

**Introduction:** Patients with lung cancer receive treatment according to National Cancer Comprehensive Network (NCCN) standards. However, disease recurrence is reported in about 30% of patients during the first five years. Our study aimed to establish independent predictors of lung cancer recurrence.

**Material and methods:** 104 patients with definitive treatment for non-small-cell lung carcinoma receiving standard adjuvant chemotherapy in the period 2014–2018 in our cancer center were retrospectively reviewed. The prognostic significance of five routine immunohistochemical (IHC) markers was examined.

**Results:** During the follow-up period disease recurrence occurred in 42 (40.4%) of the 104 enrolled patients. The median recurrence-free survival was 56.3 months, range 4–84.0 months (95% CI = 46.866–65.683). The recurrence-free survival rate was 58.8%. The frequencies of locoregional recurrence, lung recurrence, kidney, bone, lymph nodes of the neck, liver, and brain recurrence were 23.8%, 21.5%, 16.7%, 9.5%, 9.5%, 9.5% and 9.5%, respectively.

**Conclusions:** Using the Cox regression model, category T, histological differentiation, and smoking status were identified as independent predictors of disease recurrence. The studied biological markers (PD-L1, Ki67, p53, epidermal growth factor receptor, and ALK) did not help the model predict disease recurrence. For statistical reliability, it is necessary to conduct a study on a larger cohort of patients and compare the mutual influence of several biomarkers.

**Key words:** lung cancer, IHC, predictors, disease recurrence.

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# Prognostic factors for recurrence in patients with surgically resected non-small cell lung cancer

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## Introduction

Cancer incidence in 2040 will increase by 47% compared to 2020. Lung cancer is the leading cause of death from malignant neoplasms in both men and women. Therefore, diagnosis and treatment of early lung cancer through screening programs and prediction of recurrence are essential for lung cancer mortality reduction [1, 2]. Surgery is the primary treatment method for non-small cell lung cancer [3]. However, the disease recurs in about 30% of patients during the first five years. The recurrence-free period lasts on average 15.7 months [4]. According to National Cancer Comprehensive Network (NCCN) standards, adjuvant chemotherapy is recommended for stage IB–IIIA lung cancer. There is no need to prescribe cytotoxic therapy in each case. It is necessary for the presence of risk factors such as tumor size greater than 4 cm, vascular invasion, poorly differentiated tumor, marginal resection, unknown status of regional lymph nodes, and visceral pleural lesions. Mandatory chemoradiation therapy is recommended for patients with N2 [5].

In the daily routine practice at the preoperative stage, one should collect the patient's history of bad habits, chronic diseases, and social status, and additionally analyze the histology of the tumor and its differentiation. Immunohistochemistry and molecular biology for the presence of the most common mutations (epidermal growth factor receptor – EGFR, ALK, B-RAF, and ROS1) should be performed after surgical treatment [6].

There are a few methods for identifying factors related to recurrence following surgery. The first are clinical parameters and TNM (T: tumor size, N: metastases in regional lymph nodes, M: distant metastases) classification. Furthermore, the physical examination is very important, because a lower performance status and the presence of symptoms are unfavorable prognostic factors for disease-free survival [7]. An extensive pathological investigation is also essential because histological differentiation, vessel invasion, lymphatic permeation, and pleural invasion have all been reported to be poor prognostic factors for disease-free survival [8]. The other method for predicting recurrence uses molecular biological techniques. Lung cancer is a highly aggressive disease that includes different histological subtypes with distinct clinicopathological and molecular features. Therefore, the establishment of biological markers is necessary to predict disease recurrence. Immunohistochemistry is now widely used to determine the prognosis in patients with breast cancer [9], lung cancer [10], lymphoma [11], and many other types of tumors.

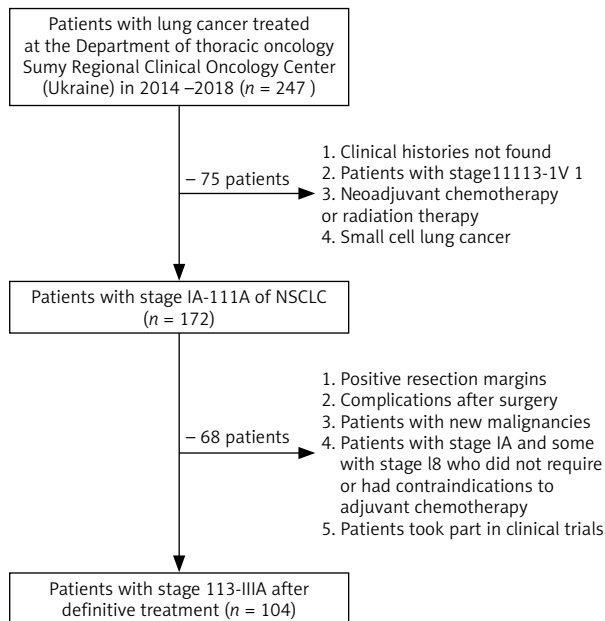


Fig. 1. Flow chart of population cohort selection

## Material and methods

### Study design

There were 104 patients selected for performing the retrospective study. They received definitive treatment according to NCCN standards [5]. The treatment was carried out in the Sumy Regional Clinical Oncology Center (Ukraine) during 2014–2018. Exclusion criteria were neoadjuvant chemotherapy or radiation therapy, positive resection margins, and the appearance of a new malignancy. The study group did not include patients with stage IA and some with stage IB who did not require or had contraindications to adjuvant chemotherapy. Patients with grade 3 or more complications after surgery, such as air leakage, atelectasis, pneumonia, chylothorax, arrhythmia, bronchopleural fistula, and cerebral infarction, were excluded from the study (Fig. 1).

Data on age, sex, smoking status, category T, category N, primary tumor size, stage of disease, lymphovascular invasion, histological variant, histological differentiation, marital status, and Eastern Cooperative Oncology Group performance score were taken from the medical records. Immunohistochemistry was performed using tumor tissue obtained from postoperative material. According to age at the time of diagnosis, patients were divided into two groups:  $\leq 60$  years and  $> 60$  years. Disease stages, categories T and N, were determined according to the eighth edition of the TNM classification [12]. According to the histology and histological differentiation, patient distribution was performed based on World Health Organization data on the classification of lung tumors (2015) [13].

### Histology

Paraffin blocks of lung cancer were taken from the archives of the Sumy Regional Clinical Oncology Center. Serial sections with a thickness of 4–5  $\mu\text{m}$  were stained with Mayer's hematoxylin and eosin.

### Immunohistochemistry

Serial sections with a thickness of 4  $\mu\text{m}$  were applied to the SuperFrost adhesive slides (Thermo Scientific, USA). The deparaffined sections were subjected to antigen unmasking by heat treatment in 0.1 M citrate buffer (pH 6.0) at 95–98°C. The UI-traVision Quanto Detection System HRP Polymer (Thermo Scientific, USA) was used to visualize the immunohistochemical (IHC) reaction. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Ultra V Block was used to prevent non-specific reaction and background staining. The reaction was amplified with Primary Antibodies Amplifier Quanto. Diaminobenzidine was used as a chromogen. The nuclei were counter-stained with Mayer's hematoxylin. Primary antibodies against PD-L1 (Master Diagnostica, clone Cal-10, dilution 1 : 50), EGFR (Thermo Scientific, clone EP38Y, dilution 1 : 100), p53 (Master Diagnostica, clone SP5, dilution 1 : 50), Ki-67 (Thermo Scientific, clone SP6, dilution 1 : 200) and ALK (Abcam, clone ab37807, dilution 1 : 100) were used. At least six different fields of view (FOV) with a diameter of 1 mm were analyzed for each sample. The immunohistochemical results were presented as the mean number of positive cells per field of view.

In cases of positive staining for PD-L1, a clear membrane response was detected in tumor cells with a complete or partial cytoplasmic environment of any intensity (Fig. 1). In our calculations, we did not consider a positive reaction in the immune cells of the tumor microenvironment (lymphocytes, macrophages). We also ignored the cytoplasmic response to PD-L1 in tumor cells.

Epidermal growth factor receptor-immunoreactivity was scored based on membranous and/or cytoplasmic staining (Fig. 1), as follows: 0, no staining or faint staining in  $< 10\%$  of tumor cells; 1+, weak staining in 10% of tumor cells; 2+, moderate staining in 10% of tumor cells; 3+, strong staining in 10% of tumor cells [14].

A positive result of ALK staining of lung tumor tissue samples was positive cytoplasmic staining of tumor cells, often in the form of lumps inside the cytoplasm and/or with a membrane component (Fig. 1). The classical H-score scale with a graduation of 0–3 with the multiplication of the percentage of positive-stained cells (range 0–300) was used for evaluation. A positive reaction was considered as 200–300 (+3) [15].

The p53 protein was found in the nuclei of lung tumor cells and was more often combined with poorly differentiated tumors with pronounced manifestations of cellular atypia (Fig. 1). Evaluation of p53 was performed by counting the percentage of stained cells in 6 different FOV with a diameter of 1 mm. Ki-67 also had nuclear localization in the case of positive staining of tumor cells (Fig. 1). The evaluation of immunohistochemistry results was similar to that for p53 protein.

### Follow-up of patients after surgical treatment and adjuvant chemotherapy

Patients were monitored according to local practice. The first computed tomography was performed one month after surgery. Subsequently, this examination was repeat-

ed every three months for the first three years, then every six months for two years. After five years after the operation, computed tomography was performed once a year. Magnetic resonance imaging of the brain, ultrasound of the neck, abdominal cavity, pelvis, and bone scans were performed as needed in case of suspicion of recurrence of the disease.

In case of recurrence of the disease, all metastases were divided into two groups: locoregional and distant. Locoregional metastases appeared in resection margins and mediastinal lymph nodes on the affected side. All other metastases were considered distant.

We calculated the recurrence-free survival of patients. The recurrence-free period was considered from diagnosis to the registration of radiological disease progression.

### Ethics committee

The study was performed retrospectively. We adhered to the Declaration of Helsinki and received the approval of the Local Ethics Commission of the Sumy Regional Clinical Oncology Center.

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### Statistical analysis

Cox regression was used to model the survival of patients with lung cancer. The main advantage of this model is that it allows one to work with categorical and censored data. In general, the model can be presented as follows:

$h(t) = h_0(t) * \exp(\beta_1 * x_1 + \beta_2 * x_2 + \dots + \beta_p * x_p)$ , where  $h_0(t)$  is the base risk;  $\beta_1, \dots, \beta_p$ —coefficients;  $x_1, x_2, \dots, x_p$  are independent variables (so-called predictors). The coefficients  $\beta_1, \dots, \beta_p$  describe the degree of influence of each variable on the probability of occurrence of the event. Thus, when the variable  $x_p$  increases by 1, the risk increases  $\exp(\beta_p)$  times. This is provided that the value of other predictors has not changed.

The standard data model was created in Excel, and the analytical model was created in the software environment IBM SPSS Statistics 27. All calculations were also made in the software environment SPSS. The risk factors were significant when the two-sided  $p$ -value was less than 0.05. The Kaplan-Meier method was used to estimate the recurrence-free survival of patients.

## Results

### Study design

Baseline characteristics of patients and the expression level of IHC markers are presented in Table 1.

### Histology

All lung cancer tissue samples belonged to non-small-cell lung carcinoma. According to histological types, 62 cases of squamous cell carcinoma, 34 cases of adeno-

carcinoma, and 8 cases of large cell carcinoma and adenocarcinoma were detected in the study group.

Squamous cell lung cancer is represented by solid areas and layers of squamous epithelium with cellular atypia: polymorphism of cells and nuclei, hyperchromatic nuclei, and pathological mitoses. A characteristic feature of differentiated forms of squamous cell carcinoma was keratin in the form of rounded horny formations called "cancer pearls". Squamous cell carcinoma of low differentiation was characterized by the diffuse arrangement of tumor cells without the formation of close connections between cells, the presence of areas of necrosis, and pronounced atypia of monomorphic tumor cells.

Adenocarcinoma was characterized by atypical glandular structures that infiltrated interstitial lung tissue (Fig. 2). Tumor cells made up of glandular and pseudoglandular structures showed signs of cellular atypia. Some tumors had a papillary pattern and contained mucin.

Large cell lung cancer consisted of large polygonal cells with vacuolated nuclei and distinct cellular atypia. Adenocarcinoma contained signs of squamous cell carcinoma and adenocarcinoma.

### Follow-up of patients after surgical treatment and adjuvant chemotherapy

Among the 104 patients included in the study, 42 had stage IB disease. They all received adjuvant chemotherapy because they had one or more risk factors, such as a primary tumor larger than 4 cm, lymphovascular invasion, poor tumor differentiation, or visceral pleural lesions. Stage II and III non-small cell lung cancer were diagnosed in 46 and 16 patients, respectively. They also received adjuvant chemotherapy. Among all patients, 14 had N2, so according to NCCN standards, they received chemoradiation therapy. During the follow-up period, recurrence of the disease occurred in 42 (40.4%) patients, and 38 (90.5%) of them died. Another 4 (9.5%) people died of other causes. The median recurrence-free survival was 56.3 months, range 4–84.0 months (95% CI = 46.866–65.683). The recurrence-free survival rate was 58.8% (Fig. 3).

Loco-regional metastases were recorded in 10 (23.8%) patients; 9 (21.5%) had distant metastases to the lungs, 7 (16.7%) to the kidneys, 4 (9.5%) in the bones, 4 (9.5%) in the lymph nodes of the neck, 4 (9.5%) in the liver, and 4 (9.5%) in the brain.

Disease recurrence and death from the underlying condition occurred in 14 patients with N2. This group of patients most often had lung metastases and local recurrences – 4 (28.7%) and 3 (21.4%). Metastases to the brain occurred in 2 (14.3%) persons, to the bones in 2 (14.3%). Another 1 (7.1%) patient had metastases in the neck lymph nodes, 1 (7.1%) in the liver, and 1 (7.1%) in the kidneys.

### Statistical analysis

Univariate Cox regression analyses revealed that sex, category T, category N, histological differentiation, and smoking status were significantly associated with disease recurrence. Multivariable Cox regression analyses identified category T, histological differentiation, and smoking

**Table 1.** Characteristics of patients and levels of expression of immunohistochemical markers

Clinical-pathological characteristics	Number of patients (%)	Clinical-pathological characteristics	Number of patients (%)
Age (years)		Tumor histology	
≤ 60	76 (73.1)	Adenocarcinoma	34 (32.7)
> 60	28 (26.9)	Adenosquamous	4 (3.8)
Sex		Squamous cell carcinoma	
Male	84 (80.8)	Large cell carcinoma	4 (3.8)
Female	20 (9.2)	Sarcomatoid	0
Smoking status		Histological differentiation	
Ever	82 (78.8)	Well	10 (9.6)
Never	22 (21.2)	Moderate	68 (65.4)
Category T		Poor	
T1	10 (9.6)	Marital status	
T2	66 (63.5)	Married	80 (76.9)
T3	28 (26.9)	Single	24 (23.1)
T4	0	PD-L (%)	
Category N		≤ 1	58 (55.8)
N0	60 (57.7)	1–49	32 (30.8)
N1	30 (28.8)	≥ 50	14 (13.4)
N2	14 (13.5)	EGFR	
Tumor size		0	10 (22.7)
≤ 4 cm	50 (48.1)	1+ or 2+	20 (45.5)
> 4 cm	54 (51.9)	3+	14 (31.8)
Pathologic TNM stage		ALK	
I	42 (40.4)	0	18 (40.9)
II	46 (44.2)	1+ or 2+	22 (50.0)
III	16 (15.4)	3+	4 (9.1)
ECOG performance score		Ki67	
0	10 (9.6)	≤ 10%	40 (38.5)
1	94 (90.4)	> 10%	64 (61.5)
Lymphovascular invasion		p53	
Present	10 (9.6)	≤ 5%	76 (73.1)
Absent	94 (90.4)	> 5%	28 (26.9)
		Surgery	
		Lobectomy	54 (51.9)
		Bilobectomy	10 (9.6)
		Pulmonectomy	40 (38.5)

ECOG – Eastern Cooperative Oncology Group, EGFR – epidermal growth factor receptor, TNM – T: tumor size, N: metastases in regional lymph nodes, M: distant metastases

status as independent predictors of disease recurrence (Table 2).

Table 3 presents the recurrence rate of lung cancer with identified independent predictors.

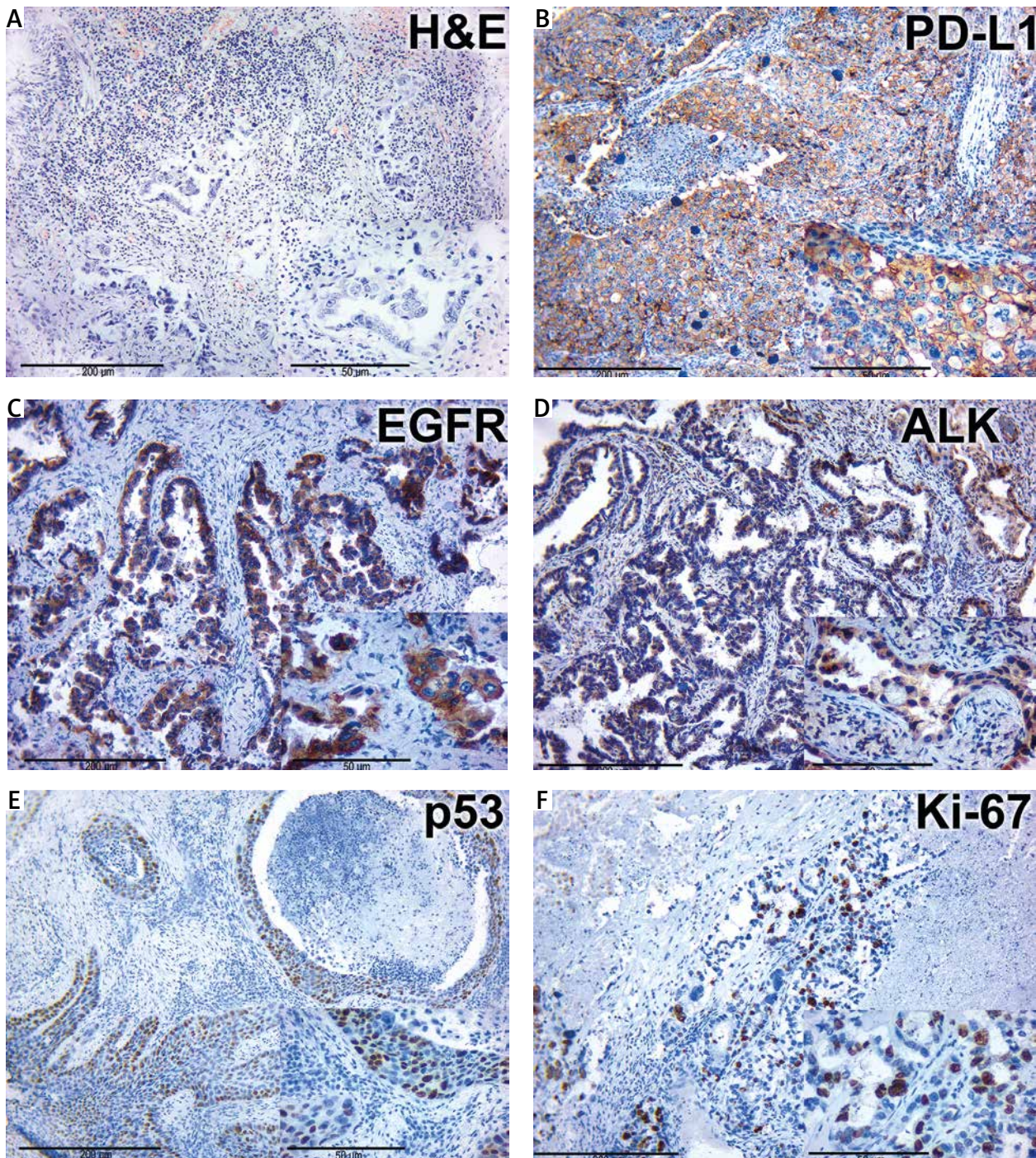
## Discussion

According to GLOBOCAN, the survival rate of patients with lung cancer five years after diagnosis is 10–20%, although, in some countries, the rates are much higher: Israel – 27%, Japan – 33%. These data apply to patients with all stages of the malignant process [16]. Data from the National Cancer Registry show that in Ukraine, the overall 5-year survival of lung cancer patients is close to 25%, which is a tolerable result [17].

The leading cause of death is recurrence of the disease. According to our study, recurrence of the disease after definitive treatment was reported in 40.4% of people, while only 3.8% of patients died from other causes.

The cohort of patients we studied had stage IB–IIIA non-small cell lung cancer. 84.6% of patients had stages I and II, which is entirely consistent with the data of the national cancer registry and our previous study. In contrast to the all-Ukrainian data, early detection of lung cancer (stage I–II) in the Sumy region is much higher (40.23% vs. 22.4%, respectively). This situation leads to decreased detection frequency of advanced stages – 22.1% vs. 35.15%. Apparently, due to the early detection of lung cancer in the population of the Sumy region, the average 1-year survival is better.





**Fig. 2.** Histology and immunohistochemistry of lung cancer tissue

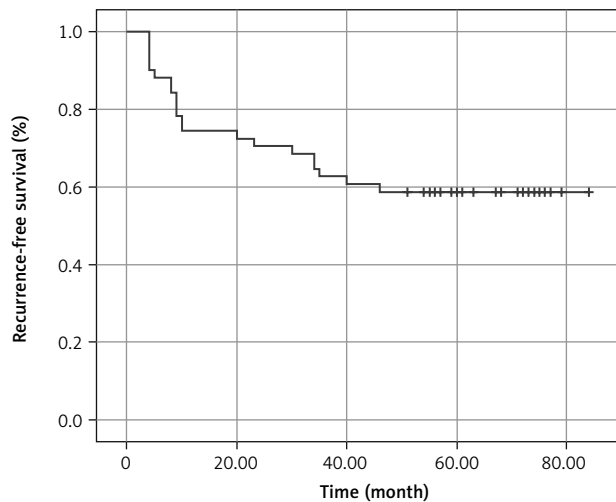
*ALK – immunohistochemical (IHC) expression of ALK, EGFR – IHC expression of epidermal growth factor receptor, H&E – hematoxylin-eosin staining, Ki-67 – IHC expression of Ki-67, PD-L1 – IHC expression of PD-L1, p53 – IHC expression of p53, The figures in the inserts correspond to the enlarged area of this sample. Magnification is indicated in the lower left corner of the image as a marker.*

At the same time, mortality up to 1 year is much lower than the Ukrainian average (46.2% vs. 55.7%) [18].

The use of Cox regression with univariate and multivariable analysis allowed us to identify independent predictors of disease recurrence – category T, histological differentiation, and smoking status – in a study group of 104 people. Each of these factors is confirmed in the scientific literature and our research.

In 2017, the Ontario Evidence-Based Cancer Care Program provided updated guidelines for treating surgically resected stage II–IIIa non-small cell lung cancer patients. Adjuvant chemotherapy has significantly improved overall survival in patients with stage II–IIIa disease [19]. Already in 2021, such a pattern was observed for the IB stage. Therefore, in the case of a primary tumor size  $\geq 4$  cm, adjuvant chemotherapy is recommended [20].





**Fig. 3.** Kaplan-Meier curve showing recurrence-free survival in patients after definitive treatment of IB-IIIa non-small-cell lung carcinoma

During our study, one of the critical predictors of recurrence of non-small cell lung cancer was category T, which we determined according to the eighth edition of the TNM classification. Category T does not depend only on the size of the primary tumor. Its formation is influenced by the spread of the primary tumor to structures such as the tracheal bifurcation, parietal and visceral pleura, chest, diaphragmatic nerve, pericardium, diaphragm, mediastinum, heart, large vessels, trachea, recurrent laryngeal nerve, esophagus, and vertebral body and the appearance of individual tumor nodes in the same lobe or an adjacent lobe of the lung.

According to the TNM classification, category T is one of the prognostic factors associated with the primary tumor [12].

Some scientists have compared the survival of patients in whom tumors belonged to one category T. The first group included the patients according to the size of the primary tumor, and the second group included the patients according to the spread to neighboring structures. It was found that the overall survival of patients in both groups was almost the same. That is why it is essential to evaluate the T category, not just the size of the primary tumor [21]. Category T is recognized as an independent factor in the prognosis of cancer recurrence of the gallbladder, vulva, thyroid, and breast [22–25].

Smoking is the leading cause of lung cancer and another independent predictor of relapse. The risk of developing a malignant lung tumor in smokers is 20 times higher than in non-smokers [26].

Researchers have found that the risk of lifelong lung cancer in women who smoke is 11.6%, and among men it is 17.2%. The rates are much lower among those who have never smoked – 1.4% and 1.3%, respectively [27]. Passive smoking is also dangerous. According to some data, smoke inhaled from the air is even more harmful [28].

At the same time, smoking cessation helps to restore the bronchial mucosa. Patients who smoke at the time of diagnosis have a worse prognosis than those who quit the habit a few years ago. People who have never smoked have better treatment outcomes than smokers [29].

According to our study, recurrence of the disease was recorded in 43.9% of patients who smoked and among

**Table 2.** Univariate and multivariable analyses of clinical-pathological factors and immunohistochemical markers associated with recurrence-free survival

Parameters	Univariate analysis		Multivariable analysis	
	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
Age	1.1 (0.54–2.2)	0.79		
Sex	2.5 (1.1–5.4)	0.023	0.85 (0.44–1.64)	0.63
Pathologic TNM stage	0.89 (0.55–1.5)	0.65		
Tumor size	0.98 (0.49–2)	0.96		
Category T	0.61 (0.43–0.87)	0.0061	0.50 (0.35–0.72)	0.0001
Category N	0.81 (0.61–0.98)	0.016	0.85 (0.61–1.17)	0.33
Histological differentiation	0.49 (0.28–0.84)	0.01	0.44 (0.30–0.64)	0.0001
Tumor histology	1.2 (0.82–1.8)	0.33		
Lymphovascular invasion	1.2 (0.76–1.9)	1		
ECOG performance status	0.99 (0.3–3.3)	0.99		
Smoking status	0.38 (0.18–0.82)	0.013	0.44 (0.23–0.81)	0.008
Marital status	1.3 (0.56–2.8)	0.58		
Surgery	0.86 (0.59–1.3)	0.43		
PD-L	0.68 (0.39–1.2)	0.16		
Ki67	0.98 (0.48–2)	0.97		
p53	1.5 (0.7–3.2)	0.3		
EGFR	1.2 (0.83–1.8)	0.32		
ALK	0.95 (0.6–1.5)	0.81		

ECOG – Eastern Cooperative Oncology Group, EGFR – epidermal growth factor receptor, TNM – T: tumor size, N: metastases in regional lymph nodes, M: distant metastases

27.3% who had never smoked. This situation reflects global trends. At the time of lung cancer diagnosis, about 86% of patients quit smoking. But after a month, most of them return to the harmful habit. Within the first year after definitive lung cancer treatment, 60% of patients return to the habit. Instead, people who have abstained from smoking for six months continue to have a healthy lifestyle [30].

One of the most significant studies of this problem was conducted by Rachel *et al.* between 2010 and 2016 on 1,124 people who received radical treatment for non-small cell lung cancer. Thus, the 1-year survival rate among smokers was 77%, among former smokers 60%, and among current smokers 57%. After adjusting for disease, age, ECOG, and gender, the former and current smokers were more likely to die within one year. The risk of death was 25% lower among those who quit smoking [31]. Among the contingent of people who managed to refrain from smoking for more than a year, married women with a high level of education predominated [32].

Histological differentiation is an independent predictor of lung cancer recurrence. This risk factor may indicate the aggressiveness of cancer, sensitivity to drug and radiation therapy, and susceptibility to metastasis and recurrence. Lung cancer tumor aggressiveness and natural growth pattern play a role in prognosis [33].

The relationship between patient survival and the histological differentiation of the tumor was studied for squamous cell carcinoma of the cervix [34]. Poorly differentiated tumors have been significantly associated with older age, larger tumor size, and lymph node metastasis. In multivariable analysis, poorly differentiated tumors are associated with low patient survival compared to moderate- and well-differentiated tumors.

The significance of histological differentiation as an independent risk factor for lung cancer recurrence was proved in a large cohort study between 1992 and 2007. Patients with stage I lung cancer with a low degree of tumor differentiation, vascular invasion, or visceral pleura have lower 5-year recurrence-free survival rates than patients with stage II lung cancer but without risk factors (63% vs. 83%, respectively) [35].

Our study traces the relationship between the histological differentiation of the tumor and the incidence of lung cancer recurrence. Thus, recurrence occurred in 20% of patients with well-differentiated tumors, 26.4% with moderately differentiated tumors, and 92.3% with poorly differentiated tumors. 75% of patients had well- and moderately differentiated tumors in the study cohort.

Immunohistochemistry played one of the leading roles in determination of the individual biological properties of tumors. However, our research and the results of research by other scientists show that only a combination of several markers can predict the prognosis of the disease. We consider this to be one of the reasons why the model did not help predict disease recurrence. A literature review suggested that every single biomarker cannot be an independent predictor of relapse-free or overall survival. Nevertheless, correlation with the category TNM has often been observed [36].

**Table 3.** Incidence of lung cancer recurrence in patients with identified independent predictors

Independent predictors	Number of patients (%)	Patients with disease recurrence (%)
Category T		
T1	10 (9.6)	4 (40)
T2	66 (63.5)	26 (39.4)
T3	28 (26.9)	12 (42.8)
T3	0	0
Histological differentiation		
Well	10 (9.6)	2 (20)
Moderate	68 (65.4)	18 (26.4)
Poor	26 (25.0)	22 (92.3)
Smoking status		
Ever	82 (78.8)	36 (4.9)
Never	22 (21.2)	6 (27.3)

Folescu *et al.* found that patients with high Ki-67 expression were more likely to have advanced stages and a low degree of histopathological gradation of the tumor. The same study revealed that co-overexpression of Ki-67 and EGFR increases the metastatic potential of the tumor. These factors lead to low recurrence-free and overall survival. Due to the small selection, the results could not be statistically significant but could justify more detailed studies with a large cohort of patients [37].

Woodard *et al.* discovered that high PD-L1 expression is associated with low overall survival in patients with lung adenocarcinomas. At the same time, PD-L1 has no significant effect on disease-free recurrence [38].

In their study, Scagliotti *et al.* observed an association between overexpression of Ki-67, p53, and a high risk of recurrence in patients with lung cancer. It is worth noting that a high level of expression of a single biomarker did not allow a poor prognosis to be suspected [39, 40].

## Conclusions

Category T, histological differentiation, and smoking status are independent predictors of recurrence in patients with surgically resected non-small-cell lung cancer. Many investigations have studied the influence of biomarkers on disease prognosis in patients with lung cancer. However, it is essential to highlight that most information was collected in small geographical areas involving small cohorts of patients. As a result, research data may differ significantly. As we can see, most scientists believe that only a combination of several markers can predict the prognosis of the disease. We consider that the main reasons biological markers (PD-L1, Ki67, p53, EGFR, and ALK) did not help the model predict the disease recurrence was a small cohort of patients and their consideration as separate predictors. At the same time, for statistical reliability, it is necessary to conduct a study on a larger cohort of patients and compare the mutual influence of several biomarkers.

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## References

- Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209-249.
- Wu FZ, Huang YL, Wu CC, et al. Assessment of selection criteria for low-dose lung screening CT among Asian ethnic groups in Taiwan: from mass screening to specific risk-based screening for non-smoker lung cancer. *Clin Lung Cancer* 2016; 17: e45-e56.
- Donington JS, Kim YT, Tong B, et al. Progress in the management of early-stage non-small cell lung cancer in 2017. *J Thorac Oncol* 2018; 13: 767-778.
- Endo C, Sakurada A, Notsuda H, et al. Results of long-term follow-up of patients with completely resected non-small-cell lung cancer. *Ann Thorac Surg* 2012; 93: 1061-1068.
- NCCN Clinical Practice Guidelines in Oncology: Nonsmall cell lung cancer. Version 1.2022-December 7, 2021. Available from: <https://www.nccn.org/professionals>.
- Kauffmann-Guerrero D, Tufman A. Rare driver alterations in nonsmall cell lung cancer: novel targeted drugs. *Curr Opin Oncol* 2022; 34: 77-82.
- Kawaguchi Y, Hanaoka J, Oshio Y, et al. Decrease in performance status after lobectomy means poor prognosis in elderly lung cancer patients. *J Thorac Dis* 2017; 9: 1525-1530.
- Liu L, Zhang J, Wang G, et al. Prognostic factors of T2aNOMO (T3-4cmNOMO, stage IB) non-small-cell lung cancer after surgery: Single-center real-world research. *Thorac Cancer* 2021; 12: 3319-3326.
- Ronchi A, Pagliuca F, Zito Marino F, Accardo M, Cozzolino I, Franco R. Current and potential immunohistochemical biomarkers for prognosis and therapeutic stratification of breast carcinoma. *Semin Cancer Biol* 2021; 72: 114-122.
- Guo W, Sun S, Guo L, et al. Elevated SLC2A1 expression correlates with poor prognosis in patients with surgically resected lung adenocarcinoma: a study based on immunohistochemical analysis and bioinformatics. *DNA Cell Biol* 2020; 39: 631-644.
- Carreras J, Kikuchi Y, Miyaoka M, et al. A single gene expression set derived from artificial intelligence predicted the prognosis of several lymphoma subtypes; and high immunohistochemical expression of TNFAIP8 associated with poor prognosis in diffuse large B-cell lymphoma. *Al. 2020*; 1: 342-360.
- Detterbeck FC, Boffa DJ, Kim AW, et al. The eighth edition lung cancer stage classification. *Chest* 2017; 151: 193-203.
- Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. Introduction to the 2015 World Health Organization classification of tumors of the lung, pleura, thymus, and heart. *J Thorac Oncol* 2015; 10: 1240-1242.
- Kim CH, Kim SH, Park SY, Yoo J, Kim SK, Kim HK. Identification of EGFR mutations by immunohistochemistry with EGFR mutation-specific antibodies in biopsy and resection specimens from pulmonary adenocarcinoma. *Cancer Res Treat* 2015; 47: 653-660.
- Savic S, Diebold J, Zimmermann A-K, et al. Screening for ALK in non-small cell lung carcinomas: 5A4 and D5F3 antibodies perform equally well, but combined use with FISH is recommended. *Lung Cancer* 2015; 89: 104-109.
- Allemani C, Matsuda T, Di Carlo V, et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet* 2018; 391: 1023–1075.
- Fedorenko Z, Michailovich Y, Goulak L. Cancer in Ukraine, 2019–2020. Incidence, mortality, prevalence, and other relevant statistics. *Bulletin of National Cancer Registry of Ukraine* 2021, 22. Available from: [http://www.ncru.inf.ua/publications/BULL\\_21/index\\_e.htm](http://www.ncru.inf.ua/publications/BULL_21/index_e.htm).
- Smorodska O, Moskalenko Y, Vynnychenko O, et al. Lung cancer prevalence: from local to global. *Art Med.* 2021; 2: 116-123.
- Bradbury P, Sivajohanathan D, Chan A, Kulkarni S, Ung Y, Ellis PM. Postoperative adjuvant systemic therapy in completely resected non-small-cell lung cancer: a systematic review. *Clin Lung Cancer* 2017; 18: 259-273.e8.
- Choi J, Oh JY, Lee YS, et al. Clinical efficacy of adjuvant chemotherapy in stage IB (< 4 cm) non-small cell lung cancer patients with high-risk factors. *Korean J Intern Med* 2022; 37: 127-136.
- Suzuki M, Yoshida S, Moriya Y, et al. Surgical outcomes of newly categorized peripheral T3 non-small cell lung cancers: comparisons between chest wall invasion and large tumors (> 7 cm). *Interact Cardiovasc Thorac Surg* 2010; 11: 420-424.
- Chen M, Cao J, Zhang B, Pan L, Cai X. A nomogram for prediction of overall survival in patients with node-negative gallbladder cancer. *J Cancer* 2019; 10: 3246-3252.
- Te Grootenhuys NC, Pouwer AW, de Bock GH, et al. Prognostic factors for local recurrence of squamous cell carcinoma of the vulva: a systematic review. *Gynecol Oncol* 2018; 148: 622-631.
- Dong W, Horiuchi K, Tokumitsu H, et al. Time-varying pattern of mortality and recurrence from papillary thyroid cancer: lessons from a long-term follow-up. *Thyroid* 2019; 29: 802-808.
- Phung MT, Tin Tin S, Elwood JM. Prognostic models for breast cancer: a systematic review. *BMC Cancer* 2019 14; 19: 230.
- Biesalski HK, Bueno de Mesquita B, Chesson A, et al. European Consensus Statement on Lung Cancer: risk factors and prevention. *Lung Cancer Panel. CA Cancer J Clin* 1998; 48: 167-176.
- Villeneuve PJ, Mao Y. Lifetime probability of developing lung cancer, by smoking status, Canada. *Can J Public Health* 1994; 85: 385-388.
- Schick S, Glantz S. Philip Morris toxicological experiments with fresh sidestream smoke: more toxic than mainstream smoke. *Tob Control* 2005; 14: 396-404.
- Warren GW, Alberg AJ, Cummings KM, Dresler C. Smoking cessation after a cancer diagnosis is associated with improved survival. *J Thorac Oncol* 2020; 15: 705-708.
- Simmons VN, Litvin EB, Jacobsen PB, et al. Predictors of smoking relapse in patients with thoracic cancer or head and neck cancer. *Cancer* 2013; 119: 1420-1427.
- Gemine RE, Ghosal R, Collier G, et al. Longitudinal study to assess impact of smoking at diagnosis and quitting on 1-year survival for people with non-small cell lung cancer. *Lung Cancer* 2019; 129: 1-7.
- Gummerson SP, Lowe JT, Taylor KL, Lobo T, Jensen RE. The characteristics of patients who quit smoking in the year following a cancer diagnosis. *J Cancer Surviv* 2022; 16: 111-118.
- Tang EK, Chen CS, Wu CC, et al. Natural history of persistent pulmonary subsolid nodules: long-term observation of different interval growth. *Heart Lung Circ* 2019; 28: 1747-1754.
- Matsuo K, Mandelbaum RS, Machida H, et al. Association of tumor differentiation grade and survival of women with squamous cell carcinoma of the uterine cervix. *J Gynecol Oncol* 2018; 29: e91.
- Maeda R, Yoshida J, Ishii G, Hishida T, Nishimura M, Nagai K. Risk factors for tumor recurrence in patients with early-stage (stage I and II) non-small cell lung cancer: patient selection criteria for adjuvant chemotherapy according to the seventh edition TNM classification. *Chest* 2011; 140: 1494-1502.
- Puderecki M, Szumilo J, Marzec-Kotarska B. Novel prognostic molecular markers in lung cancer. *Oncol Lett* 2020; 20: 9-18.
- Folescu R, Levai CM, Grigoras ML, et al. Expression and significance of Ki-67 in lung cancer. *Rom J Morphol Embryol* 2018; 59: 227-233.
- Woodard GA, Jones KD, Jablons DM. Lung cancer staging and prognosis. *Cancer Treat Res* 2016; 170: 47-75.
- Scagliotti GV, Ferrari G, Leonardo E, Marchetti A, Torri V, Flann M. Prospective evaluation of the prognostic value of p53, K-ras, Ki67 in radically resected non-small cell lung cancers randomized in a clinical trial of adjuvant chemotherapy (ALPI trial). *Lung Cancer* 1997; 18: 1.
- Ciancio N, Galasso MG, Campisi R, Bivona L, Migliore M, Di Maria GU. Prognostic value of p53 and Ki67 expression in fiberoptic bronchial biopsies of patients with non-small cell lung cancer. *Multidiscip Respir Med* 2012; 7: 29.

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