Lithuanian University of Health Sciences

MEDICAL ACADEMY

FACULTY OF MEDICINE

Nir Eshkol

Analysis of Clinical and Laboratory Data in Lung Adenocarcinoma with Activating Endothelial Growth Factor Receptor (EGFR) Mutation

Department of Pulmonology

Scientific supervisor:

Prof. Skaidrius Miliauskas

Kaunas, Lithuania

2021-2022

Table of Contents

Summary 3

Conflict of interests 5

Acknowledgment 6

Ethics committee study permission 7

Abbreviations 8

Introduction 9

The aim and the objectives of the study 10

Literature review 11

EGFR mutation: biology, epidemiology, and treatment options 11

ALK: biology, epidemiology and significance in treatment 12

KRAS: biology, epidemiology and significance in treatment 12

BRAF: biology, epidemiology and significance in treatment 13

HER2: biology, epidemiology and significance in treatment 13

ROS1: biology, epidemiology and significance in treatment 14

..... 14

Methodology 15

Results 16

Discussion 24

Conclusions 26

References 27

Summary

Author: Nir Eshkol

Title: Analysis of clinical and laboratory data in lung adenocarcinoma with activating endothelial growth factor receptor (EGFR) mutation.

Aim: To analyse the clinical and laboratory data in lung adenocarcinoma with activating endothelial growth factor receptor (EGFR) mutation.

Objectives:

1. To establish the prevalence of lung adenocarcinoma with EGFR activating mutation in Kaunas clinics in the years 2020-2021.
2. To describe the clinical characteristics (stage, age during diagnosis, gender, smoking status) in the groups of patients with mutated type (MT) EGFR gene and wild type (WT) gene.
3. To compare clinical and laboratory data of patients with I-II and III-IV stage of disease.
4. To describe and compare laboratory test results (Hb, WBC, LYM, Bilirubin, AST, ALT, and PLT) in lung adenocarcinoma patients with WT EGFR gene and MT EGFR gene.

Methodology: This research was performed at the Department of pulmonology, Hospital of Lithuanian University of Health Sciences Kaunas Clinics. A retrospective analysis of the data was performed, encompassing patients who were diagnosed with histologically confirmed various stage lung adenocarcinoma during the period of 2020-2021 years. 120 patients were included in the study. Subjects were divided to those with a mutated type (MT) EGFR mutation and those with a wild type (WT) EGFR gene. The incidence of EGFR mutation, clinical characteristics and laboratory data were described and compared. The statistical analysis was performed using “SPSS” (Statistical Package for the Social Science), version 28.0 and Microsoft Excel 2013. The statistical significance chosen for the tested hypotheses was 0.05.

Results: Out of an overall sample size of 120 patients, 13 patients without a known EGFR status were excluded from the study. 24 had a MT EGFR gene while 83 had a WT EGFR gene. Among the 24 MT EGFR patients mutations were located on the 19 exon (n=11), 21 exon (n=8), 20 exon (n=4) and in 1 patient MT EGFR was found in both 19 and 21 exons. Mean age during diagnosis with MT EGFR for men was 68.9 years for men and 66.4 years for women. Statistically significant dependence on gender and smoking was confirmed: MT EGFR was more common in women (men- 41.7%, women- 58.3%) and non-smokers

(smokers- 23.5%, non-smokers- 58.5%, past-smokers- 17,6%) while WT EGFR was more common in smoking men. Blood analysis showed significantly higher numbers of WBC and PLT in bigger tumour size, when metastatic disease and a more progressive stage of disease was confirmed. EGFR MT patients presented with a significantly lower lymphocyte count. No significant correlations between any other lab data mean values and EGFR status were found.

Conclusions:

1. During the period of 2020-2021 years most patients diagnosed with lung adenocarcinoma had a WT EGFR. Patients with MT EGFR had most commonly a 19^{th} exon mutation.

2. Significantly more non-smoking women were diagnosed with MT EGFR lung adenocarcinoma, while more smoking men had WT EGFR lung adenocarcinoma diagnosis.

3. When the more advanced stage of the disease was established, significantly higher numbers of PLT and WBC were detected.

4. LYM number was significantly lower in patients with MT EGFR compared to WT EGFR.

Conflict of interests

There is no conflict of interests

Acknowledgment

I would like to thank Professor Skaidrius Miliauskas, Head of Department of Pulmonology of the Hospital of Lithuanian University of Health Sciences Kaunas Clinics for his expert advice, guidance and suggestions that helped me to complete my master thesis. Also much gratitude to Dr. Neringa Vagulienė for her helpful advice.

Ethics committee study permission

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS

BIOETIKOS CENTRAS

Kodas 302536989, Tilžės g. 18, LT- 47181, Kaunas,tel.: (8 37) 327233, www.lsmuni.lt., el.p.: bec.registracija@lsmuni.lt

Medicinos akademija (MA) 2022.03.09 Nr. BEC-MF-297
Vientisųjų studijų programa – Medicina
VI k. studentui Nir Eshkol
Darbo vadovė prof. Skaidrius Miliauskas
LSMUL KK Pulmonologijos klinika

DĖL PRITARIMO TYRIMUI

LSMU Bioetikos centras, įvertinęs Nir Eshkol pateiktus dokumentus, studento tiriamajam darbui tema „The study of advanced lung adenocarcinoma with endothelial growth factor receptor (EGFR) activating mutation“ pritaria*.

dr. Elmantas Peikštis

* Pastaba: šis pritarimas neatleidžia tiriamąjį mokslinį darbą vykdančių asmenų nuo prievolės laikytis Bendrojo duomenų apsaugos reglamento nuostatų ir nuo atsakomybės gauti nacionalinio arba regioninio bioetikos komiteto leidimą, jei toks leidimas būtinas pagal LR Biomedicininių tyrimų etikos įstatyme numatytus reikalavimus.

Abbreviations

EGFR – Endothelial growth factor receptor

MT – Mutated type

WT – Wild type

NSCLC – Non-small cell lung caner

ADC - Adenocarcinoma

ALK – Anaplastic lymphoma kinase

HER – Human epidermoid growth factor

KRAS – Kirsten rat sarcoma

ROS1 – ROS proto-oncogene 1

TKI – Tyrosine kinase inhibitor

Introduction

Lung cancer is the leading cause of cancer death in men aged ≥40 years and women aged ≥60 years, causing far more deaths than breast cancer, prostate cancer, and colorectal cancer combined [1]. Tobacco smoking is the major cause of all major histological types of lung cancer, with the duration of smoking should be considered the strongest determinant of lung cancer risk in smokers. Occupational exposures play a significant role in lung cancer aetiology, and the risk of lung cancer is increased among workers employed in a number of industries: asbestos, silica, radon, heavy metals and polycyclic aromatic hydrocarbons. Other risk factors for lung cancer in none-smokers are air pollution, ionizing radiation, chronic inflammation as a result of other pulmonary conditions and family predisposition [2]. There are 2 main forms of lung cancer: non-small cell lung cancer (85% of patients) and small cell lung cancer (15%). The WHO has classified NSCLC into 3 main types: adenocarcinoma, squamous cell carcinoma, and large cell. The 3 types of NSCLC are adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Adenocarcinoma is the most common type of NSCLC and accounts for approximately 40% of lung cancers. Adenocarcinoma arises from alveolar cells located in the smaller airway epithelium [3]. Nearly 75% of patients with NSCLC are diagnosed at an advanced stage. The 5-year survival rate of NSCLC is approximately 15.9%, while that of advanced NSCLC is much lower, ranging from 2.8% to 14.6% [4]. The treatment of lung cancer has transformed from empirical cytotoxic chemotherapy to a more personalized, based on the genetic alterations of the patient's tumour. The identification of targetable gene alterations has transformed the management of lung cancer, with the incorporation of tumour genotyping to allow individualized therapy and leading to remarkable responses in selected patients treated with matched tyrosine kinase inhibitors (TKIs) [5]. In 2009, the first randomized clinical trial (the Iressa PanAsia Study [IPASS]) showed that, for advanced NSCLC patients with an activating EGFR mutation, initial treatment with an EGFR TKI was superior to standard platinum-based chemotherapy [6]. Since 2018, the American Society of Clinical Oncology has recommended routine mutation testing for driver genes including EGFR, ALK, ROS1 and BRAF in clinical practice for patients with metastatic NSCLC. Although there are currently no targeted drugs for Kirsten rat sarcoma *(*KRAS*)* or neuroblastoma rat sarcoma (NRAS) mutated NSCLCs, mutation testing for these genes has also been recommended due to their proven impact on clinical outcomes of NSCLC patients [7].

The aim and the objectives of the study

Aim: To analyse the clinical and laboratory data in lung adenocarcinoma with activating endothelial growth factor receptor (EGFR) mutation.

Objectives:

1. To establish the prevalence of lung adenocarcinoma with EGFR activating mutation in Kaunas clinics in the years 2020-2021.
2. To describe the clinical characteristics (stage, age during diagnosis, gender, smoking status) in the groups of patients with mutated type (MT) EGFR gene and wild type (WT) gene.
3. To compare clinical and laboratory data of patients with I-II and III-IV stage of disease.
4. To describe and compare laboratory test results (Hb, WBC, LYM, Bilirubin, AST, ALT, and PLT) in lung adenocarcinoma patients with WT EGFR gene and MT EGFR gene.

Literature review

EGFR mutation: biology, epidemiology, and treatment options

Endothelium growth factor, a transmembrane glycoprotein with cytoplasmic tyrosine kinase activity is often mutated in several cancers, including lung adenocarcinoma. Thus EGFR is an important therapeutic target for the treatment of these cancers [8]. EGFR is one of the four members of the human epidermal growth factor (HER) family transmembrane receptors (HER1/EGFR, HER2, HER3, and HER4). Each HER receptor is an inactive monomer that dimerizes with a receptor of the same type or with another member of the HER family in response to ligand binding. The receptor activation triggers a complex downstream signalling network which leads cell replication. The dysregulated receptor function or disruptions in any downstream EGFR processes may result in cell transformation and malignancy [7]. EGFR gene most common and clinically relevant mutations, accounting for approximately 80–90% of EGFR-mutated patients, occur in two hot spots in the tyrosine kinase (TK) domain: in-frame deletions in exon 19 (most frequently E746-A750) and L858R missense mutation in exon 21 leading to a base substitution of arginine to leucine at residue 858. The NSCLCs harbouring these mutations are responsive to EGFR tyrosine inhibitors and kinase inhibitors (TKIs) [9]. EGFR mutations have been reported to accumulate in young, Asian, and never-smoking patients. There is a predominance of female sex and, in addition, a higher prevalence in lepidic and micropapillary predominant ADCs [10]. EGFR mutations are observed in 10–20% of patients not of East Asian descent with NSCLC and in approximately 40% of Asian patients. EGFR gene mutations mostly occur in adenocarcinomas, younger women and girls, and never-smokers, For EGFR-mutated NSCLC patients, EGFR-TKIs have a significant advantage in terms of progression free survival (PFS), quality of life and tolerance, thus establishing the role of EGFR-TKIs in first-line setting in advanced EGFR-mutated NSCLC patients. The first-generation reversible anti-EGFR tyrosine kinase inhibitors erlotinib and gefitinib were first approved as second line therapy after cyctotoxic chemotherapy but are now recommended as first line therapy in patient with advanced EGFR positive lung cancer. Second generation irreversible TKIs, afatinib, were developed to overcome the acquired resistance of some NSCLC tumors to erlotinic and gefitinib [11].

ALK: biology, epidemiology and significance in treatment

In the past decade, researchers have identified genetic alterations involving anaplastic lymphoma kinase (ALK), which is most frequently fused with echinoderm microtubule associated protein-like 4 (EML4), as a key oncogenic driver in NSCLC, and occurs mainly in lung adenocarcinoma [12]. The *ALK* gene is located on the short arm of chromosome 2 (2p23), belongs to the insulin receptor superfamily, and encodes for the ALK protein. There are three types of *ALK* gene mutations: rearrangement (ALK-R), amplification (ALK-A), and point mutation. *ALK* gene rearrangement is a driving mutation underlying the development of NSCLC, creating an oncogenic ALK tyrosine kinase that activates many downstream signalling pathways resulting in increased cell proliferation and survival [13]. ALK gene rearrangements are observed in about 4% to 8% of the patient's with lung cancer. In general, *EML4-ALK*-mutated patients present at a younger age, in women with never or light smoking (<10 pack-years) history. Up to 55% of the patients with all NSCLC present with stage IV disease with poor prognosis [8,14].

KRAS: biology, epidemiology and significance in treatment

The KRAS proteins belong to the small guanosine triphosphate (GTP)ase family, involved in intracellular signalling. In response to extracellular signalling, KRAS proteins switch between two GTP bound "on" state and unbound "off" state. When activated a downstream signalling pathway eventually promotes cellular division and proliferation [7]. KRAS, mutated to an oncogenic form by introducing amino-acid substitutions at codons 12, 13 and 61, are detected in 25–33% of patients with lung adenocarcinomas from the United States and Europe and 8% from China [15]. The KRAS mutation was found predominantly in smoking patients, whereas the EGFR mutation occurs predominantly in female, non-smoking patients of East Asian origin [16]. Despite the successes with targeted therapy for driver mutations, personalized therapy for patients with KRAS MT is still under development. Platinum doublet chemotherapy is the standard of care [17].

BRAF: biology, epidemiology and significance in treatment

B-RAF is a serine/threonine kinase that lies downstream of RAS in the RAS-RAF-MEK-ERK signalling pathway, a key molecular cascade that regulates cell growth. Mutations in BRAF are most commonly seen in melanoma, where BRAF *V600E* is a driver mutation that can be effectively targeted with selective BRAF and/or MEK inhibitors. BRAF mutations are also detected in 1% to 3% of NSCLC. In opposed to melanoma, many of these non-V600E mutations show only intermediate or low kinase activity, and preclinical data suggest that non-V600E mutant BRAF kinases are resistant to BRAF targeted therapy, although some may be sensitive to downstream pathway inhibitors such as MEK inhibitors [18]. Most of the studies also showed no association of BRAF mutation and smoking status among the different mutations occurring in the BRAF gene, BRAF ^{V600E} is the most common. The BRAF^{V600E} mutation was significantly correlated with female and non-smoker NSCLC patients [19].

HER2: biology, epidemiology and significance in treatment

The HER2 protein product is a member of the HER/ErbB family of tyrosine kinases receptors. The common consequence of all the alterations in the HER2 gene/protein is the receptor's hyper-activation which triggers multiple signalling pathways resulting in uncontrolled cell proliferation. Three HER2 activating mechanisms have been described in NSCLC: gene mutation (1%-4% of cases), gene amplification (2%-5%) and protein overexpression (2%-30%). Exon 20 insertions affecting the kinase domain are the most frequent HER2 mutations (96%) [20]. Unlike EGFR mutations, the prevalence of HER2 mutations appears to be similar between Asian and white patient populations. Those harbouring HER2 mutations tend to be light smokers or never-smokers and are more likely to be women. Patients with tumors with HER2 mutations are younger, with a median age of approximately 60 years [21]. In contrast to breast cancer, the role of HER2-targeting drugs as monotherapy or in combination with chemotherapy remains unclear in lung cancer [22].

ROS1: biology, epidemiology and significance in treatment

The ROS1 gene is located on chromosome 6 (region 6q22.1) and generates two dominant splice variants of ROS1 encoded by either 43 or 44 exons. ROS1 fusion-positive NSCLCs are the most common of these cancers owing to the high incidence of NSCLCs in general. In NSCLCs, ROS1 fusions occur predominantly in lung adenocarcinomas in younger (median age of 50 years) never smokers (~80%). the majority of recurrent ROS1 fusions in NSCLCs result from interchromosomal translocations [23]. Chemotherapy remains a standard treatment for ROS1-driven NSCLC [24].

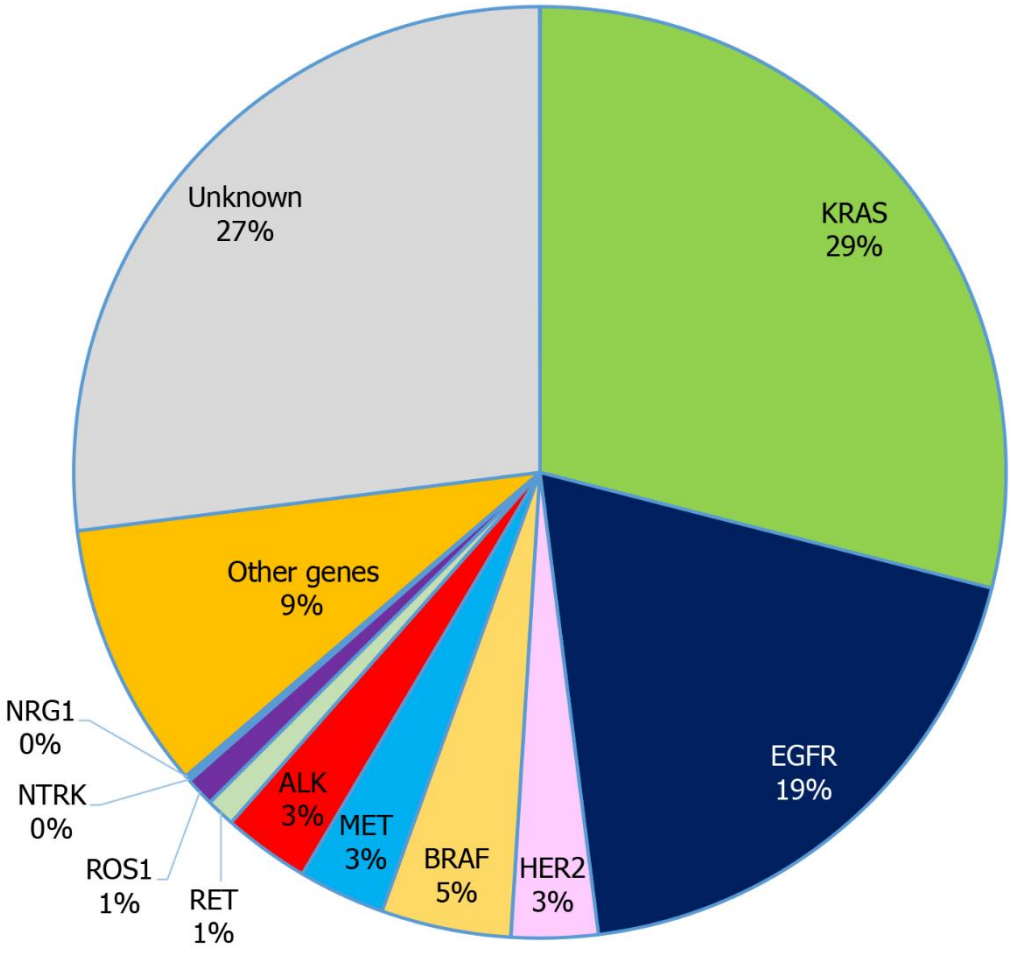


Figure 1. *Incidence of oncogenic drivers in non-small cell lung cancer (NSCLC)* [7].

Methodology

This research was performed in LUHS KK after an approval from the bioethics center was received (BEC-MF-297). A retrospective data analysis was performed, encompassing patients who were diagnosed with various stage lung adenocarcinoma at the Lithuanian University of Health Science, Kaunas Clinics, department of pulmonology during the period of 2020-2021 years.

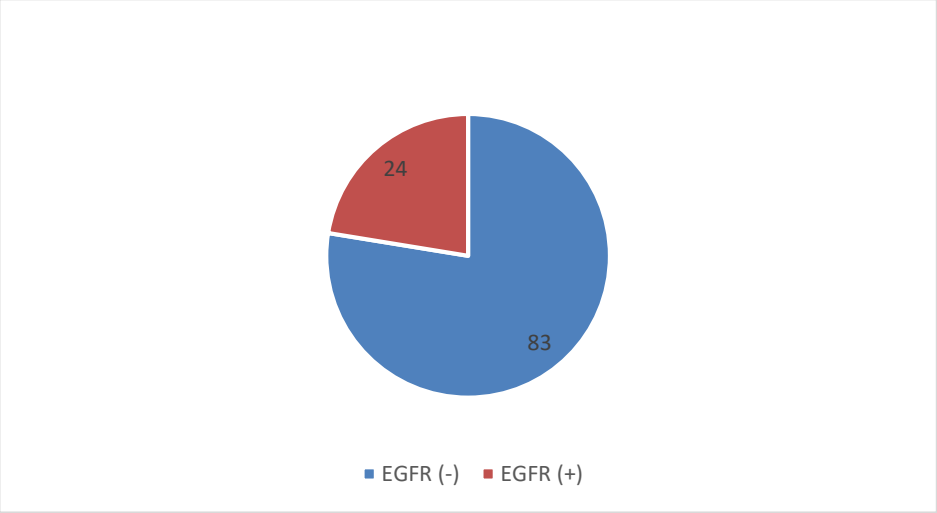
The statistical analysis was performed using "SPSS" (Statistical Package for the Social Science), version 28.0 and Microsoft Excel 2013.

The following methods of descriptive statistics were used for the investigation of the features in question: mean, standard deviation (SN). The probable distribution of the null hypothesis was assessed using the Kolmogorov-Smirnov test. Mann-Whitney U test was used for small sample size ($n<20$), qualitative sizes and when the conditions of probable distribution were not met. When > 2 groups were present – Kruskall Wallis test was applied. For qualitative data independency or homogeneity assessment exact chi-square (χ^2) test was applied. The correlation between parameters was determined using regressive analysis Pearson method. The statistical significance of the tested hypotheses was chosen to be 0.05.

Results

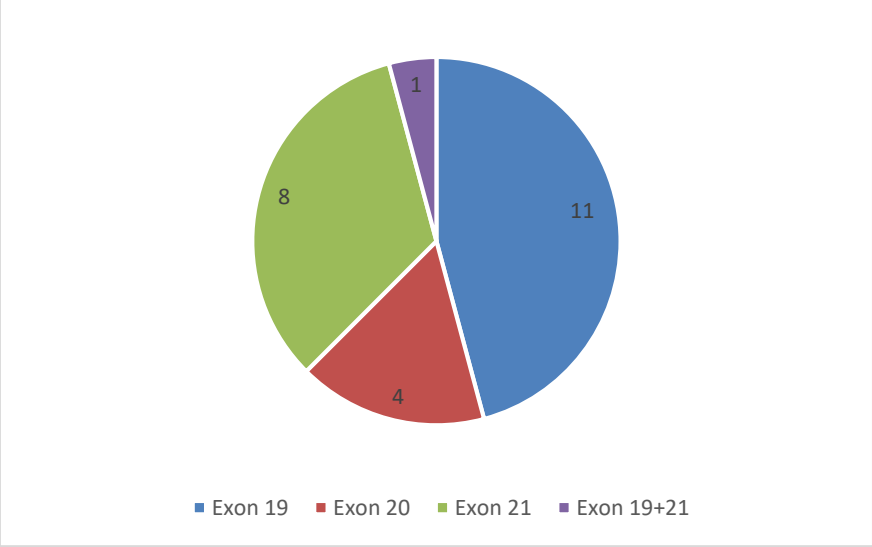
In this study cases of 120 patients diagnosed with lung adenocarcinoma in the years 2020-2021 were reviewed. 24 patients were found to be positive for EGFR activating mutation, 83 were negative. In 13 cases information regarding the EGFR status was not found. They were no longer included in the analysis. Figure 2 shows the distribution among the cases who presented with EGFR status information.

Figure 2. *Distribution of lung adenocarcinoma according to EGFR status*



Out of the 24 patients found to be carrying a MT EGFR, in 11 patients the mutation was found to be located on exon 19, in 8 patients the mutation was found on the exon 21, and in 4 patients- on the exon 20. 1 patient has EGFR mutation in both exons 19 and 21.

Figure 3. *Distribution of lung adenocarcinoma mutated type EGFR cases according to exon localization*



The mean age at the time of diagnosis for the patients found to be carrying MT EGFR was 70.3 years: men- 68.9 years, women- 66,41 years. For WT EGFR patients the mean age was found to be 67.43 years. The gender, smoking status and the stage of the disease at time of diagnosis are presented in table 1. A statistically significant relation of EGFR status to smoking status and gender was found. MT EGFR was less associated with patients who smoke or have a history of smoking.

Table 1. *Characterization of the age, gender, smoking status and disease stage among the researched cases depending on EGFR status*

Criteria		**EGFR MT n (%)**	**EGFR WT n (%)**	**p**
Age	Male mean ±SN	68.9 ±9.9	66.4 ±8.7	NS
	Female mean ±SN	71.4 ±10.7	70.3 ±10.6	
Gender	Male, n (%)	10 (41.7)	63 (75.9)	p<0.05
	Female, n (%)	14 (58.3)	20 (24.1)	
Smoking status	Smoking, n (%)	4 (23.5)	35 (50.7)	p<0.05
	Past-smoker, n (%)	3 (17.6)	16 (23.3)	
	Non-smoking, n (%)	10 (58.8)	18 (26.1)	
Stage	I	1	6	NS
	II	2	9	
	III	2	13	
	IV	18	47	

In the patients with a known EGFR status the mean value of the following laboratory data was calculated: haemoglobin (Hb), leukocytes (WBC), lymphocytes, platelets (PLT), aspartate transaminase (AST) and alanine transferase (ALT). The data reflects the last bloodwork taken before biopsy was performed and lung adenocarcinoma was diagnosed and staged.

The number of PLT showed statistically significant dependence on the size of the tumour (T), presence of metastasis (M) and the stage of the disease. Hb level showed no statistically significant dependence with any of these factors. This data is presented in table 2.

Table 2. *The mean concentration of Hb and PLT according to stage and TNM classification*

		n	Hb (g/L)	p	PLT (10*9/L)	p
T	**T1-2**	31	125.8±17.7	NS	252.4±74.5	p<0.05
	T3-4	59	130.2±19.9		309.6±123.5	
N	**N0-1**	31	129.2±20.9	NS	247.6±105.4	NS
	N2-3	59	129,1±18,2		295.4±117.0	
M	**M0**	33	124,7±15,8	NS	254.6±90.3	p<0.05
	M1	58	131,2±20,5		307.6±119.7	
Stage	**I**	6	124±13,6	NS	230.6±29.7	p<0.05
	II	10	130.0±17.0		228.3±103.5	
	III	15	125.67±13.6		269.2±99.1	
	IV	58	130.9±20.6		311.2±118.6	

The number of WBC was statistically significant dependant on the size of the tumour (T), presence of metastasis (M) and the stage of the disease. Number of LYM was no statistically significant dependant with any of these factors. This data is presented in table 3.

Table 3. *The dependence of WBC and LYM numbers on stage and TNM stage.*

		n	WBC (10*9/L)	p	LYM (10*9/L)	p
T	**T1-2**	31	7.5±2.5	p<0.05	1.6±0.6	NS
	T3-4	59	9.4±3.4		1.8±1.5	
N	**N0-1**	31	8.1±3.3	NS	1.7±0.7	NS
	N2-3	59	9.0±3.1		1.7±1.5	
M	**M0**	33	7.1±1.8	p<0.05	1.6±0.6	NS
	M1	58	9.7±3.4		1.8±1.6	
Stage	**I**	6	6.6±1.5	p<0.05	2.0±1.2	NS
	II	10	7.1±2.1		1.7±0.2	
	III	15	7.2±1.4		1.6±0.4	
	IV	58	9.7±3.5		1.8±1.6	

AST and ALT values show no statistically significant dependence neither on TNM stage nor on disease stage, as presented in table 3.

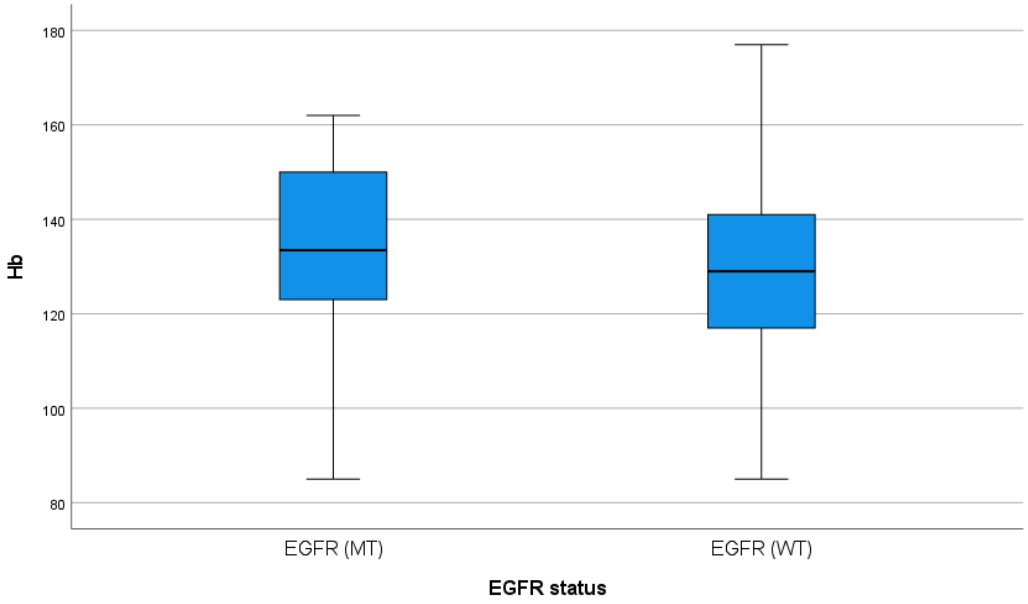
Table 3. *The dependence of mean concentration of AST and ALT on disease stage*

		n	AST (U/L)	p	ALT (U/L)	p
T	**T1-2**	31	21.6±8.5	NS	19.5±10.1	NS
	T3-4	59	23.9±10.2		26.1±24.4	
N	**N0-1**	31	21.2±6.0	NS	20.4±12.2	NS
	N2-3	59	23.6±11.0		24.7±23.4	
M	**M0**	33	20.9±8.2	NS	19.9±11.3	NS
	M1	58	24.1±10.3		25.8±24.2	

Stage	**I**	6	19.0±4.0	NS	17.0±8.1	NS
	II	10	21.0±8.6		15.0±5.5	
	III	15	22.1±8.9		21.2±11.8	
	IV	58	24.1±10.3		26.3±21.3	

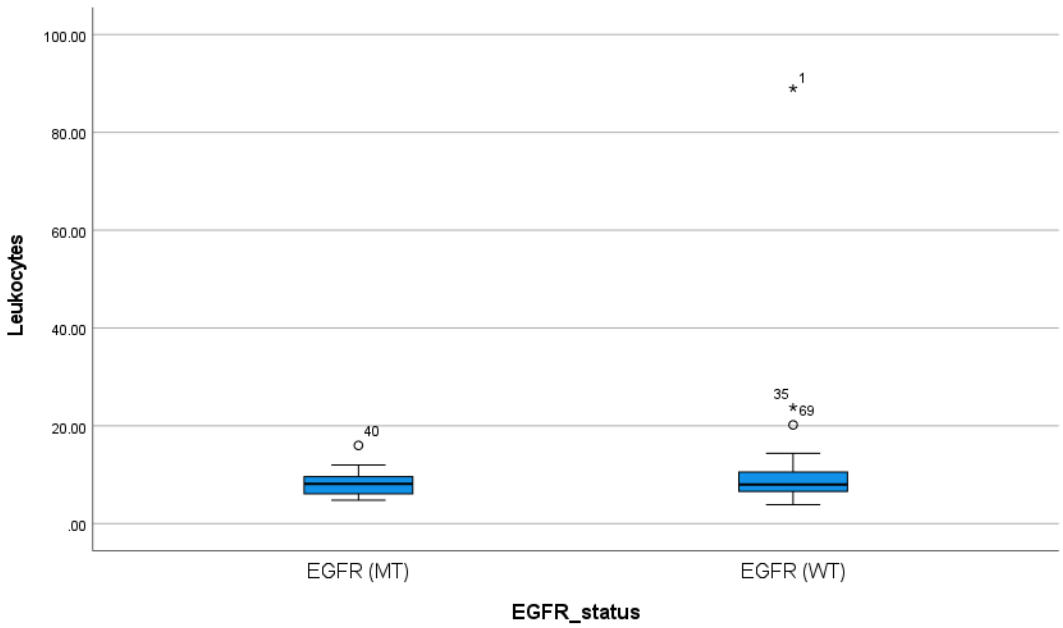
The mean Hb level calculated in patients with WT EGFR was 127.5 G/L, compared to patients with MT EGFR, with Hb mean of 133.8 G/L.

Figure 4. *The laboratory values of haemoglobin in patients with WT and MT EGFR.*



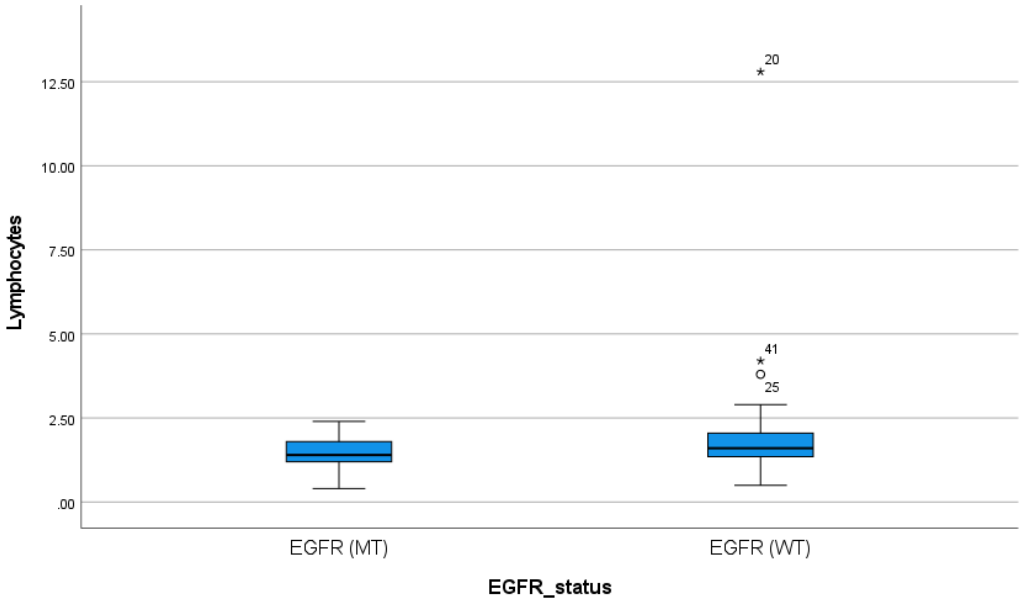
The mean WBC level calculated in patients with WT EGFR was 10.0 10*^{9}/L, compared to patients with MT EGFR, with WBC mean of 8.1 10.0 10*^{9}/L.

Figure 5. *The laboratory values of WBC in patients with WT and MT EGFR.*



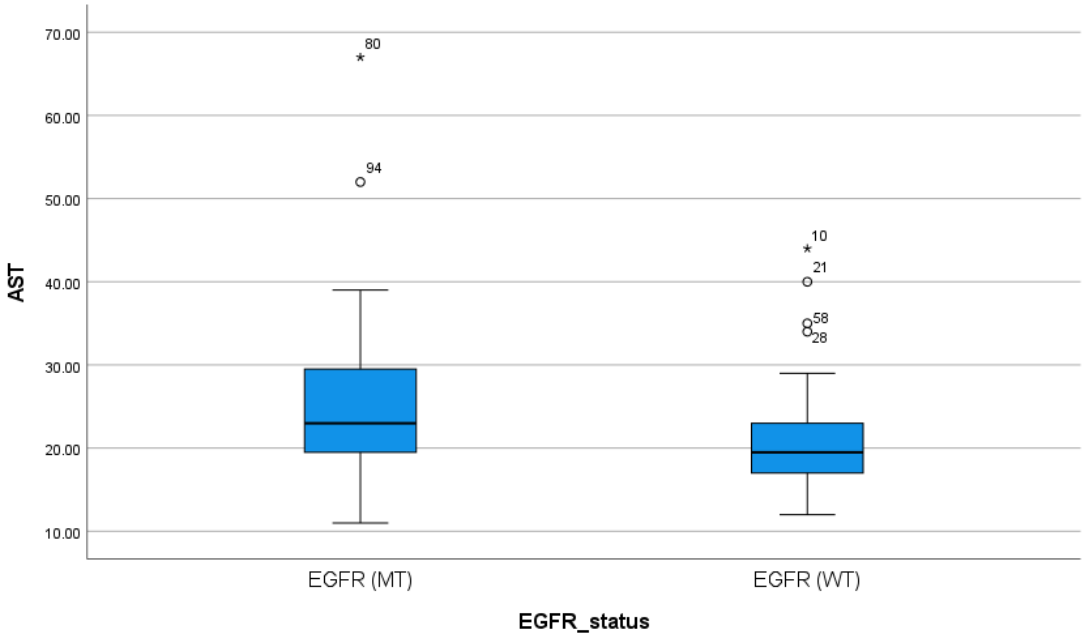
The mean LYM level calculated in patients with WT EGFR was 1.8± 10*^{9}/L, compared to patients with MT EGFR, with LYM mean of 1.4 10*^{9}/L.

Figure 6. *The laboratory values of lymphocytes in patients with WT and MT EGFR.*



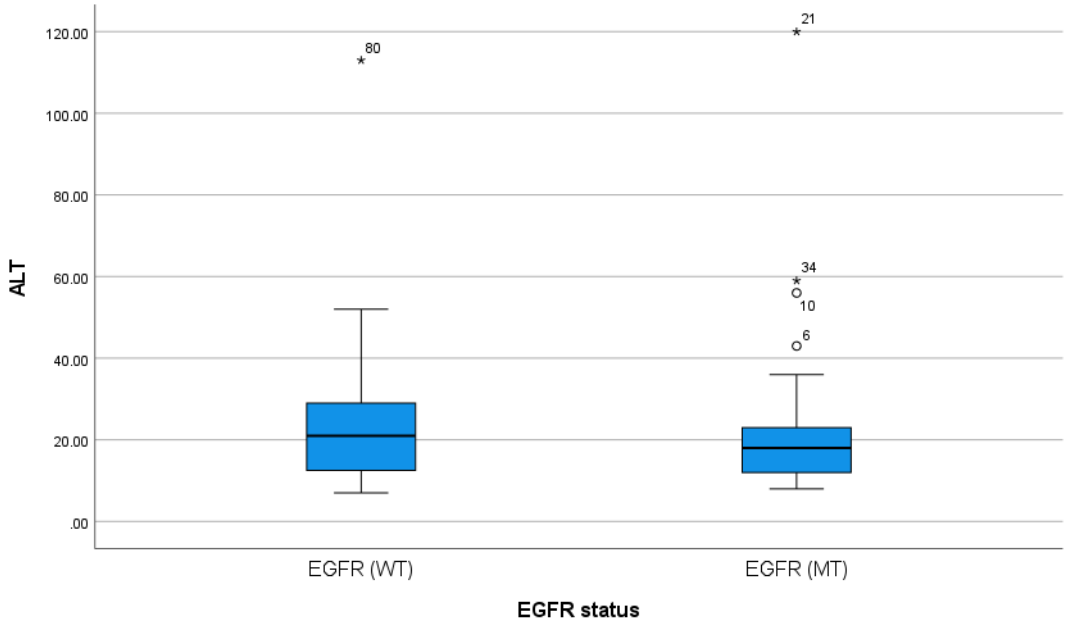
The mean AST serum level calculated in patients with WT EGFR was 21.3 U/L, compared to patients with MT EGFR, with AST mean of 26.5 U/L.

Figure 7. *The laboratory values of serum aspartate aminotransferase (AST) in patients with WT and MT EGFR.*



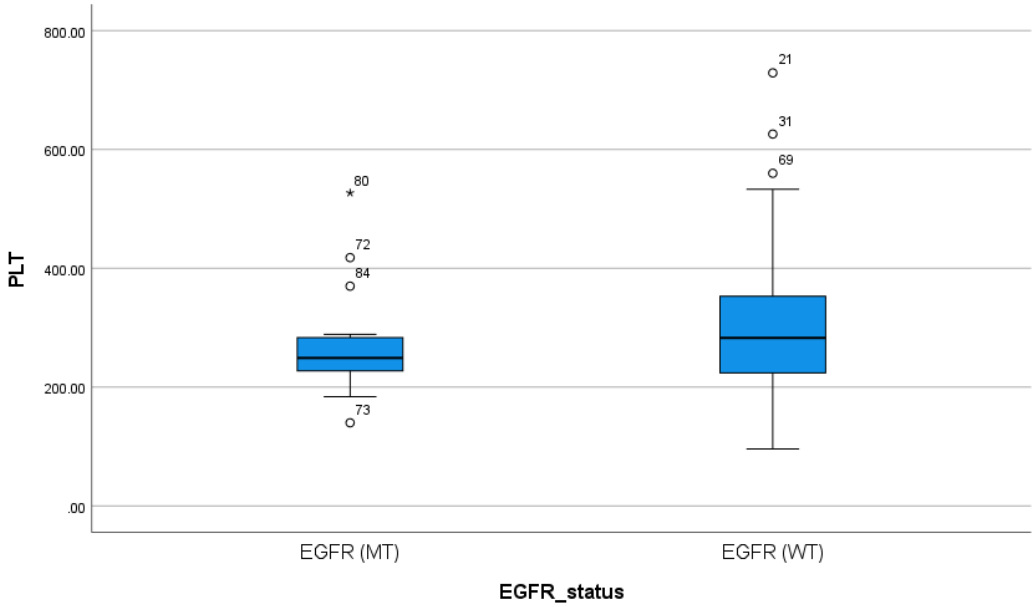
The mean ALT serum level calculated in patients with WT EGFR was 22.3 U/L, compared to patients with MT EGFR, with ALT mean of 26.4 U/L.

Figure 8. *The laboratory values of serum alanine transferase (ALT) in patients with WT and MT EGFR.*



The platelet level calculated in patients with WT EGFR was $294.3*10^9$/L, compared to patients with MT EGFR, with PLT mean of $267.2*10^9$/L.

Figure 9. *The laboratory values of platelets in patients with WT and MT EGFR.*



The mean value of the laboratory data collected in this research are all described in table 2. Mean values of the laboratory data analysed above is presented in table 4. None of the values analysed presented statistically significant dependence between EGFR status and serum levels of Hb, WBC, LYM, PLT or liver enzymes (AST, ALT).

Table 4. *Mean values of the collected laboratory data*

	EGFR MT M±SN (n)	**EGFR WT M±SN (n)**	**p**
Hb (G/L)	133.8±20.2 (20)	127.5± 18.7 (71)	NS
WBC 10 (10*^{9}/L)	8.1± 2.7 (20)	10.0± 10.1 (71)	NS
LYM (10*^{9}/L)	1.4± 0.5 (20)	1.8± 1.4 (71)	NS
PLT (10*^{9}/L)	267.2± 85.7 (20)	294.3± 118.7 (71)	NS
AST (U/L)	26.5± 13.6 (19)	21.3± 6.8 (42)	NS
ALT (U/L)	26.4± 24.4 (19)	22.4± 18.7 (45)	NS

No statistically significant correlation was detected between EGFR status and laboratory values.

Table 5. Correlation between laboratory data values and EGFR status

	EGFR status		
	n	**r**	**p**
Hb	91	0.143	NS
WBC	91	-0.079	NS
LYM	91	-0.154	NS
PLT	91	-0.128	NS
AST	61	0.213	NS
ALT	64	0.065	NS

The adenocarcinoma patients were divided into 2 groups according to the mean values of the laboratory data. The presence of EGFR mutation showed a statistically significant association with a lower lymphocyte count. Other laboratory values showed no statistically significant relations with the EGFR status in patients (table 6).

Table 6. *Laboratory data values and EGFR status*

	Mean	**EGFR MT n (%)**	**EGFR WT n (%)**	**p**
Hb	<130 (g/L)	7 (16.3)	36 (83.7)	NS
	≥130 (g/L)	13 (27.1)	35 (72.9)	
WBC	<8.1 (10^{*9}/L)	9 (20.0)	36 (80.0)	NS
	≥8.1 (10^{*9}/L)	11 (23.9)	35 (76.1)	
LYM	<1.5 (10^{*9}/L)	12 (32.4)	25 (67.6)	P<0.05
	≥1.5 (10^{*9}/L)	8 (14.8)	46 (85.2)	
PLT	<263 (10^{*9}/L)	13 (28.9)	20 (80.0)	NS
	≥263 (10^{*9}/L)	7 (15.2)	22 (61.1)	
AST	<20 (U/L)	5 (20.0)	23 (79.3)	NS
	≥20 (U/L)	14 (38.9)	21 (67.7)	
ALT	<19 (U/L)	6 (20.7)	13 (71.1)	NS
	≥19 (U/L)	10 (32.3)	39 (84.8)	

Discussion

This study is a retrospective analysis of patients diagnosed with lung adenocarcinoma in the Hospital of Lithuanian University of Health Sciences Kaunas Clinics during the period 2020-2021 years, comparing those patients who presented with a MT EGFR gene with those who presented with a WT gene. EGFR, a transmembrane glycoprotein with cytoplasmic tyrosine kinase activity is often mutated in a number of cancers, including lung adenocarcinoma. It is more common in young, non-smoking women, and with a higher prevalence in Asia (49%), and a lower one in Europe (9.5%). EGFR is an important therapeutic target for the treatment of these cancers as its tyrosine kinase domain is a target for various TKIs [8]. Out of the 107 patients with a known EGFR status, 83 were found to possess a WT EGFR gene, while only 24 held the mutated type. This can be contributed to the relatively low prevalence of EGFR mutations among Europeans.

Out of the MT cases, most mutations were located on the exon 19 (11) and exon 21 (8). 4 patients held a mutation on the exon 20 and one had both exons 19 and 21 mutated. The location of the mutation may also be a predictor of prognosis. A study by Xiuzhi Zhou et al. found not only a statistically higher survival time for patients with EGFR mutation in compared to those without, but also better survival for those possessing an exon 19 deletion, compared to patients with exon 21 L858R mutation. It was contributed to the probable differences in the sequences and structures of exons 19 and 21, and the differences in TKI activity in patients with different mutations [25].

The dependence of EGFR status on the age during diagnosis was found to be statistically insignificant. This may be explained by the relatively small sample size, as in the literature EGFR is attributed to younger patients. On the other hand gender dependence was significant, as EGFR mutation was more common in female patients (M=10, F=14), whereas WT EGFR was more dominant in male patients (M=63, F=20). Smoking status also showed significant results when described in EGFR MT patients – among EGFR MT patients 58.8% were non-smokers and only 23.5% were smokers. The opposite is true for WT EGFR, where 50.7% were smokers, while only 18% were non-smokers. The remaining cases are past-smokers. There was no dependence between the stage of the disease and the EGFR status.

Among the laboratory data analysed in this study and its relation to the TNM stage of the disease, the only values who were found to be statistically significant were platelets and leukocytes. These values were found more increased with bigger tumor size (T3-4), presence

of metastasis (M1) and a more advanced stage of the disease (stages III-IV). No dependence of any value was found with the degree of spreading to lymph node (N status). When the same laboratory data values were examined for dependence on EGFR status, lymphocyte count was significantly lower (<1.5 10^{*9}/L) in MT EGFR patients when compared to WT EGFR cases where most patients had a higher LYM (>1.5 10^{*9}/L) number. A study performed by Jason Y. Y. Wong et al demonstrated a positive relationship between a higher WBC count and a bigger risk for lung cancer, especially in never-smoking women. Albeit, the results were mainly driven by elevated neutrophils, rather than LYM [26]. A high PLT count can be attributed to worse prognosis, as malignant cancer cells demonstrate an ability to aggregate platelets, which enable them to evade anti-tumor immune activity and facilitates faster growth and spread [27], thus explaining their abundance in advanced stages. No statistically significant correlation was detected between EGFR status and laboratory values.

Conclusions

1. During the period of 2020-2021 years most patients diagnosed with lung adenocarcinoma had a WT EGFR. Patients with MT EGFR had most commonly an exon 19 mutation.

2. Significantly more non-smoking women were diagnosed with MT EGFR lung adenocarcinoma, while more smoking men had WT EGFR lung adenocarcinoma diagnosis.

3. When the more advanced stage of the disease was established, significantly higher numbers of PLT and WBC were detected.

4. LYM number was significantly lower in patients with MT EGFR compared to WT EGFR.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021 Jan;71(1):7-33. doi: 10.3322/caac.21654. Epub 2021 Jan 12. Erratum in: CA Cancer J Clin. 2021 Jul;71(4):359. PMID: 33433946.

[2] Malhotra J, Malvezzi M, Negri E, La Vecchia C, Boffetta P. Risk factors for lung cancer worldwide. Eur Respir J. 2016 Sep;48(3):889-902. doi: 10.1183/13993003.00359-2016. Epub 2016 May 12. PMID: 27174888.

[3] Duma N, Santana-Davila R, Molina JR. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. Mayo Clin Proc. 2019 Aug;94(8):1623-1640. doi: 10.1016/j.mayocp.2019.01.013. PMID: 31378236.

[4] Chen HJ, Shen JM, Lin YN, Du W, Zhou JP, Li QY. Long-term survival of advanced lung adenocarcinoma by maintenance chemotherapy followed by EGFR-TKI: A case report. Medicine (Baltimore). 2021 Feb 12;100(6):e24688. doi: 10.1097/MD.0000000000024688. PMID: 33578601.

[5] Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018 Jan 24;553(7689):446-454. doi: 10.1038/nature25183. PMID: 29364287.

[6] Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, Jenkins RB, Kwiatkowski DJ, Saldivar JS, Squire J, Thunnissen E, Ladanyi M. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol. 2013 Jul;8(7):823-59. doi: 10.1097/JTO.0b013e318290868f. Erratum in: J Thorac Oncol. 2013 Oct;8(10):1343. PMID: 23552377; PMCID: PMC4159960.

[7] Chevallier M, Borgeaud M, Addeo A, Friedlaender A. Oncogenic driver mutations in non-small cell lung cancer: Past, present and future. *World J Clin Oncol*. 2021;12(4):217-237. doi:10.5306/wjco.v12.i4.217

[8] Tfayli A, Rafei H, Mina A, Khalil M, Fakhreddin N, Mahfouz R, Hamouri S, Farhat F, Salem Z, Dbouk H, Rabee H, Saghir N, Shamseddine A, Makarem J, Bitar N, Mougharbil A, Assi H, Temraz S, Mukherji D, Matalka I, Zaatari G. Prevalence of EGFR and ALK

Mutations in Lung Adenocarcinomas in the Levant Area - a Prospective Analysis. Asian Pac J Cancer Prev. 2017 Jan 1;18(1):107-114. doi: 10.22034/APJCP.2017.18.1.107. PMID: 28240017; PMCID: PMC5563085.

[9] Zito Marino, Federica et al. "Intratumor Heterogeneity of ALK-Rearrangements and Homogeneity of EGFR-Mutations in Mixed Lung Adenocarcinoma." *PloS one* vol. 10,9 e0139264. 30 Sep. 2015, doi:10.1371/journal.pone.0139264

[10] Warth A, Penzel R, Lindenmaier H, Brandt R, Stenzinger A, Herpel E, Goeppert B, Thomas M, Herth FJ, Dienemann H, Schnabel PA, Schirmacher P, Hoffmann H, Muley T, Weichert W. EGFR, KRAS, BRAF and ALK gene alterations in lung adenocarcinomas: patient outcome, interplay with morphology and immunophenotype. Eur Respir J. 2014 Mar;43(3):872-83. doi: 10.1183/09031936.00018013. Epub 2013 Aug 29. PMID: 23988776.

[11] Yuan M, Huang LL, Chen JH, Wu J, Xu Q. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. Signal Transduct Target Ther. 2019 Dec 17;4:61. doi: 10.1038/s41392-019-0099-9. PMID: 31871778; PMCID: PMC6914774.

[12] Song L, Zhu Z, Wu H, Han W, Cheng X, Li J, Du H, Lei J, Sui X, Song W, Jin ZY. Individualized nomogram for predicting ALK rearrangement status in lung adenocarcinoma patients. Eur Radiol. 2021 Apr;31(4):2034-2047. doi: 10.1007/s00330-020-07331-5. Epub 2020 Nov 4. PMID: 33146791.

[13] Shaw AT, Engelman JA. ALK in lung cancer: past, present, and future. *J Clin Oncol*. 2013;31(8):1105-1111. doi:10.1200/JCO.2012.44.5353

[14] Gupta R, Amanam I, Rahmanuddin S, Mambetsariev I, Wang Y, Huang C, Reckamp K, Vora L, Salgia R. Anaplastic Lymphoma Kinase (ALK)-positive Tumors: Clinical, Radiographic and Molecular Profiles, and Uncommon Sites of Metastases in Patients With Lung Adenocarcinoma. Am J Clin Oncol. 2019 Apr;42(4):337-344. doi: 10.1097/COC.0000000000000508. PMID: 30741758; PMCID: PMC7597761.

[15] Li S, Li L, Zhu Y, Huang C, Qin Y, Liu H, Ren-Heidenreich L, Shi B, Ren H, Chu X, Kang J, Wang W, Xu J, Tang K, Yang H, Zheng Y, He J, Yu G, Liang N. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts. Br J Cancer. 2014 May 27;110(11):2812-20. doi: 10.1038/bjc.2014.210. Epub 2014 Apr 17. PMID: 24743704; PMCID: PMC4037826.

[16] Pan W, Yang Y, Zhu H, Zhang Y, Zhou R, Sun X. KRAS mutation is a weak, but valid predictor for poor prognosis and treatment outcomes in NSCLC: A meta-analysis of 41 studies. *Oncotarget*. 2016;7(7):8373-8388. doi:10.18632/oncotarget.7080

[17] Brady AK, McNeill JD, Judy B, et al. Survival outcome according to KRAS mutation status in newly diagnosed patients with stage IV non-small cell lung cancer treated with platinum doublet chemotherapy. *Oncotarget*. 2015;6(30):30287-30294. doi:10.18632/oncotarget.4711

[18] Cardarella S, Ogino A, Nishino M, Butaney M, Shen J, Lydon C, Yeap BY, Sholl LM, Johnson BE, Jänne PA. Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. Clin Cancer Res. 2013 Aug 15;19(16):4532-40. doi: 10.1158/1078-0432.CCR-13-0657. Epub 2013 Jul 5. PMID: 23833300; PMCID: PMC3762878.

[19] Chen D, Zhang LQ, Huang JF, et al. BRAF mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis. *PLoS One*. 2014;9(6):e101354. Published 2014 Jun 30. doi:10.1371/journal.pone.0101354

[20] Riudavets M, Sullivan I, Abdayem P, Planchard D. Targeting HER2 in non-small-cell lung cancer (NSCLC): a glimpse of hope? An updated review on therapeutic strategies in NSCLC harbouring HER2 alterations. ESMO Open. 2021 Oct;6(5):100260. doi: 10.1016/j.esmoop.2021.100260. Epub 2021 Aug 31. PMID: 34479034; PMCID: PMC8414039.

[21] Pillai RN, Behera M, Berry LD, et al. HER2 mutations in lung adenocarcinomas: A report from the Lung Cancer Mutation Consortium. *Cancer*. 2017;123(21):4099-4105. doi:10.1002/cncr.30869

[22] Mazières J, Barlesi F, Filleron T, Besse B, Monnet I, Beau-Faller M, Peters S, Dansin E, Früh M, Pless M, Rosell R, Wislez M, Fournel P, Westeel V, Cappuzzo F, Cortot A, Moro-Sibilot D, Milia J, Gautschi O. Lung cancer patients with HER2 mutations treated with chemotherapy and HER2-targeted drugs: results from the European EUHER2 cohort. Ann Oncol. 2016 Feb;27(2):281-6. doi: 10.1093/annonc/mdv573. Epub 2015 Nov 23. PMID: 26598547.

[23] Drilon A, Jenkins C, Iyer S, Schoenfeld A, Keddy C, Davare MA. ROS1-dependent cancers - biology, diagnostics and therapeutics. Nat Rev Clin Oncol. 2021 Jan;18(1):35-55.

doi: 10.1038/s41571-020-0408-9. Epub 2020 Aug 5. PMID: 32760015; PMCID: PMC8830365.

[24] Moliner, Laura & Arriola, Edurne. (2021). ROS1 non-small cell lung cancer patients treatment approach. Precision Cancer Medicine. 4. 10.21037/pcm-20-38.

[25] Zhou X, Cai L, Liu J, et al. Analyzing EGFR mutations and their association with clinicopathological characteristics and prognosis of patients with lung adenocarcinoma. *Oncol Lett*. 2018;16(1):362-370. doi:10.3892/ol.2018.8681

[26] Wong JYY, Bassig BA, Loftfield E, Hu W, Freedman ND, Ji BT, Elliott P, Silverman DT, Chanock SJ, Rothman N, Lan Q. White Blood Cell Count and Risk of Incident Lung Cancer in the UK Biobank. JNCI Cancer Spectr. 2019 Dec 12;4(2):pkz102. doi: 10.1093/jncics/pkz102. PMID: 33313477; PMCID: PMC7083262.

[27] Jurasz P, Alonso-Escolano D, Radomski MW. Platelet--cancer interactions: mechanisms and pharmacology of tumour cell-induced platelet aggregation. *Br J Pharmacol*. 2004;143(7):819-826. doi:10.1038/sj.bjp.0706013.