammatory immune phenotype are more common in women; high STAT6 and an immune exclusion phenotype in men. Females and patients with high CD8+ expression in tumor clusters, low CD163+ in tumor stroma, and low STAT6 expression have better overall survival. Conclusions: STAT6 overexpression is associated with immunosuppressive microenvironment and has a negative impact on recurrence-free survival and overall survival in NSCLC patients. To obtain more accurate results, it is necessary to conduct a study that includes a larger cohort of patients, in particular, female patients.

Keywords: lung cancer; survival; gender; immune phenotype; FOXP3; CD163; CD8.

Introduction

Lung cancer is the leading cause of death from cancer in Ukraine and worldwide (Leiter et al., 2023). Surgery, chemotherapy, radiation therapy, personalized immunotherapy, and targeted therapy have prolonged overall survival in patients with non-small cell lung cancer (NSCLC). Despite improved clinical outcomes, some patients cannot achieve long-term disease control. Therefore, finding new biomarkers for targeted therapy is a primary goal (Deshpand et al., 2022). Approaches focusing on the tumor microenvironment and overcoming immunological resistance are the most promising directions in clinical oncology.

Among the tumor microenvironment cells, tumor-associated macrophages M2 type have the most potent immunosuppressive properties. These cells stimulate angiogenesis, tumor growth, and spreading to other organs. M2 macrophages can produce inhibitory cytokines (TGFβ1 and IL10), stimulate angiogenesis through VEGF production, and suppress the antitumor properties of other immune cells (Xu et. al., 2021). Therefore, the inhibition of M2 macrophages is the main point of attention of many scientists (Cortese et al., 2019; Miyauchi et al., 2023).

A new potential way of impacting M2 macrophages is the signaling protein and activator of transcription 6 (STAT6). STAT6 is a protein belonging to the STAT family of transcription factors. The negative impact on patient survival is related to the transmission of IL-4/STAT6 signals and increased M2 macrophages (Fu et al., 2019). STAT6 is expressed in many tumors. STAT6 overexpression is often associated with radioresistance and immunosuppression (Rahal et al., 2018). Pastuszak-Lewandoska et al. (2017) established that STAT6 overexpression is observed in 54.0% of patients with NSCLC. The expression level is higher in squamous cell lung carcinomas than in adenocarcinomas. Significantly, the activation of transcription factor STAT6 in macrophages is associated with polarization towards the M2-type. M2 macrophages are closely interacted with other cells of the tumor microenvironment. Effector T cells (CD8+) and regulatory T cells (T_{reg}), along with M2 macrophages, contribute to the immune landscape, affecting NSCLC patient survival (Backman et al., 2023).

Recent advances in scientific research have led to a deeper understanding of the intricate structure, function, and interaction of transcription factors with cofactors and other proteins (Ma et al., 2020; Huang et al., 2021). Furthermore, significant progress has been made in elucidating the precise binding mode of these transcription factors to DNA. These breakthroughs have paved the way for novel medical treatments targeting transcription factors, offering promising avenues for more effective and tar-

geted therapeutic interventions in various cancer types (Chai et al., 2022; Shu et al., 2024).

In recent years, inhibiting STAT proteins has garnered significant attention as a promising strategy in cancer research and therapeutics (Faida et al., 2023). This focus has intensified following the discovery of oncogenes and their association with cancer progression. Multiple studies have provided compelling evidence that the overexpression of STAT proteins not only promotes carcinogenesis but also significantly influences the prognosis of cancer patients (Nie et al., 2023). The intricate signaling pathways involving STAT proteins are pivotal in tumor progression, rendering them attractive targets for drug development and innovative cancer therapy approaches (Chen et al., 2022).

The aim of our study was to evaluate the role of STAT6 in formating an immunosuppressive microenvironment and determining the prognosis of patients with radically treated NSCLC.

Materials and methods

Study design and bioethics approval. The research was carried out in compliance with the principles of medical ethics and the protection of patients' rights, human dignity and moral and ethical norms, in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, the laws of Ukraine. The study was approved by the Local Ethics Committee of the Sumy Regional Clinical Oncology Center (protocol No. 21, dated December 25, 2023). All participants of this study signed informed consent forms.

Forty-two Sumy Regional Clinical Oncology Center patients who received radical treatment for NSCLC were enrolled in the study. Inclusion criteria were IA-IIIB stages of the disease, age over 18 years, absence of severe pulmonary and cardiovascular pathology, and radical surgery of lung cancer ((bi)lobectomy or pneumonectomy). Exclusion criteria were stage IV NSCLC, comorbidities that could lead to the patient's death in the coming years, neoadjuvant radiation or chemotherapy, and complications after surgery. Patients with IB-IIIB stages received adjuvant platinum-based chemotherapy. Patients with category N2 underwent radiation therapy (total dose of 30 Gray). Pathological stages were evaluated according to the 8th edition of the TNM classification. Data on the sex, age, and histological variant of the tumor were taken from the primary medical records.

Follow-up period. The follow-up period began immediately after the surgical treatment. Computed tomography was performed according to local practice. For the first two years after the surgery, imaging tests were repeated every three months, after that – every six months. After a 5-year follow-up period, a chest X-ray was performed every year. In cases of suspicion of disease recurrence, appropriate examinations were performed unscheduled. Long-term follow-up continued for at least five years. Recurrence-free survival was considered the interval between the surgery and the date of disease recurrence. Overall survival was considered the interval between the surgery and the patient's death. The cancer registry of the Sumy Regional Clinical Oncology Center was used as the primary source for data on patient deaths. Data collection was completed on July 1, 2024.

Histological and immunohistochemical (IHC) examination. IHC was performed with subsequent counting of immune cells to determine immune phenotypes. Antibodies against CD8+ (clone C8/144B, Dako, Glostrup, Denmark, 2023) were used to visualize cytotoxic T cells. Antibodies against forkhead box P3 (FOXP3, clone EP340, Cell Marque, Rocklin, CA, USA, 2023) were allowed to determine the regulatory T cells (Treg). Macrophages of the M2 type were determined using antibodies to CD163+ (Master-Diagnostica, Spain, 2023). The number and distribution of CD8+ and CD163+ were investigated in tumor clusters and stroma. For FOXP3, the total expression level was assessed. The number of immune cells was determined per square millimeter. Samples were analysed by blinded pathology.

The sample belonging to a certain phenotype was determined according to the criteria of Chen and Mellman (2013). A low number of lymphocytes in tumor clusters and stroma characterized the immune desert (ID) phenotype. The immune exclusion (IE) phenotype demonstrated a low number of lymphocytes in the tumor clusters and a significant stroma infiltration. In samples with an inflammatory (Inf) phenotype, lymphocytes actively infiltrated the tumor tissue. The semi-quantitative method was used to determine the degree of infiltration by CD8+ cells and the phenotype of the studied samples. The cut-off value for stratification of infiltration as high or low was 9 for CD8+ in the clusters and 24 per square millimeter for CD8+ in the peritumor stroma.

To evaluate the effect of STAT6 on survival in patients with NSCLC, we used antibodies against STAT6 (clone EP325, Cell Marque, USA, 2023) to determine the expression of this biomarker in tumor cells and stroma. The STAT6 expression was calculated using a semiquantitative method on a scale from 0 to 9. We used the formula to calculate the immunohistochemical score: intensity of staining × staining extent. The staining extent was assessed by the percentage of positive cells: 0% – negative; from 1% to 25% – 1+; from 26% to 50% – 2+; >50% – 3+. The staining intensity was determined on a scale from 0 to 3 (0, negative; 1, weak; 2, moderate; 3, strong). The cut-off value for dividing patients into groups with high and low STAT6 was determined by the mean values method.

Statistical analysis. The normality of the distribution was determined by the Shapiro-Wilk test. Spearman's rank correlation coefficient was used to establish the relationship between the STAT6 expression and clinicopathological characteristics of patients and their tumor microenvironment. The significance of the difference between the two studied groups was assessed by the Student's t-test (for parametric variables) and the Mann-Whitney test (for non-parametric variables). The results of STAT6 expression in samples with different phenotypes were compared using the Kruskal-Wallis H test. The effect on survival was assessed by Cox regression analysis. The results were considered statistically significant at $P \leq 0.05$. Statistical analysis was performed using Stata V.18.0 software (StataCorp, Texas, USA; www.stata.com; 2024).

Results

Histology and immunohistochemistry. Histological examination showed characteristic features of adenocarcinoma and squamous cell lung cancer. We performed IHC to divide NSCLC tissue samples into Inf, ID, and IE phenotypes. The Inf immune phenotype was the most common and was found in 12 adenocarcinomas and 8 squamous cell carcinomas (Fig. 1). IHC examination of NSCLC tissue with antibodies against CD8+ showed the presence of a significant number of cytotoxic lymphocytes in the tumor clusters and stroma in the form of lymphocyte-like cells with a positive membrane reaction (Fig. 1a). IHC determination of the quantitative indicators of M2-type macrophages showed moderate infiltration of tumor clusters and stroma (Fig. 1b). IHC examination of Tregs also showed a small number of Foxp3+ cells both in the stroma and in the tumor clusters (Fig. 1c). IHC expression of the STAT6 marker among the tumor stroma and parenchyma of NSCLC of the Inf immunophenotype was also moderate (Fig. 1d). We found an ID phenotype in 5 adenocarcinomas and 1 squamous cell lung cancer sample. This immunophenotype was characterized by a low number of CD8+ lymphocytes in the tumor clusters and stroma (Fig. 2a), a moderate number of CD163-positive macrophages in the tumor stroma (Fig. 2b), increased expression of Foxp3 and STAT6 markers in the tumor stroma and parenchyma (Fig. 2 c-d).

The IE phenotype was detected in 3 adenocarcinoma and 13 squamous cell carcinoma samples. We found a significant number of cytotoxic CD8-positive lymphocytes (Fig. 3a) and CD163-positive macrophages (Fig. 3b) in the tumor stroma, high expression of Foxp3 and STAT6 in the tumor clusters and stroma of NSCLC (Fig. 3c-d).

Characteristics of the patients. The median age of the patients was 58.47 ± 1.30 years. According to the expression levels, patients were divided into groups with low STAT6 (<6) and high STAT6 (≥6). High expression of STAT6 was detected in 50.0% of patients with NSCLC. A moderate correlation was found between STAT6, gender, and tumor histology. More details about the clinicopathological characteristics of the patients are presented in Table 1.

STAT6 expression in patients with different histological types of NSCLC. Adenocarcinoma and squamous cell carcinoma were diagnosed in 20 patients and 22 patients, respectively. High expression of STAT6 was registered predominantly in squamous cell carcinomas ($P = 0.0001$). Thus, in 6/20 (30.0%) patients with adenocarcinomas and 15/22 (68.2%)

patients with squamous cell carcinomas, the immunohistochemical score was ≥6. At the same time, STAT6 was expressed mainly in stromal cells, which was similar in distribution to CD163+ cells. The study of the expression of immune cells demonstrated a higher number of M2 macrophages in the stroma of squamous cell carcinomas compared to lung adenocarcinomas. This finding supports the impact of STAT6 on the polaripolarization of M2 macrophages (Table 2).

Tumor microenvironment and the risk of metastasis to lymph nodes (LNM). The frequency of regional LNMs was compared depending on the immune phenotype and histology of NSCLC. Regional LNMs were registered in 35.0% of patients with adenocarcinomas and 50.0% with squamous cell carcinomas.

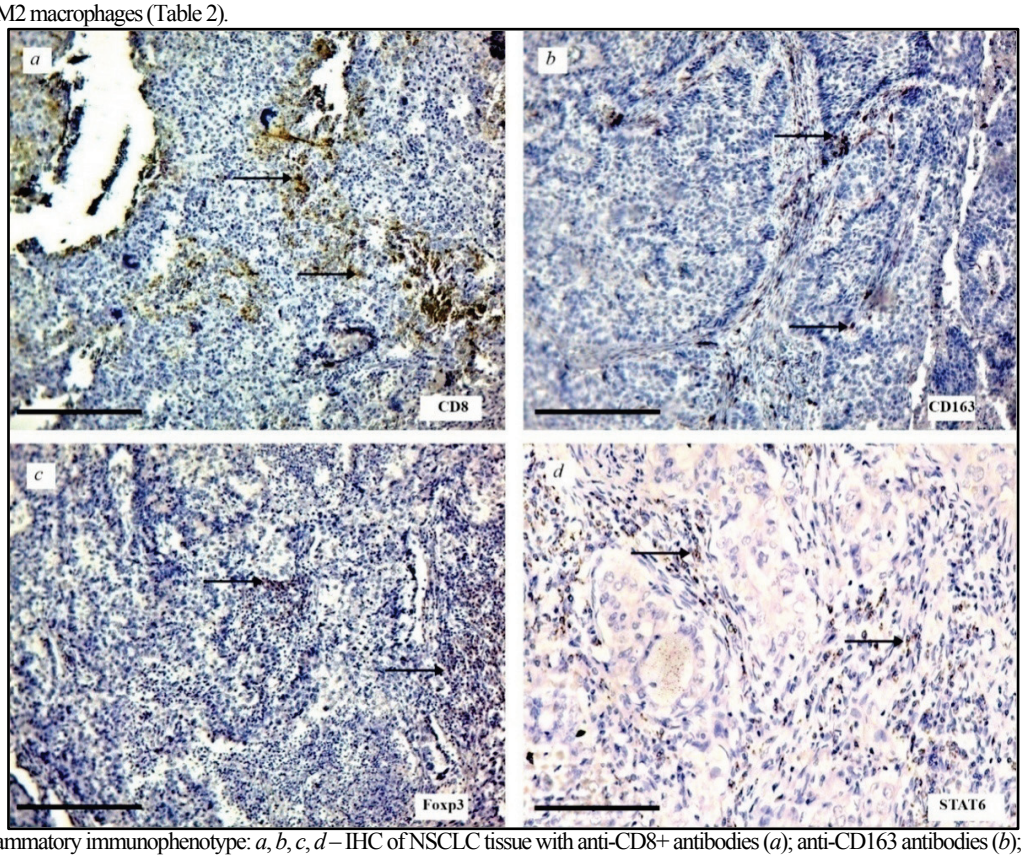

Fig. 1. Inflammatory immunophenotype: *a*, *b*, *c*, *d* – IHC of NSCLC tissue with anti-CD8+ antibodies (*a*); anti-CD163 antibodies (*b*); anti-Foxp3 antibodies (*c*); anti-STAT6 antibodies (*d*); scale bar indicated the distance of 100 μm; arrows indicated appropriate cells of tumor microenvironment

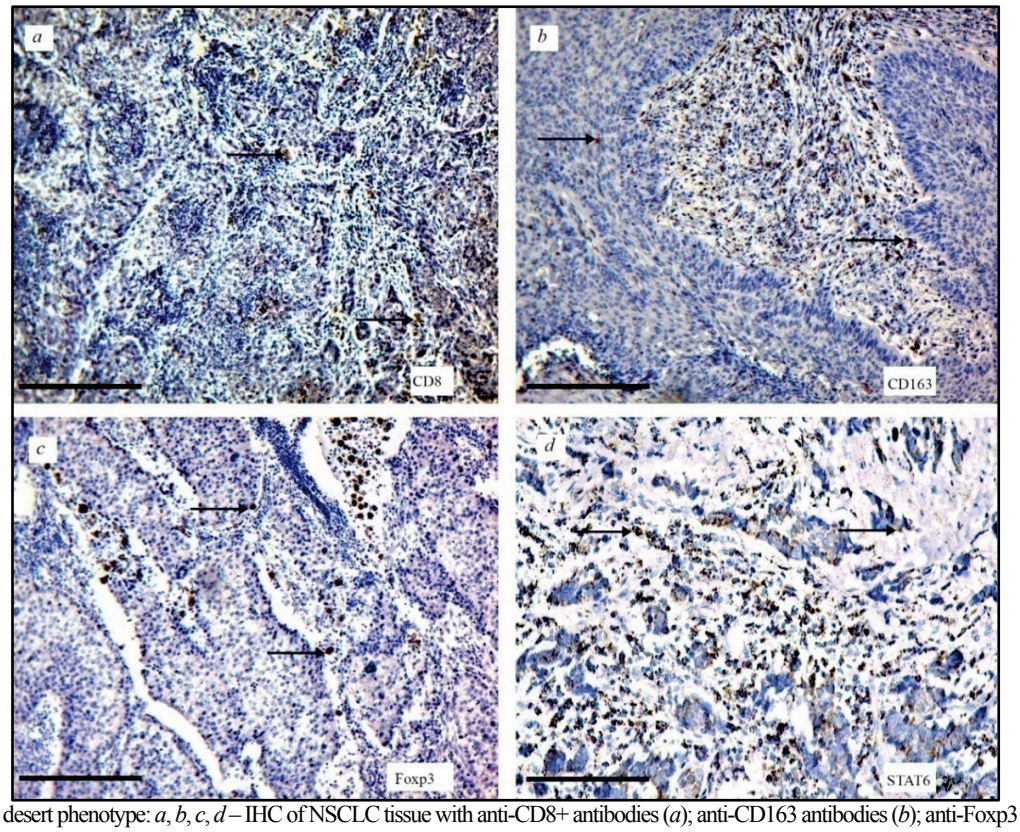

Fig. 2. Immune desert phenotype: *a*, *b*, *c*, *d* – IHC of NSCLC tissue with anti-CD8+ antibodies (*a*); anti-CD163 antibodies (*b*); anti-Foxp3 antibodies (*c*); anti-STAT6 antibodies (*d*); scale bar indicated the distance of 100 μm; arrows indicated appropriate cells of tumor microenvironment

Table 1
Baseline clinicopathological characteristics of patients according to the STAT6 expression

Baseline clinicopathological characteristics	Total (%), n = 42	Low STAT6 (<6), (%) n = 21	High STAT6 (≥6), (%) n = 21	Correlation between STAT6 and baseline clinicopathological characteristics $r \pm s_r$, P-value
Age, n (%):				
Median	58	59	58	
Range	29–75	29–73	40–75	
< 60	22 (52.4)	10 (47.6)	12 (57.1)	-0.095 ± 0.158
≥ 60	20 (47.6)	11 (52.4)	9 (42.9)	(0.5436)
Sex, n (%):				
Female	8 (19.0)	8 (38.1)	0 (0.0)	0.485 ± 0.138
Male	34 (81.0)	13 (61.9)	21 (100.0)	(0.0010)
Stage, n (%):				
IA-IIA	15 (35.7)	8 (38.1)	7 (33.3)	0.049 ± 0.157
IIB-IIIB	27 (64.3)	13 (61.9)	14 (66.7)	(0.7523)
Histology, n (%):				
Adenocarcinoma	20 (47.6)	14 (66.7)	6 (28.6)	0.381 ± 0.146
Squamous cell carcinoma	22 (52.4)	7 (33.3)	15 (71.4)	(0.0140)
CD163+ in clusters, n (%):				
<13 (low)	27 (64.3)	15 (71.4)	12 (57.1)	0.149 ± 0.156
≥13(high)	15 (35.7)	6 (28.6)	9 (42.9)	(0.3426)
CD163+ in stroma, n (%):				
<24 (low)	21 (50.0)	12 (57.1)	9 (42.9)	0.142 ± 0.156
≥24(high)	21 (50.0)	9 (42.9)	12 (57.1)	(0.3624)
CD8+ in clusters, n (%):				
<9 (low)	13 (40.0)	8 (38.1)	5 (23.8)	-0.154 ± 0.159
≥9 (high)	29 (60.0)	13 (61.9)	16 (76.2)	(0.3259)
CD8+ in stroma, n (%):				
<24 (low)	20 (47.6)	10 (47.6)	10 (47.6)	0.001 ± 0.158
≥24(high)	22 (52.4)	11 (52.4)	11 (52.4)	(1.0000)
Regulatory T-cells (FOXP3), n (%)				
<23 (low)	18 (42.9)	9 (42.9)	9 (42.9)	0.001 ± 0.158
≥23 (high)	24 (57.1)	12 (57.1)	12 (57.1)	(1.0000)

Table 2
Relationship between the expression of immune cells and histology of non-small cell lung cancer

Immune cells	Adenocarcinoma, n = 20	Squamous cell carcinoma, n = 22	P-value
M2 macrophages (CD163+):			
Tumor clusters	11.8 (6–31)	13.5 (6–24)	0.1924
Tumor stroma	19.7 (9–36)	27.6 (11–50)	0.0060
Cytotoxic T-cells (CD8+):			
Tumor clusters	22.0 (5–73)	13.7 (3–58)	0.0692
Tumor stroma	31.8 (8–79)	30.6 (6–84)	0.9197
Regulatory T-cells (FOXP3+)	24.9 (7–72)	30.1 (18–49)	0.1654

The most common type of immune microenvironment in patients with adenocarcinoma was Inf phenotype (12/20; 60%). The ID (5/20; 25.0%) and IE (3/20; 15.0%) phenotypes were determined less often. On the other hand, squamous cell carcinomas showed a phenotype of IE, with T-cytotoxic lymphocytes predominantly present in the stroma and low infiltration of tumor clusters (13/22; 59.1%).

Among adenocarcinoma samples, the most common was an Inf immunophenotype without LNM (8 out of 20 samples, 40.0%). IE phenotype with LNM (8 out of 22 samples, 36.4%) was typical for squamous cell carcinomas. An ID phenotype was observed in only 1 of 22 patients (4.6%) with squamous cell carcinomas and in 5 of 20 patients (25.0%) with lung adenocarcinomas (Table 3).

In addition, the number and distribution of CD163+ and FOXP3+ were evaluated depending on the immunophenotype of the samples. Inf immunophenotype was associated with lung adenocarcinomas. A trend toward higher expression of CD163+ and FOXP3+ was observed in samples with an IE phenotype, but no significant difference was found between groups (P = 0.3732 and P = 0.1862, respectively; Table 4).

Relationship between STAT6 expression, immune phenotypes, and gender. We previously established that STAT6 expression correlates with patient gender. High expression of STAT6 is more common in men than in women (P = 0.0001).

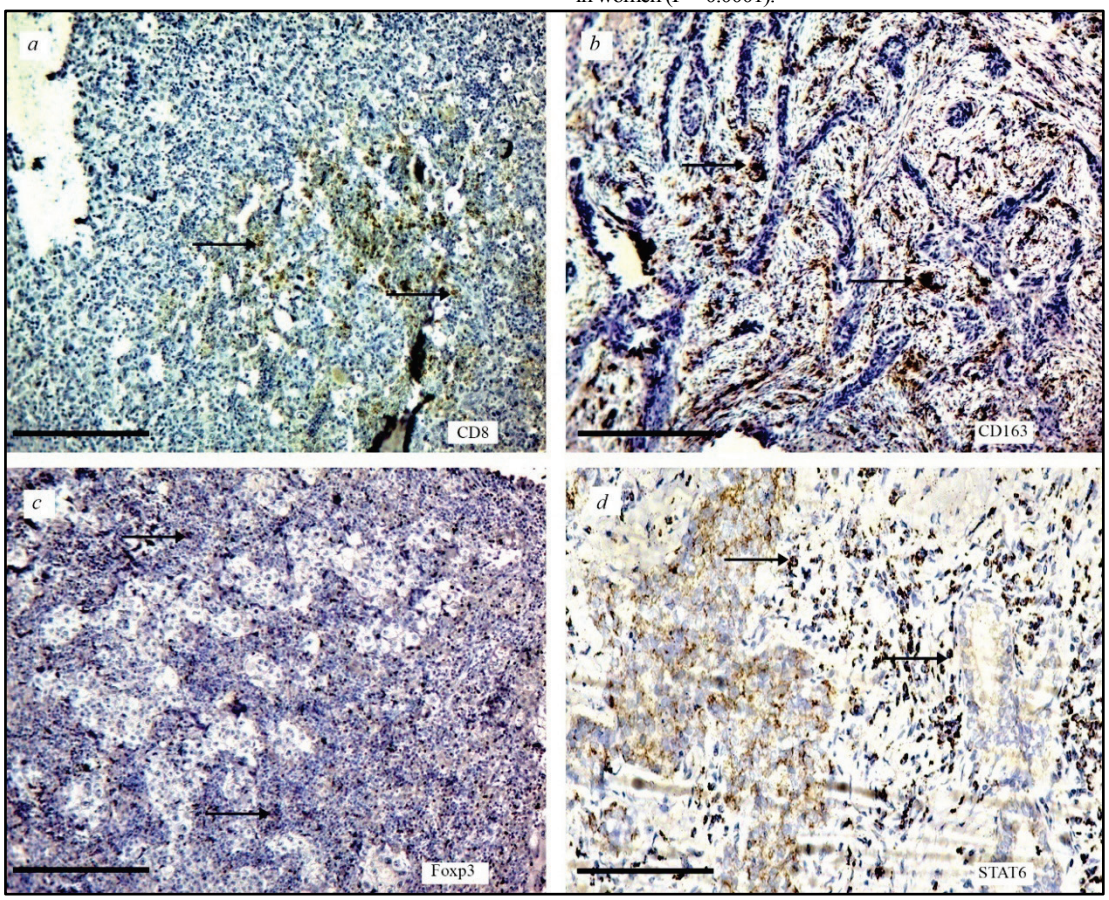

Fig. 3. The immune exclusion phenotype: *a*, *b*, *c*, *d* – IHC of NSCLC tissue with anti-CD8+ antibodies (*a*); anti-CD163 antibodies (*b*); anti-Foxp3 antibodies (*c*); anti-STAT6 antibodies (*d*); scale bar indicated the distance of 100 μm; arrows indicated appropriate cells of tumor microenvironment

Table 3
Distribution of immune phenotypes in patients with non-small cell lung cancer

Phenotype of tumor microenvironment	Adenocarcinoma, n (%)	Squamous cell carcinoma, n (%)	Total, n (%)
ID	5	1	6
– LNM	2 (10.0)	0	2 (4.8)
– no LNM	3 (15.0)	1 (4.6)	4 (9.5)
IE	3	13	16
– LNM	1 (5.0)	8 (36.4)	9 (21.4)
– no LNM	2 (10.0)	5 (22.7)	7 (16.7)
Inf	12	8	20
– LNM	4 (20.0)	3 (13.6)	7 (16.7)
– no LNM	8 (40.0)	5 (22.7)	13 (30.9)
Total	20	22	42

Table 4
Distribution of immune cells according to immune phenotype

Immune cells	ID phenotype	IE phenotype	Inf phenotype	P-value
M2 macrophages (CD163+):				
Tumor clusters	12.8 (6–31)	12.8 (6–24)	12.7 (6–26)	0.7818
Tumor stroma	19.7 (9–36)	26.0 (10–39)	23.3 (12–50)	0.3732
Cytotoxic T-cells (CD8+):				
Tumor clusters	6.8 (4–9)	16.0 (3–12)	28.8 (13–73)	0.0001
Tumor stroma	9.0 (6–11)	28.2 (13–71)	39.9 (15–84)	0.0002
Regulatory T-cells (FOXP3+)	19.8 (9–34)	30.4 (18–49)	27.8 (7–72)	0.1862

Low expression of STAT6 was registered in all women of the investigated group (8 out of 8 patients, 100%). Most men, on the contrary, had high expression of STAT6 (21 of 34 patients, 62.8%). Only two immune phenotypes were registered among female samples: Inf (87.5%) and ID (12.5%). No woman had an IE phenotype. In men, high expression of STAT6 was associated with an IE phenotype. This phenotype was observed in 16 out of 34 male samples (47.1%, Fig. 4).

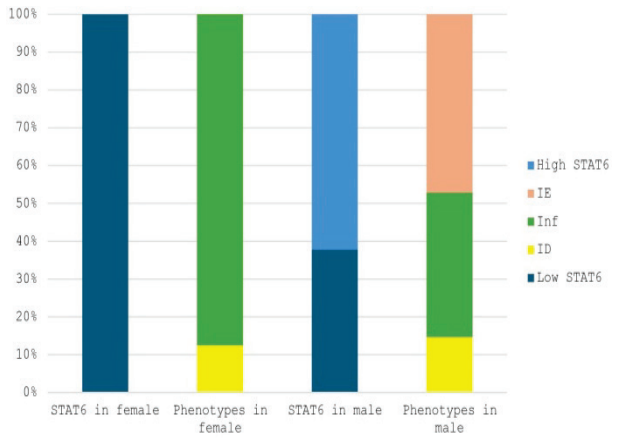

Fig. 4. Relationship between STAT6 expression, immune phenotypes, and gender of non-small cell lung cancer patients

The relationship between STAT6 expression and survival in patients with radically treated NSCLC. The median follow-up period for the studied cohort was 57.9 ± 4.2 months. Disease recurrence was registered in 19 out of 42 patients (45.2%). As a result of lung cancer progression, 18 patients (42.9%) died, and one patient died due to other causes. Cox regression analysis demonstrated that gender, CD8+ expression in tumor clusters, and STAT6 expression are independent predictors of recurrence-free survival. Female gender, high CD8+ expression in tumor clusters, and low STAT6 expression were associated with better recurrence-free survival (Table 5).

Gender, expression of CD163+ in the stroma, the number of CD8+ in tumor clusters, and the level of STAT6 expression were determined as independent predictors of overall survival. Females and patients with high CD8+ expression in tumor clusters, low stromal CD163+ cells, and low STAT6 expression had better overall survival (Table 6).

Table 5
Multivariate analysis of the impact of clinicopathological characteristics and immune cells of the tumor microenvironment on the recurrence-free survival of patients with non-small cell lung cancer

Clinicopathological characteristics	Hazard ratio	95% CI	P-value
Age (<60 versus ≥60)	1.19	0.66–2.12	0.553
Sex (female versus male)	3.21	1.05–9.80	0.040
Stage (IA–IIA versus IIB–IIIB)	1.55	0.73–3.27	0.245
Histology (adenocarcinoma versus squamous cell carcinoma)	1.32	0.62–2.81	0.470
CD163+ in tumor clusters (low versus high)	0.78	0.40–1.54	0.487
CD163+ in tumor stroma (low versus high)	0.59	0.28–1.24	0.169
CD8+ in tumor clusters (high versus low)	0.33	0.13–0.86	0.024
CD8+ in tumor stroma (high versus low)	1.27	0.66–2.42	0.462
FOXP3+ (low versus high)	1.31	0.65–2.62	0.444
STAT6 (low versus high)	0.32	0.14–0.72	0.006

Table 6
Multivariate analysis of the influence of clinicopathological characteristics and immune cells of the tumor microenvironment on the overall survival of patients with non-small cell lung cancer

Clinicopathological characteristics	Hazard ratio	95% CI	P-value
Age (<60 versus ≥60)	1.27	0.71–2.27	0.413
Sex (female versus male)	10.12	2.45–41.74	0.001
Stage (IA–IIA versus IIB–IIIB)	1.47	0.68–3.18	0.318
Histology (adenocarcinoma versus squamous cell carcinoma)	1.18	0.58–2.41	0.642
CD163+ in tumor clusters (low versus high)	0.96	0.47–1.95	0.927
CD163+ in tumor stroma (low versus high)	0.49	0.23–1.02	0.050
CD8+ in tumor clusters (high versus low)	0.35	0.13–0.90	0.031
CD8+ in tumor stroma (high versus low)	1.43	0.72–2.84	0.298
FOXP3+ (low versus high)	0.97	0.49–1.92	0.941
STAT6 (low versus high)	0.29	0.12–0.65	0.003

Discussion

STAT6 promotes the progression of many types of malignancies, including breast (Salguero-Aranda et al., 2019), pancreatic (Yu et al., 2022), colorectal (Li et al., 2022), and lung cancer (He et al., 2023). STAT6 is stimulated by interleukin (IL)-4 and IL-13. As a result, activated T-helpers type 2 (Th2) change the tumor microenvironment (Karpathiou et al., 2021). In addition, activating the JAK/STAT signaling pathway leads to the translocation of a specific protein to the nucleus and the transcription of Fizz1 genes, which are responsible for increasing the population of M2 macrophages (Stütz et al., 2003). Our study showed that NSCLC cells actively express STAT6. Overexpression is observed in 50.0% of cases. We established that STAT6 correlates with the histology of NSCLC and the gender of patients. The study of the expression of immune cells in the tumor microenvironment, depending on the histology of NSCLC, demonstrated a significantly higher number of M2 macrophages in the stroma of squamous cell carcinomas. This supports the theory of a stimulating effect of STAT6 on macrophage polarization.

Some authors have studied the mechanisms of polarization of M2 macrophages in detail. Zhou et al. (2020) found that tumor necrosis factor receptor-associated factor 6 (TRAF6) is essential in IL-4 signaling and maintaining STAT6 stability. TRAF6 acts as a positive regulator of M2 macrophage polarization. In addition, the inhibition of the HIF1α/NF-κB pathway due to the activating effect of Krüppel-like factor 4 (KLF4) on STAT6 causes the blocking of the activation of M1 macrophages. It stimulates the polarization of M2 macrophages (Liao et al., 2011).

In the initial stages of tumor development, macrophages exhibit antitumor properties. They destroy tumor cells directly or involve other immune cells for this. STAT6 works as a key transcription factor that leads to Th2 dominance in the tumor microenvironment and a shift of the immune system to a humoral condition. In turn, the weakening of the cytotoxic response associated with Th1 effects is accompanied by tumor progression and metastasis (Lopez-Yrigoyen et al., 2021). Interestingly, in mouse models, animals without STAT6 expression have resistance to some types of malignant tumors, including prostate (Kacha et al., 2000) and breast can-

cer (Jensen et al., 2003). Such animals are visually indistinguishable from others but tend to neutrophilia (Valladao et al., 2016) and higher levels of pro-inflammatory cytokines such as tumor necrosis factor-α, macrophage inflammatory protein-2, and IL-6 (Lee et al., 2021). The protective effect of STAT6 deficiency and the absence of significant immunological and laboratory abnormalities in experimental animals make STAT6 an attractive target for cancer therapy.

The current study examined the immune phenotypes of adenocarcinomas and squamous cell carcinomas and their association with regional LNM. Caruana et al. (2018) described three immune phenotypes of solid tumors based on the distribution of CD8+ lymphocytes. The ID phenotype reflects the absence of antitumor immunity, as CD8+ cells are absent in tumor clusters and stroma. The main factors that lead to the appearance of such a phenotype may be a low mutational burden and insufficient presentation or release of tumor antigen. In samples with an IE phenotype, CD8+ cells actively infiltrate the peritumoral stroma but do not reach the tumor clusters. A low level of chemokine receptors and a deficiency of chemokines and cytokines lead to disrupting CD8+ recruitment mechanisms in tumor clusters (Allen et al., 2022).

Inf immunophenotype is associated with high infiltration of tumor clusters and stroma by CD8+ cells. The current study found that adenocarcinoma samples are mostly associated with Inf phenotype without LNM. In contrast, squamous cell carcinoma samples are often related to IE phenotype and LNM. The strong association between STAT6 expression and the number of M2 macrophages in the stroma of squamous cell carcinomas allows us to suspect their stimulatory effect on tumor spreading to regional lymph nodes. In our previous study, we evaluated the effect of STAT6 on the tumor microenvironment and LNM in patients with papillary thyroid cancer and obtained similar results (Sulaieva et al., 2020).

We found that low STAT6 expression is more common in women than men. In addition, 87.5% of samples belonging to women had an Inf phenotype with high infiltration of CD8+ tumor clusters and stroma. This suggests a positive effect of low STAT6 and high CD8+ expression on survival in women. The scientific literature has no data on the relationship between STAT6 expression, immune phenotypes of the tumor microenvironment, and patient gender. However, in several publications, the authors concluded that there is a higher level of inflammation in the airways of women. Zhao et al. (2019) established gender differences in the effect of STAT6-induced IL-33 on the level of airway inflammation. Increased inflammation in women is associated with intensive production of IL-13s, an effector cytokine of IL-33. Ray et al. (2022) confirm that eosinophilic inflammation is more common in women and associated with the activation of alveolar macrophages.

Multivariable regression analysis demonstrated that female sex, high CD8+ expression in tumor clusters, and low STAT6 expression were associated with better recurrence-free survival. In turn, predictors of high overall survival are female gender, low expression of CD163+ in the stroma, high expression of CD8+ in tumor clusters, and low STAT6.

High STAT6 expression is associated with a poor prognosis in many types of cancer, including Hodgkin's lymphoma (Yang et al., 2022), oropharyngeal carcinoma (Lee et al., 2024), acute myeloid leukemia (Liu et al., 2020), and primary CNS lymphoma (Mondello et al., 2020). Data on the impact of STAT6 on the survival of patients with NSCLC are limited. Faida et al. (2023) established that activating STAT proteins leads to anti-apoptotic reactions, uncontrolled cell proliferation, and angiogenesis. Together, these factors worsen the survival of patients with lung cancer. Yu et al. (2022) investigated the antitumor and antimetastatic effect of chitinase 3-like 1 (Chi3L1) in a mouse lung cancer model. The inhibitory effect of this humanized antibody was associated with an effect on STAT6-dependent polarization of M2 macrophages. This experiment demonstrated that blocking STAT6 is a potential promising area of targeted therapy for lung cancer.

Although we consider the obtained results important and reliable, the study's main limitations were the small sample size and the absence of molecular genetic testing of tumor tissue.

Conclusions

50.0% of patients with NSCLC have high expression of STAT6. STAT6 overexpression occurs predominantly in squamous cell carcinomas than in adenocarcinomas. Inf immune phenotype is most common in adenocarcinomas. IE phenotype is more typical for squamous cell carcinomas. Regional LNM is associated with an IE phenotype and is mediated by high infiltration of M2 macrophages into the tumor stroma. Low STAT6 and an Inf immune phenotype are more common in women, and high STAT6 and an IE phenotype in men. High STAT6 predicts poor recurrence-free survival and overall survival in patients with NSCLC. To obtain more accurate results, it is necessary to conduct a study that includes a larger cohort of patients, in particular, female patients.

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The authors declare they have no conflict of interest.

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