” CAROL DAVILA” UNIVERSITY OF MEDICINE AND PHARMACY,

BUCHAREST

DOCTORAL SCHOOL

OBSERVATIONAL STUDIES IN ADVANCED NON-SMALL CELL LUNG CANCER WITH "REAL WORLD" DATA ON MULTIMODAL THERAPY IN SECOND AND THIRD LINE NIVOLUMAB AND TARGETED THERAPY WITH ERLOTINIB

PhD Supervisor:

RESEARCHER DEGREE I DR. GRIGORESCU ALEXANDRU CĂLIN

Ph D Student:

ALBU (TEODORESCU) MIHAELA

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Summary

1 ACTIUAL STATE OF KNOWLEDGE

1.1 Epidemiology

Cancers represent a major challenge in the health field, ranking as the second leading cause of death worldwide in 2018, according to data provided by the World Health Organization (WHO). With approximately 9.6 million deaths, cancer is responsible for one in six deaths globally. Bronchopulmonary tumours dominate the list of incidence and mortality, especially in men, with 2.09 million cases and 1.76 million deaths in 2018, according to GLOBOCAN **[1]**

1.2 Carcinogenesis in pulmonary epithelium

1.2.1 Steps of carcinogenesis

Overall, the process of carcinogenesis involves a complex series of molecular and cellular events, with multiple stages and interactions between environmental and genetic factors, providing a basis for understanding and intervening in lung cancer. [2]

1.3 Morphological, genetic, and epigenetic alterations in non-small cell lung cancer

1.3.1 Chromosomal aberrations

Chromosomal alterations in DNA are common in human cancers, including bronchopulmonary cancer. Allelic losses on certain chromosomes suggest the presence of tumour suppressor genes. The incidence and mortality of lung cancer have increased globally. Loss of heterozygosity at certain loci is associated with lung cancer, and the frequency of this loss varies among histological types of lung cancer. [3]

1.3.2 p53 mutation

Mutations in the tumour suppressor gene p53 are among the first identified in human tumour cells. These mutations, concentrated in the DNA-binding domains, generate mutant forms of p53, affecting DNA binding. [4], [5]

1.3.3 K-RAS mutation

The K-RAS gene, a crucial human oncogene, regulates cell proliferation through transmembrane signalling. RAS mutations, especially K-RAS, present in 30% of cancers, are common in lung, pancreatic, and colorectal tumours. K-RAS mutation is predominant in smokers, suggestive of the influence of tobacco carcinogens on cellular signalling. [6]

1.3.4 EGFR mutation

The EGFR gene, located on chromosome 7p11.2, encodes a transmembrane receptor protein with 28 exons. Exons 5-7 and 13-16 encode the ligand-binding domain, exons 18-24 encode the tyrosine kinase domain, and exons 25-28 induce autophosphorylation. Frequent mutations in exons 18-21 are associated with lung cancer. [7]

1.3.5 ALK mutation

Anaplastic Lymphoma Kinase (ALK) is a tyrosine kinase receptor protein expressed in neoplasms. ALK mutations are present in neuroblastoma, anaplastic lymphoma, myofibroblastic tumours, and non-small cell lung cancers. [8]

1.3.6 ROS1 mutation

ROS1, a member of the insulin receptor family, is a tyrosine kinase protein. Involved in chromosomal translocations in various neoplasms, including non-small cell lung cancers, it is found on chromosome 6q22. [8]

1.3.7 BRAF mutation

The BRAF gene encodes a serine/threonine kinase found on chromosome 7q34. Activating mutations, like V600E, are present in malignant melanoma and lung carcinoma, with an incidence of 1%-4%. [9]

1.3.8 NTRK mutation

The NTRK genes encode tropomyosin receptor kinases (TRK), which are essential in neural development. These receptors bind to neurotrophins initiating cellular signalling. [10]

1.3.9 MET mutation

The MET gene encodes the c-MET protein, located on chromosome 7. c-MET forms a heterodimer bound to the extracellular domain and activates tyrosine kinase signalling upon binding with the Hepatocyte growth factor, triggering signalling pathways, regulating cellular proliferation and invasiveness. [11]

1.3.10 HER mutation

Overexpression or amplification of HER2 is present in 6-30% of patients, suggesting a potential therapeutic target. Anti-HER therapies are under investigation. [12]

1.4 Epigenetic alterations

1.4.1 DNA base methylation

Abnormal methylation of cytosine in gene promoters regulates gene expression in lung cancer. Genes such as p16, APC, CDH1, CDH13, MGMT, and PTEN are affected, influencing proliferation, apoptosis, and cellular mobility. [13]

1.4.2 Chromatin remodelling

Chromatin remodelling inactivates DNA transcription. Epigenetic alterations, including methylation of MGMT and p16, are potential biomarkers for early diagnosis, while APC, CDH1, MLH1, and PTEN are for the prognosis of lung cancer. [14]

1.4.3 NFIB factor

NFIB facilitates chromatin accessibility and promotes a pro-metastatic neuronal state in metastases of small cell lung cancers. These findings open new therapeutic perspectives for non-small cell lung cancers. [15]

1.5 Diagnosis of lung cancers

1.5.1 Clinical diagnosis

Patients with lung tumours present with various symptoms, including cough, haemoptysis, paraneoplastic syndromes, and symptoms associated with disease extension or metastases. Diagnosis involves history-taking, physical examination, and detailed paraclinical evaluation, including chest X-ray and PET-CT. Suspecting lung tumour is essential in patients with risk factors and associated symptoms. [16]

1.5.2 Imaging diagnosis

Imaging diagnostic methods for lung tumours include chest X-ray, chest CT, bone scintigraphy, and PET/CT. Chest X-ray is commonly used but may be limited in detecting nodal invasions. Chest CT is preferred for nodal invasion details and staging. [17]

1.5.3 Bronchology diagnosis

Bronchoscopy, essential in the diagnosis and staging of lung tumours, employs various methods such as transbronchial biopsy, EBUS, or mediastinoscopy. From the 18th century to the present, technological advancements have transformed bronchoscopy into a crucial tool,

enabling not only diagnosis and treatment but also research into the carcinogenesis process and assessment of response to targeted therapies. [18] , [19]

1.5.4 Pathology, immunohistochemical and molecular-genetic diagnosis

1.5.4.1 Pathology diagnosis

Accurate diagnosis of bronchopulmonary cancer is crucial for selecting optimal treatment and has a significant impact on prognosis. Tissue biopsy provides essential information about histology and immunohistochemistry, necessary for tumour classification and identifying subsequent therapies. [20]

1.5.4.2 Immunohistochemical diagnosis

Immunohistochemistry is essential in the diagnosis and classification of lung cancer, providing crucial information to distinguish between different cell types and guide appropriate therapy. Its use minimizes diagnostic errors and identifies specific markers of the cell lineage, essential for excluding the metastatic origin. [21], [22]

1.5.4.3 Molecular-genetic diagnosis

In the treatment of non-small cell lung tumours, genetic mutation testing is essential and validated, guiding molecular therapy. Targeted therapies, directed towards specific mutations, reduce toxicity, and improve survival compared to standard chemotherapy. [23]

1.6 Staging of lung tumours

Staging of bronchopulmonary tumours (TNM) is essential for initiating appropriate treatment. Stages range from 0 to IV, based on the correlation of descriptors T, N, and M. [24]

1.7 Therapeutic management of non-small cell lung cancer

1.7.1 Surgical Methods

Surgical resection represents the therapeutic standard for early-stage bronchopulmonary carcinomas, with survival rates of 60-80% for stage I and 30-50% for stage II. Lobectomy with mediastinal lymph node dissection is recommended, with VATS as an alternative to reduce complications. Studies highlight that complete resection (R0) is crucial for a favourable prognosis of overall survival. [25], [26]

1.7.1.1 Central nervous system

Cerebral metastasis is common (20-30%) in non-small cell carcinoma. Studies show long-term survival (1-30%) with aggressive treatment. Synchronous therapy for brain metastases recommends surgical or radiological ablation. [27], [28]

1.7.1.2 Bone tissue

Studies suggest that bone metastases are not considered oligometastatic disease, with low survival rates even with surgical and radiotherapy interventions. Unfavourable outcomes indicate that these metastases do not have a favourable long-term prognosis, regardless of their location. [29]

1.7.1.3 Adrenal glands

Metastasis to the adrenal glands is common in non-small cell tumours. For patients with resectable primary tumours, it is recommended to first perform resection of the primary tumour and then adrenalectomy. [30]

1.7.1.4 Lungs

Surgeons face a dilemma between metastases and multifocal processes in separate lung lesions. The Martini and Melamed criteria are helpful but debated. Resection of both lesions is preferred in the absence of affected mediastinal lymph nodes. [31]

1.7.2 Radiotherapy methods

Radiation therapy for lung tumours has evolved significantly, moving from simulator-based planning to three-dimensional techniques and intensity-modulated radiation therapy (IMRT), improving precision and minimizing adverse effects. 4DCT and CBCT imaging allow visualization of tumour motion and more precise planning. IMRT and VMAT allow dose shaping around the tumour and reduction of doses to healthy organs. [32]

1.8 Systemic therapy for non-small cell lung cancer

1.8.1 Chemotherapy

Patients with non-small cell lung tumours benefit from curative surgical intervention, but recurrences are common. Adjuvant chemotherapy, although effective, is selectively applied, considering factors such as stage, age, comorbidities, etc. Side effects and treatment resistance are challenges. Chemotherapy remains the gold standard, although the risk-benefit ratio is considered for each patient. [33], [34]

1.8.2 Immunotherapy

Immunotherapies focus on stimulating the immune response against tumours. Current approaches include anti-tumour vaccines, immune cell manipulation, and inhibition of tumour evasion mechanisms, especially through immune checkpoint inhibitors (ICI) such as CTLA-4 and PD-1. [35]

1.8.3 Targeted therapy

In the treatment of lung adenocarcinoma, the AKT/mTOR, EGFR, RAS, and ROS1 pathways are essential, and targeted therapies have proven effective in clinical studies. Examples include AKT/mTOR inhibitors (everolimus), ALK inhibitors (crizotinib), EGFR inhibitors (erlotinib, afatinib, gefitinib, osimertinib), and NTRK inhibitors (entrectinib). [36]

1.8.4 Gene therapy

Gene therapy, while promising in theory, faces technical obstacles in practice. The use of viral vectors is preferred in delivering transgenes, but improvements are needed in identifying vectors and increasing their efficiency. [37]

1.8.4.1 Adenoviral vectors

Modifiable viral vectors, especially adenoviral ones, are used for efficient and safe genetic transfer. Despite their advantages and disadvantages, they remain important for various therapeutic and research applications. [38]

1.8.4.2 Oncolytic viruses

Oncolytic viruses, such as T-VEC, act by directly killing tumours and stimulating the immune response. They could provide a safe therapy and can be combined with other treatments for increased efficacy. [39]

1.8.4.3 Vaccinia viral vectors

Vaccinia vectors, like adenoviral vectors, can transduce a variety of cells, resulting in transient expression but with inevitable cellular lysis, affecting dendritic cell transduction. [40]

1.8.4.4 Plasmid vectors and retroviruses

Plasmid vectors have low transduction efficiency, especially in vivo. Unlike viral vectors, they are less immunogenic but offer inferior transgenic expression. [41]

2 Personal Contributions: Original studies with "real-world" data regarding second-line chemotherapy, immunotherapy, and targeted therapy in non-small cell lung cancers (NSCLC).

Purpose and objectives

"Real-world" data used in medical research are information obtained from electronic medical records or patient observation files. These data encompass clinical examinations, imaging studies, and therapeutic indications. They record side effects and patient progression. Additionally, they are useful for medical research and can aid in the implementation of new drugs in current practice by confirming or refuting the results of clinical studies. "Real-world" studies can more faithfully reproduce the conditions encountered by physicians in their daily practice and can contribute to optimizing patient treatment. In this study, the effectiveness and efficiency of various therapies for bronchopulmonary tumours were evaluated using "real-world" data collected from medical institutions in Bucharest between 2015-2018. These data are important to determine whether the results of clinical studies are relevant and applicable in real medical practice in the country.

2.1 Aspects of advanced non-small cell lung cancer therapy through real-world data: a study conducted on 77 patients treated at the Bucharest Oncology Institute.

2.1.1 Introduction

Non-small cell lung cancer represents a major challenge in oncology, being among the most common forms of cancer globally. Recent research has brought progress in anti-tumour therapies, improving survival and quality of life. However, survival remains low for advanced stages. Targeted therapies and immunotherapy offer new perspectives but require further research to better understand their impact and prognosis.

2.1.2 Study hypothesis

The study hypothesis stemmed from the observation that most advanced non-small cell lung cancers (NSCLC) underwent complex therapy involving surgery, radiotherapy, and systemic therapy, highlighting the necessity of a multidisciplinary treatment approach. We particularly focused on the therapeutic impact of systemic therapy, considering the perspective of medical oncologists. The goal was to evaluate response and survival in the context of multimodal therapy. We aimed to monitor patient progress to understand the effects of conventional therapy and the introduction of immunotherapy and targeted therapy.

2.1.3 Study motivation

The study aimed to demonstrate the necessity of implementing multidisciplinary therapy in non-small cell lung cancer (NSCLC) on one hand, and the importance of introducing new systemic therapies by evaluating the impact of immunotherapy and targeted therapies on the other. Essential aspects addressed include the need for a deeper understanding of the effectiveness of these therapies in diverse clinical settings, factors influencing patient response and survival, and comparing results with existing literature. The goal is to improve survival outcomes and reduce the impact of non-small cell lung cancer on society.

2.1.4 Study objectives

- Comparing overall survival duration between patients treated with multiple therapies, including immunotherapy and targeted therapies, and those treated with chemotherapy alone, in relation to data from previous clinical studies.
- Analysing the duration of disease-free survival between patients treated with immunotherapy and those treated with chemotherapy, comparing them to data from previous clinical studies.
- Identifying correlations between various patient characteristics (gender, performance status, disease stage, age, smoking status, histopathologic type) and overall and disease-free survival, based on the various therapies applied.
- Evaluating the safety profile of treatments to assess associated side effects and toxicity grades, alongside therapeutic efficacy.

2.1.5 Method and materials

2.1.5.1 Participants

The study, conducted between January 2015 and December 2018 at the Bucharest Oncology Institute, had a real-world, single-center, retrospective, and observational design. The oncological records and observation sheets of 77 discharged patients were examined, meeting criteria such as ECOG performance status 0, 1, 2, and the presence of adenocarcinoma or squamous cell carcinoma. Enrolled patients had received at least two lines of therapy, and therapeutic response was documented in their medical records.

2.1.5.2 Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Age >18 years	Age <18 years

Histologically non-small cell lung cancer (NSCLC): squamous cell carcinoma and non-squamous cell carcinoma.	Small cell histology
The performance of immunohistochemical tests.	The absence of immunohistochemical tests
Administration of at least two lines of therapy.	Administration of just one line of therapy
Diagnostic, palliative, or curative surgical intervention.	No surgical intervention
Palliative or curative radiotherapy	No radiotherapy
Stage III and IV of disease	Stages I and II of disease
Performance status of ECOG 0, 1 and 2	Performance status of ECOG >2

Table 2.1.1: enrolled patients characteristics

2.1.5.3 Statistical methods

To evaluate overall survival (OS) and progression-free survival (PFS) in the absence of death certificates, a surrogate marker was used by calculating the difference between the last visit and the oncologic registration date. OS and PFS were estimated using descriptive statistics and graphically represented by Kaplan-Meier curves. PFS was defined as the time from the start of therapy to disease progression/death. Therapy response assessment was based on RECIST 1.1 and CTCAE 4.0 criteria. The analysis was conducted in R 3.6.2, and data distribution was assessed using the Shapiro-Silk test. For comparisons, t-tests, Wilcoxon-Mann Whitney tests, ANOVA, and Kruskal-Wallis tests were used. Log-rank and chi-square tests were used for survival and proportions. The limitation of the dataset was its small sample size.

2.1.6 Results

The study included 77 patients, of whom 34% were female and 66% were male, with an average age of 62 years, and a minority originating from rural areas (35%). The majority (71%) had adenocarcinoma, while 23% had squamous cell carcinoma. ECOG performance status was predominantly 1 (65%). Metastases were observed in 43% of cases, with pleural and pulmonary metastases being the most common. 55% were tested for EGFR mutation status, predominantly without detected mutations (45%). All patients received first-line chemotherapy or targeted therapy based on molecular test results. Most patients (27) underwent two lines of chemotherapy. Immunotherapy was administered as third-line treatment or beyond to 31% of participants. Targeted therapies, including TKIs, were administered to 47% of patients. Overall survival was estimated at 24 months, with a median of 50 months. 40.2% died. Survival analysis showed a median of 50 months (95% CI: 28 - infinity).

Survival analysis in relation to different variables is a crucial aspect of medical studies, directly impacting patient management and treatment. In a real-world study, age and associated comorbidities are particularly relevant factors.

To assess the influence of age, patients were divided into two groups: those under 65 years and those 65 years and older. The results indicated a median survival of 50 months for those <65 and 42 months for those >65. However, the dataset was unbalanced, with fewer patients in the over 65 group. Statistical analysis did not show significant differences between the two groups.

Another variable considered was gender. The median survival for women could not be calculated due to a small number of events, while for men, it was 31 months. Additionally, the dataset was unbalanced, with fewer women included. However, the analysis revealed significant differences between genders.

ECOG status, expressed binary, demonstrated significant differences in survival between those with an ECOG score of 2 and those with ECOG scores of 1 and 0. The analysis indicated significantly lower survival for those with an ECOG score of 2.

Histopathological outcome was also associated with differences in survival. However, the analysis did not reveal significant differences between patient groups.

EGFR mutation analysis did not show significant differences in survival between different mutations, although there were variations in median survival. Finally, the number of metastases was not associated with significant differences in survival, according to statistical analysis. Regarding chemotherapy treatment administration, we correlated the number of chemotherapy lines administered with survival, which was significant: as the number of chemotherapy lines increased, so did survival in months (p-value = 0.004).

The duration of survival in cases of immunotherapy + targeted therapy combination was much higher than those who did not receive either treatment: 36.71 vs 21.86 months.

In conclusion, survival analysis in relation to various variables provides a complex picture of disease progression and treatment efficacy, with significant implications for patient management.

2.1.7 Discussions

The study included 77 patients with non-small cell lung cancer, mostly men with an average age of 62 years, primarily from urban areas, and with ECOG performance status between 0 and 1. An average overall survival of 24 months and a median survival of 22 months were observed. Patients under 65 years of age had a higher average survival than those over

65, and those with ECOG between 0 and 1 had better survival than those with ECOG = 2. Adenocarcinoma was associated with higher survival than squamous cell carcinoma.
A significant correlation was observed between the number of chemotherapy lines administered and patient survival. Administration of targeted therapy and/or immunotherapy led to significantly higher survival, especially the combination of immunotherapy and targeted therapy. The duration of survival in the case of the combination of immunotherapy and targeted therapy was significantly higher than in patients who did not receive either of these treatments.

2.1.8 Conclusions

Our retrospective study, conducted between January 2015 and December 2018, highlights similar results to other international research, such as the study by Gong J. et al. (2015) [42] and the studies of Debiuvre (2017) [43]. In these studies, the average age of participants was approximately 58 years, with the majority being male. Median survival varied between 14.3 and 27.91 months, depending on disease stage and treatment line.

The results highlight that combined therapies were more effective than monotherapy. Combinations of targeted therapy and immunotherapy showed increased efficacy in treating non-small cell lung cancer. A Canadian study confirmed these findings, reporting a median overall survival of 9.2 months for patients treated with systemic chemotherapy alone compared to 20.2 months for those receiving immunotherapy and targeted therapy.

Additionally, the importance of immunohistochemical testing and mutational status assessment in patients is crucial, as these factors influenced survival. Patients not evaluated for these aspects had lower survival rates.

Our study provides relevant insights into treatment effectiveness in a real-world context but acknowledges its limitations and the need for further investigation on a larger sample. We conclude that each treatment line contributes to prolonging overall survival, and appropriate assessment of tumour characteristics can guide patient management and prognosis.

2.2 Real-world evidence with nivolumab in advanced non-small cell lung cancer – second line and beyond.

2.2.1 Introduction

Immunotherapy with immune checkpoint inhibitors has revolutionized the treatment of advanced and metastatic non-small cell lung cancers. Five-year survival rates, previously between 7% and 19%, have now significantly improved. PD-L1 and PD-1 inhibitors are pivotal, offering comparable or superior benefits to standard chemotherapy. Advanced

diagnostic techniques and therapies, including radiotherapy and immunotherapy, extend survival prospects. PD-1, also known as "programmed cell death protein-1," regulates immune recognition and interactions with tumor cells. Anti-PD-1/PD-L1 antibodies enhance therapeutic responses by activating cytotoxic T lymphocytes. The FDA has approved five types of antibodies, including nivolumab and pembrolizumab, for the treatment of lung cancers, consolidating progress in the fight against these conditions. [44]

2.2.2 Study hypothesis

The study aims to assess the effectiveness and efficiency of nivolumab immunotherapy in treating patients with advanced and metastatic lung tumors, considering real-world data from our country. The goal is to confirm or refute the results of previous pivotal studies and to expand understanding of the use of this treatment beyond the initial stages of the disease and the limitations of clinical research.

2.2.3 Study motivation

The study aimed to evaluate real-world survival with nivolumab as second-line treatment and beyond, comparing the results with previous clinical studies. Objectives included identifying prognostic factors related to overall survival and response rate to nivolumab immunotherapy.

2.2.4 Study objectives

1. Establishing the therapeutic efficacy of nivolumab as second line and beyond treatment in patients with advanced and metastatic non-small cell lung cancer.
2. Estimating overall survival.
3. Estimating progression-free survival.
4. Comparing the data with those from pivotal studies that led to the approval of these innovative therapies.

2.2.5 Method and materials

2.2.5.1 Participants

The study examined the records of 34 patients with advanced lung cancer treated at the "Prof. Dr. Alexandru Trestioreanu" National Institute of Oncology in Bucharest. Therapeutic efficacy, safety, survival benefits, and progression-free interval were evaluated. The dose of nivolumab used in 2018 was 1 mg/kg every two weeks, different from the current standard of 240 mg every two weeks.

It was a single-centre, open-label, retrospective observational study conducted between January 2018 and December 2020, analysing the effectiveness and safety of nivolumab in the treatment of metastatic lung cancer. Patients were monitored for overall survival (OS) and progression-free survival (PFS), comparing them with standard chemotherapy. The safety of nivolumab was also assessed. OS and PFS were calculated using descriptive analyses and graphically represented. OS was defined as the time from the start of nivolumab treatment to death, and PFS represented the time from the start of nivolumab to disease progression or death. Treatment response was evaluated clinically and radiographically according to CTCAE and RECIST 1.1 standards.

2.2.5.2 Exclusion and inclusion criteria

Inclusion criteria	**Exclusion criteria**
Age > 18 years	Age <18 years
Non-small cell carcinoma histology	Small cell lung cancer histology
Advanced non-small cell lung carcinoma (IIIB-IIIC)	Early stages of disease (IA-IIIA)
Metastatic non-small cell lung cancer (IV)	Pregnancy and/or breastfeeding
Progression of the disease during platinum-based chemotherapy.	Poor performance status ECOG =3
Disease recurrence within 6 months of platinum-based therapy.	Concomitant therapy for another cancer

2.2.5.3 Statistical methods

The data analysis was performed using R 3.6.2 software and Excel application from the Microsoft 365 for Enterprise package. To evaluate the data distribution, the Shapiro-Wilk test was employed, considering a significance level of 0.05. The independent t-test for two samples was used for normally distributed data, while the Wilcoxon-Mann Whitney test was applied for non-normally distributed data. For more than two subgroups, ANOVA or Kruskal-Wallis test was used, depending on the data distribution. Proportion comparison was conducted using the chi-square test or Fisher's test. Kaplan-Meier curve and log-rank test were employed for survival analysis and comparisons between groups.

2.2.6 Results

In our study, the distribution of patients by gender was as follows: we included 7 females and 27 males. Statistical analysis did not show significant differences in age between the two genders: W = 115, p-value = 0.3934.

Regarding the distribution of patients based on smoking status, we observed that there were 27 smokers and 7 non-smokers. The proportion of smokers was much higher in males than in females, and this difference was statistically significant: p-value = 0.02044.

Regarding the histopathological type identified on anatomopathological examination, we had 15 patients with squamous cell carcinoma and 19 patients with non-squamous cell carcinoma. Statistically, the difference between these two types of cancer was not significant: X-squared = 0.52941, df = 1, p-value = 0.4669.

In clinical practice, most patients present significant comorbidities, especially cardiovascular and metabolic, associated with an unhealthy lifestyle, obesity, smoking, and cardiovascular diseases. Except for patients with cardiovascular comorbidities (50% of patients), all other cases (gastroenterological comorbidities - 26%, pulmonary - 21%, metabolic - 9%, psychiatric - 6%, oncological - 3%) showed statistically significant differences compared to the general distribution of patients.

All patients included in the study received first-line therapy containing platinum (34 patients), followed by second-line therapy (29%), third-line therapy (9%), and fourth-line treatment (6%). The distribution of therapeutic choices: most patients received gemcitabine + carboplatin, followed by those with alimta + carboplatin and then those with paclitaxel + carboplatin. Vinorelbine + carboplatin and vinorelbine monotherapy were used equally.

All patient observation sheets documented the administration of immunotherapy for advanced and metastatic non-small cell lung cancer in the second line and beyond. Nivolumab immunotherapy was relatively well-tolerated, with 24 patients having no adverse events, while 10 patients reported at least one type of adverse event. No patient achieved a complete response (CR), half of the patients had disease progression (PD), the remaining patients had stable disease (SD), and only one patient achieved a partial response (PR). The maximum number of cycles administered was 31, and the minimum was one. On average, approximately 10 cycles/patient were administered.

Overall survival (OS) was calculated using a surrogate marker, with a mean of 32.57 months and a median of 24.43 months. 75% of patients had an OS of less than 45.55 months. Kaplan-Meier method was used for survival analysis. The median OS was 61.2 months. There were no significant differences between the survival functions of females and males. Progression-free survival (PFS) was 16.87 months for deceased patients and 12.081 months for those with lost follow-up. Overall survival for Nivolumab therapy had a median of 72.3 weeks, with a 95% confidence interval between 12.5 months and infinity.

Survival analysis and Kaplan-Meier summary showed significant differences between genders and between smokers and non-smokers. Additionally, differences were observed between histological types of lung cancer regarding survival and PFS with Nivolumab therapy.

The median for non-squamous was 17.3 weeks (4.3 months), while for squamous it was not reached (NA). Chisq = 0 on 1 degree of freedom, p = 0.9.

2.2.7 Discussions

Nivolumab, the first immune checkpoint inhibitor in its class, has been approved for the therapy of advanced non-small cell lung cancer in the second line of treatment. Randomized controlled studies have shown improvements in survival compared to standard chemotherapy in pre-treated advanced/metastatic non-small cell lung cancer. The presented research was conducted on a small, unique cohort of patients from the same physician, receiving treatment according to therapeutic guidelines to ensure uniformity of selection criteria and elimination of differences in associated supportive therapy. This research represents a positive feasibility study, but larger samples are needed to consolidate the results.

Overall survival (OS) was calculated as the difference between the date of the last recorded visit and the date of oncological registration. The OS and progression-free survival (PFS) results were like those in the pivotal CheckMate 017 [45] and 075 studies [46]

In the CheckMate 017 and 057 studies, inclusion criteria were restricted, with a small proportion of patients over 65 years old, while our sample had a median age of 65.5 years. The positive results of nivolumab were not influenced by advanced age. Our study included patients with multiple comorbidities and possible contraindications for therapy, representing a significant challenge.

Favourable responses to therapy were associated with good performance status, male sex, smoker status, non-squamous histology, and a reduced number of comorbidities, confirming the results of other studies. No positive outcomes were identified for hyperprogression or complete response to immunotherapy, consistent with reviews in the specialized literature. [47]

In conclusion, the results suggest that nivolumab is effective in the treatment of advanced non-small cell lung cancer, however, therapy resistance can be an obstacle and requires a clear scientific definition. Further studies are needed to confirm these findings and to develop more efficient management strategies.

2.2.8 Conclusions

Nivolumab, the first immune checkpoint inhibitor approved for the treatment of non-small cell lung cancers, has shown improvements in survival compared to standard chemotherapy in the second-line treatment for advanced/metastatic stages. A pilot study conducted on an extensive database from Romania, between 2018 and 2020, evaluated the efficacy and tolerability of nivolumab under these conditions.

The results indicated a median survival of 32.5 months, suggesting that nivolumab may represent a promising therapeutic option, especially for patients with limited treatment options. Factors associated with a more favourable outcome included male gender, smoker status, good health status, and non-squamous histology.

The tolerability of nivolumab was good, with only one interruption of therapy due to a severe adverse event, indicating that this treatment can be administered with manageable adverse events in clinical practice.

This study provides real-world evidence validating findings from randomized controlled trials, essential for understanding treatment effectiveness and cost management in daily clinical practice. However, the small sample size underscores the need for larger-scale studies to confirm these findings and to investigate mechanisms of resistance to immunotherapy, aiming to improve outcomes in metastatic non-small cell lung cancer.

2.3 Real-world data study regarding the efficacy of second line and beyond tyrosine kinase inhibitors in patients with advanced and metastatic non-small cell lung tumours.

2.3.1 Introduction

Targeted therapies introduced in the treatment of bronchopulmonary cancer represent a significant advancement in improving therapeutic response, increasing progression-free survival, overall survival, and finally, the quality of life of patients diagnosed with advanced non-small cell lung carcinoma. Targeted therapy is applied in the first line of treatment, following the identification of mutations in non-small cell lung cancers: EGFR, ALK, ROS. Upon progression, in subsequent lines of treatment, targeted therapy with small molecule TKIs can be administered regardless of the presence of mutations, as their administration demonstrates efficacy and a favourable toxicity profile. The therapeutic decision should primarily consider the patients' preferences and the side effects of the medications used. The most studied molecule in this regard is represented by erlotinib, a tyrosine kinase inhibitor for the epidermal growth factor receptor (EGFR).

2.3.2 Study hypothesis

Targeted therapy with erlotinib in non-small cell lung cancer, in pre-treated patients, is a subject of debate. The study by P. Neumair and L. Joos [48] shows similar efficacy of erlotinib and chemotherapy in these patients. In our research, we aim to evaluate the efficacy of erlotinib in this patient population.

2.3.3 Study motivation

The main goal in managing advanced and metastatic bronchopulmonary tumours is to improve overall survival and other aspects such as recurrence-free period and progression-free survival. While the first-line treatment is clear, the effectiveness of erlotinib treatment in subsequent therapeutic lines and in elderly patients with comorbidities is being investigated to provide real-world data.

2.3.4 Study objectives

1. Establishing the efficacy and effectiveness of tyrosine kinase inhibitor therapy in previously treated patients with non-small cell bronchopulmonary tumors who do not have activating mutations that previously required this therapeutic line.
2. Determining the toxicity profile associated with tyrosine kinase inhibitor therapy in pre-treated patients presenting with significant associated comorbidities.
3. Establishing correlations between targeted therapy administration with overall survival, progression-free survival, as well as disease-free interval..

2.3.5 Methods and material

2.3.5.1 Participants

This was a retrospective, open-label observational cohort study conducted at a single centre from January 1, 2015, to December 31, 2020. Based on data from a single physician, it employed an open methodology to minimize bias. A total of 79 patients with advanced non-small cell lung cancer were selected, with criteria including ECOG status, histological type of tumour, and previous therapy. The evaluations aimed to assess effectiveness, safety, and survival.

2.3.5.2 Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
age > 18 yeas	age <18 years
Non-small cell lung cancer histology: squamous cell carcinoma, non-squamous cell carcinoma	Small cell carcinoma histology
"wild type" EGFR status	EGFR mutation present
Advanced disease (stages IIIA/IIIB/IIIC)	Early stages of disease (IA-IIIA)
stage IV non-small cell lung cancer	Pregnancy and/or breastfeeding
Standard first line therapy according to guidelines (chemotherapy)	Poor performance status of ECOG > 2
Second line and beyond with tyrosine kinase inhibitors (erlotinib)	Concomitant therapy for another cancer
Number of chemotherapy cycles (at least 4)	First line therapy with tyrosine kinase inhibitors (erlotinib)
At least two comorbidities	

Tab.2.3.1: enrolled patients characteristics

2.3.5.3 Statistical methods

The participants were followed for overall survival (OS) and progression-free survival (PFS) in treatment with erlotinib and standard chemotherapy, and the safety of erlotinib was assessed. OS and PFS were estimated and graphically represented by Kaplan-Meier curves. Treatment response was evaluated clinically and radiographically. The analysis was conducted using the R programming language, and some graphs and tables were created in Excel. Parametric and non-parametric tests were used for statistical evaluations, and Kaplan-Meier curves and the log-rank test were employed for survival analysis. The dataset was unbalanced due to the small sample size.

2.3.6 Results

The study included 79 patients, predominantly male smokers, with a mean age of 59.9 years. Most of them came from urban areas and presented with non-squamous forms of non-small cell lung cancer, with adenocarcinomas being the most common. Comorbidities, especially cardiovascular and metabolic ones, were present in a significant proportion, reflecting the association of lung disease with an unhealthy lifestyle. In stage IV of the disease, most patients were exposed to second-line or subsequent therapies.

Overall survival (OS) and progression-free survival (PFS) were followed as primary objectives. The mean OS was 26.40 months for deceased patients and 45.38 months for those still alive. The mean PFS was 26.43 months. The administration of erlotinib in the second or subsequent line did not indicate significant differences in PFS. Survival analysis by sex and age showed significant differences, with longer survival in women and those under 60 years old. However, the analysis did not reveal significant differences based on the histopathological type of cancer or the timing of erlotinib administration in the treatment line.

2.3.7 Discussions

The study reveals that the administration of erlotinib in the second and third lines led to a significant improvement in progression-free survival in patients with lung cancer in Romania. The results indicate a higher median survival compared to other similar studies, highlighting the therapeutic efficacy of erlotinib in these lines of treatment. Additionally, it is noted that patients with adenocarcinoma histology showed higher survival following treatment with erlotinib, suggesting the benefits of this treatment in this subpopulation.

The study mentions that there are studies that have obtained similar results, such as a prospective multicenter study from France [49] and another study from Switzerland [50]. However, it is important to note that some authors have found that the duration of overall response for unselected patients remains limited. Additionally, the results of other studies are also discussed, such as the TAILOR [51], DELTA [52] and CTONG0806 [53], which have investigated the benefit of tyrosine kinase inhibitors in the second or third line. These studies have yielded varied results, and some have contradicted the findings of the current study. For example, the TAILOR study identified a survival benefit in favour of docetaxel, while the DELTA and CTONG0806 studies obtained different results regarding overall survival and progression-free survival.

In conclusion, the study demonstrates that erlotinib may be a valuable therapeutic option for patients with lung cancer in Romania; however, further research is needed to confirm and strengthen these findings, as well as to better identify the characteristics of patients who would benefit most from this treatment.

2.3.8 Conclusions

Our real-world observational study on the effectiveness of erlotinib in pre-treated non-small cell lung cancer (NSCLC) demonstrates that erlotinib is a viable treatment option. Erlotinib can be administered to patients with impaired performance status due to its favourable toxicity profile. The study included patients regardless of EGFR status and found a median overall survival of 26.40 months for deceased patients, with a median of 20.75 months. Overall survival was higher in women compared to men. However, this must be interpreted in the context of a small sample size, limiting statistical significance.

Erlotinib represents a feasible treatment option for second-line therapy and becomes important in the third-line setting, especially considering the emergence of immunotherapies for second-line treatment. Furthermore, patients with adenocarcinoma histology also had higher survival rates.

In conclusion, this study provides real-world information on the use of erlotinib in advanced non-small cell lung cancer, regardless of squamous or non-squamous histology. The results also suggest that erlotinib may provide benefits in progression-free survival. Additionally, our results overall align with findings from randomized controlled trials and confirm the therapeutic value of erlotinib in the second and third lines for selected patients. We believe that second and third-line therapy represents a challenge for oncologic research.

2.4 Final conclusions and personal contributions

In the first study, we presented an overview of therapeutic management of NSCLC in Romania, analysing systemic treatment administered to patients with advanced or metastatic non-small cell lung cancer at the Bucharest Oncology Institute between 2015 and 2019. The aim was to establish a reference standard for comparison with specific therapies, such as targeted therapies and immunotherapy. The conclusions drawn from the first study indicate that chemotherapy still has a significant impact on prolonging the survival of patients with advanced or metastatic NSCLC. However, currently, combinations of chemotherapy, targeted therapy, and immunotherapy have proven to be the most effective. Additionally, monotherapy with immunotherapy or targeted therapy has entered current practice with notable results and reduced toxicity compared to chemotherapy. During the period in which the patients included in this study were treated, there were no data available in Romania, nor are there currently, regarding conjugated monoclonal antibodies.

Personalizing treatment based on individual patient characteristics is essential for optimizing outcomes. Furthermore, targeted therapies and immunotherapy, especially in combination, can offer significant benefits, with a positive association with survival. The study supports the relevance of results from randomized controlled trials in real-world contexts, highlighting the importance of continuing research on a larger sample.

The specific use of nivolumab in the second-line treatment of advanced NSCLC was evaluated in the second study, which demonstrated, through real-world data, its effectiveness, yielding similar results to clinical trials. Additionally, the study data confirm that nivolumab is well-tolerated and can be safely administered in a real-world setting, suggesting broad clinical applicability.

Although the results are promising, a larger sample size is necessary to confirm and objectify the findings. This study reiterates the importance of real-world research for validating findings from clinical trials. The conclusions of the study on the use of erlotinib in the second-line treatment of NSCLC, and even in the third line, demonstrate its effect on disease progression and a trend towards improved survival. Real-world data in this case showed a modest but existing response even in patients without detected EGFR mutation, making erlotinib a last-resort medication for patients with advanced NSCLC. This result suggests that erlotinib may be a valuable therapeutic option in these lines of treatment, without adding additional toxicity. The study confirmed that patients with adenocarcinoma histology had higher survival rates. However, from the perspective of current therapy, we know that only

EGFR determination can lead to therapeutic indication with TKI inhibitors. Overall, studies on nivolumab or erlotinib therapy in the second line and beyond, with real-world data, reveal that these therapies can be used for selected patients. These studies highlight the importance of real-world data studies, which have been relatively recently approved by the FDA for assessing the effectiveness of an innovative drug to be introduced to the market.

Another conclusion of the presented studies is that real-world data should be improved to have all the necessary details for a study, as patient selection for these studies was burdened by the absence of many data (treatment evolution data, date of death, adverse reactions, etc.), leading to the selection of a relatively small number of patients who had all the necessary study data. Of course, extensive research on a larger number of patients would provide more robust conclusions. However, the finding that our data, collected from a relatively small group of patients, confirms the results of international studies indicates that the results are close to reality.

List of scientific articles published

1. ”Real-world and clinical trials for assessing the effectiveness of new drugs in oncology. The role of observational studies with exemplification in non-small cell lung cancer (NSCLC)”
   Mihaela Teodorescu, Alexandru Grigorescu: ”Oncolog – Hematolog ”, Year XVI • No. 60 (3) 2022 • DOI: 10.26416/OnHe.60.3.2022

2. ”Cardiac toxicity of checkpoint inhibitors (CPI) used in cancer immunotherapy”
   Alexandru Grigorescu, Mihaela Teodorescu, ”Oncolog – Hematolog ” Year XVII • No. 62 (1) 2023 • DOI: 10.26416/OnHe.62.1.2023

3. ”Real-world evidence with nivolumab in advanced non-small cell lung cancer – second line and beyond. Our experience in Romania following straightaway reimbursement of healthcare costs”.
   Mihaela Teodorescu, Alexandru C. Grigorescu, ”Oncolog – Hematolog ” Year XVII • No. 62 (1) 2023 • DOI: 10.26416/OnHe.62.1.2023

4. „How to integrate medical oncology and palliative care?”
   Mihaela Teodorescu, Alexandru C. Grigorescu, ”Integrative Cancer Science ”
   October 26, 2019• Volume 6 1-2 DOI: 10.15761/ICST.1000322

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