

**Retrospective analysis of the influence of TP53, STK11, CDKN2A,
KEAP1 co-mutations in EGFR or KRAS G12C mutated non-small cell
lung cancer (NSCLC) on clinical outcome parameters**

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von Herrn Massimo Privitelli

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Gutachter: Prof. Dr. Marc-Philip Hitz, PhD

Zweitgutachter: Prof. Dr. Cornelius Waller

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Abstract

Introduction: Non-small lung carcinoma (NSCLC) is the most prevalent subtype of lung cancer and remains one of the leading causes of cancer-related mortality worldwide. Advances in molecular diagnostics enabled the identification of targetable driver mutations, such as EGFR and KRAS G12C, significantly improving patient outcomes through targeted therapy. However, the prognostic impact of co-mutations, particularly TP53, STK11, CDKN2A, and KEAP1, in driver-mutated NSCLC is not yet fully understood. This retrospective study aimed to investigate the influence of these co-mutations on clinical outcomes, such as overall survival (OS), progression-free survival (PFS), and overall response rate (ORR), in patients with KRAS G12C- or EGFR-mutated NSCLC. **Material and Methods:** 190 NSCLC patients treated at the Pius-Hospital Oldenburg between June 2016 and February 2024 were analysed, of which 162 were diagnosed with stage IV NSCLC. Molecular and clinical data were obtained from routine diagnostics and pseudonymized for evaluation. Subgroup analyses were performed to assess the impact of TP53, STK11, CDKN2A, and KEAP1 co-mutations on clinical endpoints. Statistical analyses included Kaplan-Meier survival estimation, log-rank testing, and univariate Cox regression to determine hazard ratios and 95% confidence intervals. **Results:** Patient characteristics of the investigated cohort correspond to the data of NSCLC patients in Germany. Among EGFR-mutated NSCLC, 43.4% (n=23/53) harboured a TP53 co-mutation. When treated with a tyrosine kinase inhibitor (TKI) these patients showed a non-significantly shorter median OS of 22 months (n=17) compared to 27 months (n=11) in EGFR-only mutated patients ($p \leq 0.228$). In the subgroup receiving first-line Osimertinib, median OS was 28 months for TP53 co-mutated patients (n=10) and was not reached for TP53 wild-type (n=7) ($p \leq 0.551$). Median OS in patients with a KRAS G12C mutation was 12 months (n=21), whereas patients with a KRAS G12C mutation and a TP53, STK11, CDKN2A, or KEAP1 co-mutation had a median OS of 18 months (n=42) ($p \leq 0.851$). In KRAS G12C-mutated NSCLC, TP53 co-mutations were present in 42.9% (n=30/70) and were associated with a median OS of 20 months (n=27), compared with 16 months in KRAS G12C-only patients (n=27) ($p \leq 0.292$). Among patients with KRAS G12C-mutated NSCLC harbouring at least one of the investigated co-mutations, those treated with combined immune checkpoint inhibitor (ICI) therapy and chemotherapy (n=23) demonstrated a numerically longer median OS of 20 months compared with those receiving ICI monotherapy (n=11; median OS 9 months; $p=0.502$). In the first-line ICI plus chemotherapy setting, median OS was not reached in KRAS G12C/TP53 co-mutated patients (n=14), compared with 16 months in KRAS G12C-mutated, TP53-wild-type patients (n=13;

p=0.092). STK11 co-mutations occurred in 20% (n=14/70) and were linked to a median OS of 18 months (n=13) versus 16 months without (n=41) (p≤0.203). Patients harbouring a CDKN2A co-mutation (8.6%, n=6/70) had a median OS of 9 months (n=6) compared to 16 months in the KRAS G12C-only cohort (n=48) (p ≤0.898). KEAP1 co-mutations were detected in 11.4% (n=8/70) and resulted in a median OS of 11 months (n=6) compared to 17 months without co-mutation (n=48) (p≤0.805). **Conclusion:** This study provides valuable insights into the complex role of TP53, STK11, CDKN2A, and KEAP1 co-mutations on clinical outcomes in EGFR- or KRAS G12C-mutated NSCLC. While TP53 co-mutation in EGFR-mutated NSCLC was associated with a trend toward shorter survival, statistical significance was not reached. In KRAS G12C-mutated NSCLC, co-mutations exhibited heterogeneous effects on overall survival. A key finding was the improved OS in KRAS G12/TP53-mutated patients treated with a combination of ICI and chemotherapy compared to ICI monotherapy, suggesting a potential synergistic effect. Moreover, the evaluation of PD-L1 expression seems to be particularly relevant in this subgroup, as this subgroup may demonstrate a more favourable response to immunotherapy. These results emphasize the importance of comprehensive molecular profiling for optimizing personalized treatment strategies for advanced NSCLC and warrant validation in larger, multicentric cohorts.

Zusammenfassung

Einleitung: Das nicht-kleinzellige Lungenkarzinom (NSCLC) ist die häufigste Subgruppe des Lungenkrebses und weiterhin eine der führenden Ursachen krebssbedingter Mortalität weltweit. Fortschritte in der molekularen Diagnostik haben zur Identifizierung therapierbarer Treibermutationen, wie EGFR und KRAS G12C, geführt und somit die Behandlungsergebnisse durch gezielte Therapien deutlich verbessert. Der prognostische Einfluss von Komutationen, insbesondere in TP53, STK11, CDKN2A und KEAP1, bei NSCLC mit Treibermutationen ist jedoch bislang nicht vollständig geklärt. Ziel dieser retrospektiven Studie war es, den Einfluss dieser Komutationen auf klinische Endpunkte wie Gesamtüberleben (OS), progressionsfreies Überleben (PFS) und objektive Ansprechrates (ORR) bei Patientinnen und Patienten mit KRAS G12C- oder EGFR-mutiertem NSCLC zu untersuchen. **Material und Methoden:** In die Analyse wurden 190 NSCLC-Patientinnen und -Patienten eingeschlossen, die zwischen Juni 2016 und Februar 2024 am Pius-Hospital Oldenburg behandelt wurden. 162 Patientinnen und Patienten wiesen ein NSCLC im Stadium IV auf. Molekulare und klinische Daten wurden aus der Routinediagnostik erhoben und pseudonymisiert ausgewertet. Subgruppenanalysen erfolgten zur Beurteilung des Einflusses von TP53-, STK11-, CDKN2A- und KEAP1-Komutationen auf die klinischen Endpunkte. Die statistische Auswertung umfasste Kaplan-Meier Überlebenszeitanalysen, Log-Rank-Tests sowie univariate Cox-Regressionsanalysen zur Bestimmung von Hazard Ratios und 95%-Konfidenzintervallen. **Ergebnisse:** Die Patientencharakteristika der untersuchten Kohorte entsprachen den Daten von NSCLC-Patientinnen und -Patienten in Deutschland. Unter den EGFR-mutierten Fällen wiesen 43,4% (n=23/53) eine TP53-Komutation auf. Bei Behandlung mit einem TKI zeigte sich ein nicht signifikant kürzeres medianes OS von 22 Monaten (n=17) im Vergleich zu 27 Monaten bei ausschließlich EGFR-mutierten Patientinnen und Patienten (n=11; $p \leq 0,228$). In der Subgruppe, die eine Erstlinientherapie mit Osimertinib erhielt, lag das mediane OS bei 28 Monaten für TP53-komutierte (n=10) und war bei TP53-Wildtyp (n=7) nicht erreicht ($p \leq 0,551$). Bei KRAS G12C-mutiertem NSCLC betrug das mediane OS 12 Monate (n=21), während bei zusätzlicher TP53-, STK11-, CDKN2A- oder KEAP1-Komutation ein medianes OS von 18 Monaten (n=42) beobachtet wurde ($p \leq 0,851$). TP53-Komutationen traten in 42,9 % der Fälle auf (n=30/70) und waren mit einem medianen OS von 20 Monaten (n=27) gegenüber 16 Monaten bei alleiniger KRAS G12C-Mutation (n=27) assoziiert ($p \leq 0,292$). Bei Vorliegen mindestens einer der untersuchten Komutationen zeigte sich unter ICI plus Chemotherapie (n=23) ein numerisch längeres medianes OS von 20 Monaten als

unter ICI-Monotherapie (n=11; 9 Monate; p=0,502). In der Erstlinie wurde unter ICI plus Chemotherapie das mediane OS bei KRAS G12C/TP53-komutierten Fällen (n=14) nicht erreicht, verglichen mit 16 Monaten bei KRAS G12C-mutiertem TP53-Wildtyp (n=13; p=0,092). STK11-Komutationen traten bei 20% (n=14/70) auf und waren mit einem medianen OS von 18 Monaten (n=13) im Vergleich zu 16 Monaten ohne STK11-Komutation (n=41; p≤0,203) verbunden. Patientinnen und Patienten mit einer CDKN2A-Komutation (8,6%, n=6/70) wiesen ein medianes OS von 9 Monaten (n=6) gegenüber 16 Monaten in der KRAS G12C Kohorte (n=48; p≤0,898) auf. KEAP1-Komutationen wurden in 11,4% (n=8/70) nachgewiesen und führten zu einem medianen OS von 11 Monaten (n=6) gegenüber 17 Monaten ohne Komutation (n=48; p≤0,805). **Fazit:** Diese Studie liefert wichtige Erkenntnisse über die komplexe Rolle von TP53-, STK11-, CDKN2A- und KEAP1-Komutationen in EGFR- oder KRAS G12C-mutiertem NSCLC. Während TP53-Komutationen bei EGFR-mutierten Fällen mit einem Trend zu kürzerem Überleben assoziiert waren, erreichte dieser Unterschied keine statistische Signifikanz. Bei KRAS G12C-mutiertem NSCLC zeigten Komutationen heterogene Effekte auf das OS. Ein wesentliches Ergebnis war das verlängerte OS bei KRAS G12C/TP53-komutierten Patientinnen und Patienten unter kombinierter ICI- und Chemotherapie im Vergleich zur ICI-Monotherapie, was auf einen möglichen synergistischen Effekt hinweist. Zudem erscheint die Evaluation der PD-L1-Expression in dieser Subgruppe besonders relevant, da diese Patientinnen und Patienten möglicherweise besser auf eine Immuntherapie ansprechen. Diese Ergebnisse unterstreichen die Wichtigkeit einer umfassenden molekularen Charakterisierung zur Optimierung personalisierter Behandlungsstrategien beim fortgeschrittenen NSCLC und sollten in größeren, multizentrischen Kohorten validiert werden.

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List of abbreviations

ACCP – American College of Chest Physicians
AJCC – American Joint Committee on Cancer
ALK – Anaplastic Lymphoma Kinase
CCI – Charlson Comorbidity Index
CNS – Central nervous system
CDKN2A – Cyclin-Dependent Kinase Inhibitor 2A
CRISP – Clinical Research platform Into molecular testing, treatment and outcome of (non-) Small cell lung carcinoma Patients
CTLA-4 - Cytotoxic T Lymphocyte Antigen-4
DKG – Deutsche Krebsgesellschaft
DNA – Deoxyribonucleic Acid
ECOG - Eastern Cooperative Oncology Group
EGFR – Epidermal Growth Factor Receptor
ESMO – European Society for Medical Oncology
ICI – Immune Checkpoint Inhibitor
IASLC – International Association for the Study of Lung Cancer
KEAP1 – Kelch-like ECH-associated Protein 1
KRAS – Kirsten Rat Sarcoma Viral Oncogene Homolog
LDCT – Low-dose computed tomography
LOF - Loss-of-function
MET – Mesenchymal-Epithelial Transition factor
NCCN – National Comprehensive Cancer Network
NGS - Next-Generation Sequencing
NSCLC – Non-Small Cell Lung Cancer
NTRK -Neurotrophic Tyrosine Receptor Kinase
OMD – Oligometastatic Disease
ORR – Overall Response Rate
OS – Overall Survival
PD-L1 – Programmed Death Ligand 1
PFS – Progression-Free Survival
RNA – Ribonucleic Acid
ROS1 – ROS Proto-Oncogene 1, Receptor Tyrosine Kinase
RTK - Rreceptor tyrosine kinase
SCLC – Small Cell Lung Cancer

STK11 – Serine/Threonine Kinase 11

TKI – Tyrosine Kinase Inhibitor

TNM – Tumor, Node, Metastasis

TTF1 - Thyroid Transcription Factor 1

TP53 – Tumor Protein 53

UICC – Union for International Cancer Control

WHO – World Health Organization

1. Introduction

In Germany lung carcinoma is one of the most common malignancies representing the 3rd most common malignant tumor in women and 2nd in men. Generally, lung cancer is divided into small (SCLC) and non-small lung cancer (NSCLC), whereby NSCLC is the more common entity. In the years 2017 to 2019 NSCLC was accounting for 79% of lung cancer cases in Germany (1).

The main risk factor for developing lung cancer is smoking, which is responsible for the occurrence of 85% to 90% of all lung cancer cases (2). Lung cancer remains a malignant disease with a poor prognosis (3). However, due to medical advances, many different treatments are now available based on tumor histology, molecular changes, tumor stage and patient performance (1).

In recent decades, numerous driver mutations have been discovered, and appropriate targeted therapies have been developed. This has proven to be very valuable in improving the quality of treatment and has led to significantly improved clinical outcomes compared to a standard chemotherapy regimen (1).

However, the impact of co-mutations in driver-mutated NSCLC on clinical outcomes is still not fully understood and data regarding the topic is scarce. While the association of TP53 co-mutations with primary resistance to targeted therapies and poorer clinical outcome in EGFR-mutated NSCLC has been proven (3–5). The impact of other co-mutations on clinical outcome is yet to be investigated.

In this retrospective analysis, data from patients with advanced NSCLC treated at the certified lung cancer center Oldenburg, Pius-Hospital, is evaluated to assess the relationship between the presence of DNA-based biomarkers obtained from routine diagnostics, the administered treatments, and their impact on clinical outcome parameters defined as overall response rate (ORR), progression free survival (PFR), and overall survival (OS).

Chapter 2 presents the current state of research and describes the various molecular changes. The chapter concludes with the formulation of the research questions of the study. Chapter 3 provides a comprehensive description of the study design, study population, and data sources used for data extraction. This is followed by a description of the diagnostic procedures used to obtain both histological and molecular data. Then the statistical methods used in this study are described. Chapter 4 is a concise presentation of the results of the research, featuring various figures and tables designed to visualize the responses to the questions formulated in the introduction. In chapter 5, the discussion section, the obtained

results are being put into a larger context by comparing them with the conclusions of other studies performed in the same field of research. The final chapter serves to summarize the investigation. This dissertation mentions common characteristics of lung cancer, but the investigation is performed exclusively on patients diagnosed with NSCLC.

2. State of research

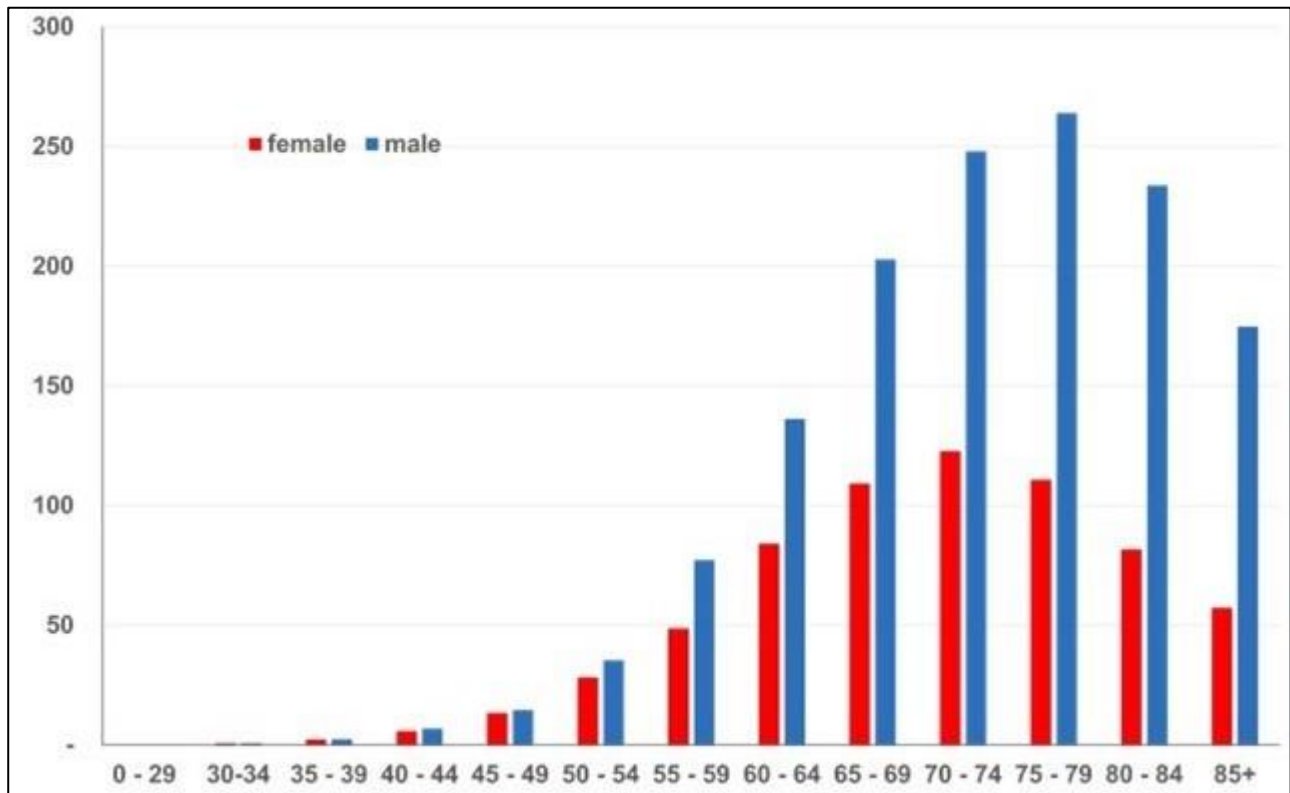
2.1 Epidemiology

In the year 2020 in Germany around 56.700 new cases of lung cancer were reported (6). Most lung cancer cases are NSCLC, and men are more frequently affected. In Germany, 14,500 women and 29,500 men are diagnosed with NSCLC every year. Lung cancer is not only one of the most common tumor diseases, but also one of the most common causes of death: 45,200 patients died of lung cancer in Germany in 2022. Lung cancer has an age-specific incidence that rises until the eighth decade of life. The median age at which the disease occurs is 69, with only around 2% of patients developing lung cancer before the age of 50 (figure 1) (1,6).

Since the end of the 1990s, the age-standardized morbidity and mortality rates for both genders have developed in opposite directions. For men, they are decreasing continuously, while for women they are rising steadily, so that they are approaching each other significantly. This trend appears to be due to a change in smoking behaviour among both genders and is likely to continue in the future (figure 2) (6).

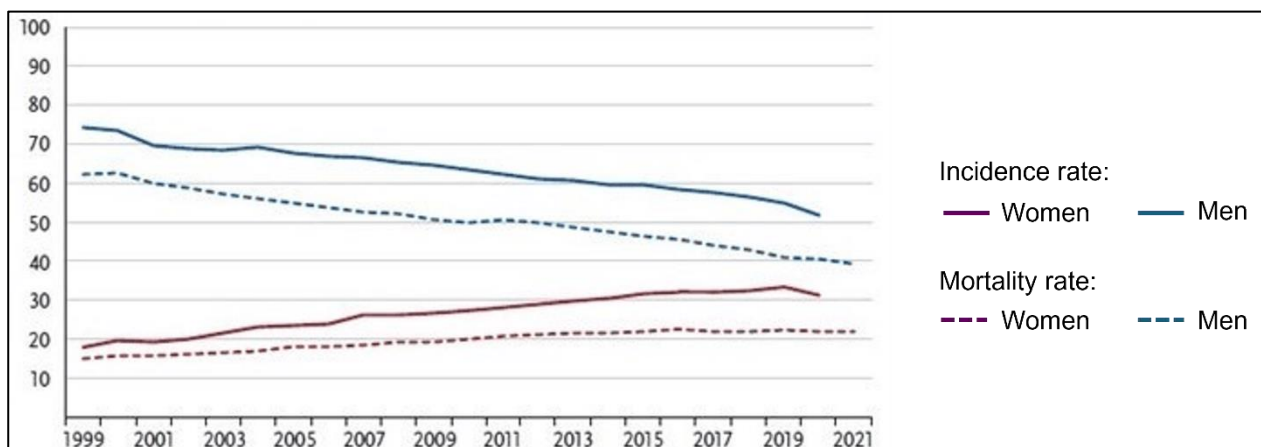
Lung cancer remains to be one of the cancers with an overall unfavourable prognosis with a relative 5-year survival rate of around 25% in females and 19% in males and a 10-year relative survival rate of 19% in females and 14% in males (6).

Figure 1 Annual incidence rates of NSCLC per 100.000 persons, by age and sex



Reference: Onkopedia (1)

Figure 2 Age-standardized morbidity and mortality rates



Reference: RKI 2023 (6)

2.2. Etiology & Risk factors

While lung cancer is primarily associated with tobacco smoking and accounts for over 80% of lung cancer cases in the United States and other countries, the association between smoking and cancer varies depending on the histologic subtype of NSCLC. This association is stronger in cases of lung squamous NSCLC and SCLC, compared to non-squamous

NSCLC. In fact, in never smokers non-squamous NSCLC is the most common histological subtype (7). Over the last three decades, there has been a shift in prevalence from squamous cell carcinoma to non-squamous cell carcinoma in smokers. This seems to be due to changed smoking behaviour as well as changes in the production of cigarettes. Especially the creation of filter cigarettes and so-called “light” cigarettes, which allow smokers to inhale deeper, so that the toxins contained in the smoke are able to enter deeper regions of the lung to the alveoli, thus generating adenocarcinoma (8).

With the rise of alternative methods of smoking, namely electrical cigarettes, and vaping liquids, which are gaining popularity especially amongst younger generations, it should be highlighted that these devices also contain several known and suspected oncogenic substances, such as derivatives of nicotine, heavy metals, and aldehydes. Despite the fact that research on this topic is still inconclusive, there is good reason to be concerned about the potential carcinogenicity of e-cigarettes and e-liquids.(9).

Except smoking, there are various other external factors that can increase the risk of developing lung cancer. Exposure to air pollution and environmental radon gas contribute to the development of NSCLC (1,8). Other forms of ionizing radiation, such as radiation exposure for medical purposes, as well as exposure to particulate dust or asbestos also increase the risk of developing lung cancer. Chronic lung infections or chronic lung diseases (e.g. COPD, emphysema) can also increase the risk of lung cancer. Individuals with one or more first-degree relatives who have had lung cancer are also at a higher risk of developing the disease themselves (1,10).

2.3 Histological subtypes

In order to categorize the various histological subtypes of lung cancer the World Health Organization has developed a classification. The latest one is the WHO Classification of thoracic tumors from 2021. While the previous edition from 2015 prioritized the use of immunohistochemistry for more precise tumor classifications, in the new edition the emphasis lies on more specific molecular and genetic profiling of tumors for improved tumor identification (table 1) (11).

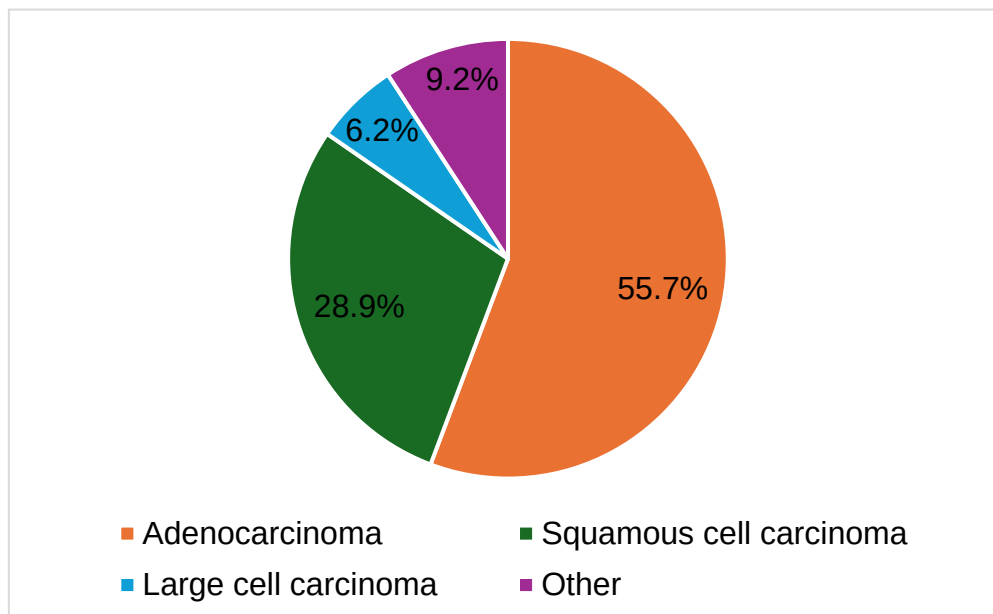
Table 1 The 2021 WHO Classification of thoracic tumors

The 2021 WHO Classification of thoracic tumors	
Squamous cell carcinoma	keratinizing
	nonkeratinizing
	basaloid
Adenocarcinoma	Preinvasive
	minimally invasive -nonmucinous -mucinous
	invasive nonmucinous -lepidic -acinar -papillary -micropapillary -solid
	invasive mucinous
	Mixed invasive mucinous and nonmucinous
	Further variants
Large cell carcinoma	
Neuroendocrine tumors	Carcinoid -typical carcinoid -atypical carcinoid
	Small cell carcinoid
	Large cell neuroendocrine carcinoma

Reference: Nicholson AG, Tsao MS, Beasley MB et al. 2021 (11)

Lung cancer is divided into NSCLC and SCLC, whereas NSCLC is responsible for ~80% of all lung malignancy cases (1). The main types of NSCLC are adenocarcinoma, squamous NSCLC, and large cell carcinoma. In Germany, from 2017 to 2019, adenocarcinoma was the most prevalent histological subtype, accounting for 55.7% of all NSCLC cases. Squamous NSCLC made up 28.9%, while large cell carcinoma accounted for 6.2% of the cases. A gender-based discrepancy in the prevalence of adenocarcinoma in NSCLC is observed. Among females, adenocarcinoma represented 64.7% of all NSCLC cases, while only accounting for 50.3% in males (12).

Figure 3 Characteristics of NSCLC cases recorded in Germany from 2017 - 2019 (annual average)



Reference: Epidemiologie des nichtkleinzelligen Lungenkarzinoms in Deutschland – ein Update. Z Pneumologie. 2023 (12)

2.4 Screening

Although the use of low-dose computed tomography (LDCT) has significantly reduced lung-cancer-specific mortality (13,14), still, over 50% of the patients are diagnosed with stage IV NSCLC. For most patients at this advanced stage, there is no curative treatment available (15–20). Screening with LDCT is most beneficial for high-risk populations, such as smokers and individuals with occupational exposure to fumes or toxic substances. In the USA, smokers with 20 to 30 pack years currently are recommended to begin screening at 55. Nevertheless, studies have demonstrated that LDCT screening has a false-positive rate of 81%, requiring further imaging or testing to validate the results (21).

Despite this, LDCT significantly reduces lung cancer-specific mortality, as shown in the US National Lung Cancer Screening Trial (NLST) and the NELSON trial from Belgium and the Netherlands. The NELSON trial reported a statistically significant decrease in lung cancer mortality after 9 years, particularly among women (hazard ratio 0.52), but also among men (hazard ratio 0.76) (1). A seven-year smoking cessation period has a similar mortality reduction effect, suggesting that combining screening programs with smoking cessation efforts can improve outcomes (22).

Currently, there are no accepted protocols for early detection in the German-speaking countries of Europe, except for the Swiss Accident Insurance Fund (SUVA) recommendation for individuals with exposure to asbestos and the German Statutory Accident Insurance for individuals with hazard exposure at work (1). However, low dose CT screening for lung cancer will be established in Germany by April 2026 for the high-risk group defined by smoking and age (23). Examining possible predictive biomarkers in 5000 high-risk individuals and screening for cardiac and pulmonary diseases, the HANSE trial is a comprehensive prospective study in Germany, aiming to assess the feasibility of lung cancer screening. A mobile CT truck facilitates the screening by moving between three certified lung cancer centres (13).

2.5 Clinical features

Spiro et al. observed cough as the most common initial symptom of lung cancer (8-75%), followed by weight loss (0-68%), dyspnea (3-60%), and chest pain, occurring in 20-49% of the patients (24). A detailed overview prevalence of early symptoms is shown in table 2 below. While small nodules in the lung often do not cause symptoms, larger lesions more commonly lead to pulmonary symptoms, such as cough, chest pain, or dyspnea. Often patients will experience vague or sharp chest pain, also known as pleuritic pain, which warrants careful consideration as it may be a sign of pleural invasion (25).

An observational study conducted in Spain between 2016 and 2019, involving 9876 patients, showed that the prevalence of symptoms increased with advancing tumor stages. In stage I and II 59.3% and 42.5% of patients, respectively, were asymptomatic. In stage III only 30.2% and in stage IV 27.7% of patients were without symptoms. However, the absence of symptoms should not rule out a diagnosis of lung cancer, as over a quarter of stage IV patients were asymptomatic at the time of diagnosis (26).

A more recent Danish study from 2023, which included over 11,000 patients with lung cancer, demonstrated that 17.4% of those with stage I-II disease were asymptomatic, while only 5.4% of those with stage III-IV cancer had no symptoms (25). Additionally, the absence of symptoms has been associated with a better prognosis (24,27).

Table 2 Occurrence of initial symptoms of lung cancer

Occurrence of initial symptoms of lung carcinoma	
Symptoms and signs	Frequency, %
Cough	8-75
Weight loss	0-68
Dyspnea	3-60
Chest pain	20-49
Haemoptysis	6-35
Bone pain	6-25
Digital clubbing	0-20
Fever	0-20
Weakness	0-10

Reference: Spiro et al. 2007 (24)

2.6 Diagnostics

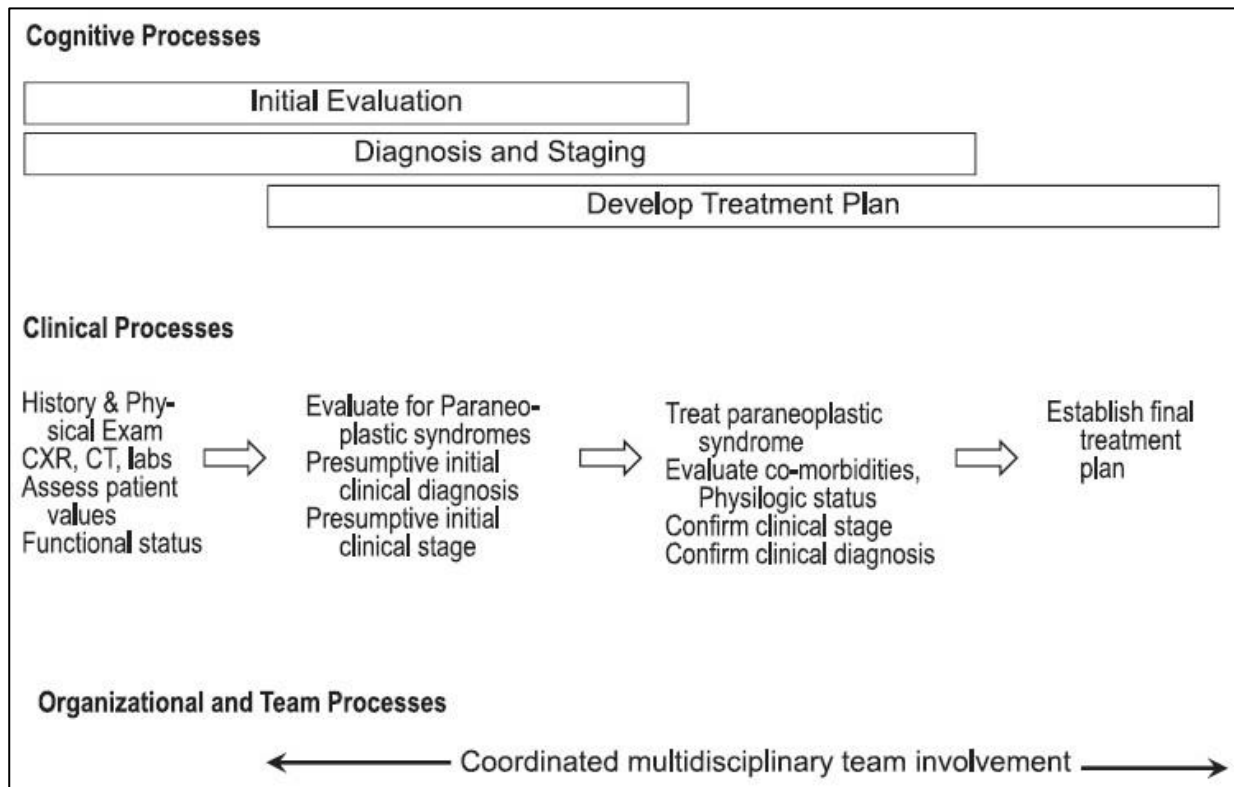
The American College of Chest Physicians (ACCP) has released the 3rd edition of its guidelines, showing the initial assessment and diagnostic procedures required for lung cancer diagnosis. Emphasizing a multidisciplinary approach, the guideline advocates for a systematic approach beginning with comprehensive medical history-taking and physical examination, completed by medical imaging, laboratory analyses, as well as functional assessments. Subsequent steps are screening for paraneoplastic syndromes, evaluating comorbidities, and employing additional imaging methods for tumor staging. Ultimately, highlighting the importance of formulating a personalized treatment plan (25).

According to the current German S3-guideline, every stage IV NSCLC patient should receive a molecular analysis of the tumor tissue regarding relevant molecular mutations before starting a first-line therapy. Currently included on this list are EGFR 18-21 mutations, BRAF V600 mutations, ALK-fusions, ROS1-fusions, RET-fusions, NTRK1-3 fusions, KRAS mutations, MET amplifications, MET Exon 14 Skipping mutations, NRG1-fusions, HER2 amplifications and HER2-Exon 20-mutations (16).

According to Frost et al., maintaining up-to-date guidelines and implementing them might be difficult. These challenges result from the rapid advancements in lung cancer diagnostics and treatments, alongside the time-consuming nature of developing consensus-based, thoroughly reviewed, evidence-based guidelines. Therefore, other sources like Onkopedia provide more frequently updated guidelines and are valuable in clinical practice. It is recommended that newly diagnosed patients undergo clinical evaluation and treatment planning

at DKG-certified lung cancer centres in Germany, where cases are reviewed by a multidisciplinary tumor board (13).

Figure 4 Diagnostic Work-up of a suspected lung cancer patient



Reference: 3rd Edition ACCP Guidelines. 2013 (25)

2.7 NSCLC classification – The TNM-Staging

Between 2010 and 2016, the staging of lung cancer was performed according to the 7th edition of the tumor, node, and metastasis (TNM) classification (15). The 8th edition was introduced and used from 2017 until the end of 2023. This revision was performed by the International Association for the Study of Lung Cancer (IASLC), the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC), (1). All patients included in this investigation have been classified based on UICC7/UICC8 staging. The 8th edition introduces tumor size as a factor in all T categories. Furthermore, the classification of stage IV has been altered by limiting the M1b category to cases with a single, distant metastasis, and by adding M1c for all cases in which there are >1 distant metastases. Also, stage IV was sub-categorized into stage IVA and IVB, where M1a and M1b fall into stage IVA and M1c is classified as stage IVB (15). Also, all solitary metastases, independent from their location, are classified as oligometastatic disease (OMD), (1).

For cancer staging, the UICC TNM classification is essential for assessing disease severity, planning treatments, estimating prognosis, and providing a standardized, internationally accepted format for comparison (28). The exact staging criteria of the UICC 8 TNM classification are shown in table 3 and table 4 below.

As of 2024, the 9th edition of the TNM classification of lung cancers is in effect. The changes have been made based on a database with 124.581 patients, of which 87.043 cases remained after the exclusion of incomplete data sets. More than half of the collected data comes from Asia and Australia (56%), followed by Europe (24.7%), North America (15.7%), and the rest from South & Central America, Africa, and the Middle East. The data base included lung cancer patients diagnosed between 2011 and 2019 (29).

Table 3 Description of TNM stages according IASLC Lung Cancer Staging Project UICC 8

Category	Stage	Brief description
T (Tumor)	T0	No primary tumor
	Tis	Carcinoma in situ (squamous or adenocarcinoma)
	T1	Tumor ≤3 cm
	T1mi	minimally invasive adenocarcinoma
	T1a	Superficial spreading tumor in central airways*, tumor ≤1 cm
	T1b	Tumor >1 but ≤2 cm
	T1c	Tumor >2 but ≤3 cm
	T2	Tumor >3 but ≤5 cm or tumor involving: visceral pleura,** main bronchus (not carina), atelectasis to hilum**
	T2a	Tumor >3 but ≤4 cm
	T2b	Tumor >4 but ≤5 cm
	T3	Tumor >5 but ≤7 cm or invading chest wall, pericardium, phrenic nerve; or separate tumor nodule(s) in the same lobe
	T4	Tumor >7cm or tumor invading: mediastinum, diaphragm, heart, great vessels (v. cava, aorta, pulmonary artery, pulmonary vein intrapericardially), recurrent laryngeal nerve, carina, trachea, oesophagus, spine; or tumor nodule(s) in a different ipsilateral lobe
N (lymph node)	N0	No regional lymph node metastasis
	N1	Metastasis in ipsilateral pulmonary or hilar nodes
	N2	Metastasis in ipsilateral mediastinal or subcarinal nodes
	N3	Metastasis in contralateral mediastinal, hilar, or supraclavicular lymph nodes
M (Metastasis)	M0	No distant metastasis
	M1a	Malignant pleural or pericardial effusion*** or pleural or pericardial nodules or separate tumor nodule(s) in a contralateral lobe
	M1b	Single extrathoracic metastasis
	M1c	Multiple extrathoracic metastases (1 or > 1 organ)

*Superficial spreading tumor of any size but confined to the tracheal or bronchial wall.

**Atelectasis or obstructive pneumonitis extending to hilum; such tumors are classified as T2a if >3 and ≤4 cm, T2b if >4 and ≤5 cm

***Pleural effusions are excluded that are cytologically negative, nonbloody, transudative, and clinically judged not to be due to cancer.

Reference: JTCVS (30)

Table 4 Classification of tumor stages according to UICC 8

T/M	Subcategory	N0	N1	N2	N3
T1	T1a	IA1	IIB	IIIA	IIIB
	T1b	IA2	IIB	IIIA	IIIB
	T1c	IA3	IIB	IIIA	IIIB
T2	T2a	IB	IIB	IIIA	IIIB
	T2b	IIA	IIB	IIIA	IIIB
T3	T3	IIB	IIIA	IIIB	IIIC
T4	T4	IIIA	IIIA	IIIB	IIIC
M1	M1a	IVA	IVA	IVA	IVA
	M1b	IVA	IVA	IVA	IVA
	M1c	IVB	IVB	IVB	IVB

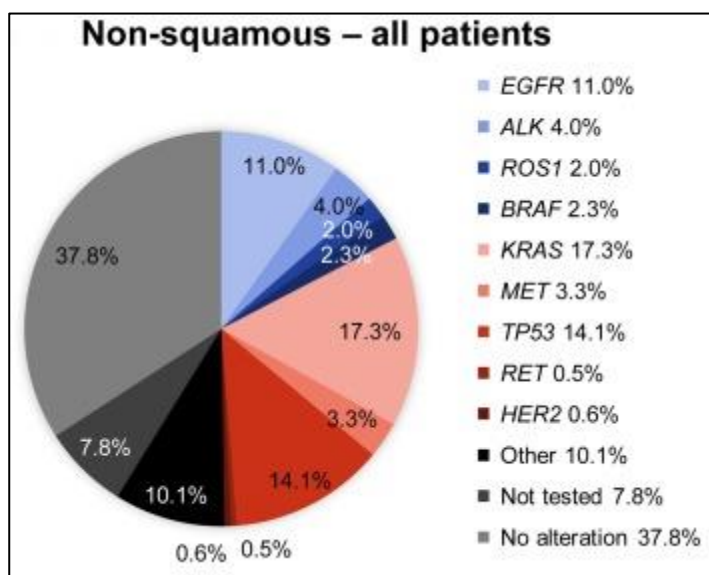
Reference: JTCVS (30)

2.8 Molecular subgroups – Driver mutations

NSCLC is the most common type of lung cancer, and research has shown that a significant number of cases involve mutations in specific genes, promoting oncogenesis, known as oncogenic drivers. In recent years, the identification of additional oncogenic drivers has led to the development of targeted therapies - drugs designed to specifically target these mutations. Targeted therapies offer significant improvements in both the quality and quantity of life for patients, providing better outcomes compared to traditional chemotherapy (31,32).

In Germany the prospective, nation-wide CRISP registry (AIO-TRK-0315) collects representative data on the frequency and results of biomarker testing of advanced NSCLC patients. Griesinger et al. demonstrated that 90.5% of patients with advanced NSCLC enrolled in the registry underwent biomarker testing, with testing rates progressively increasing over the study period. Notably, 17.7% of patients exhibited actionable genetic alterations. This indicates that while testing rates in Germany are generally high and might be adequate when compared to other countries, a sizable number of patients remain untested, despite potential benefits, underscoring the need for additional efforts to increase testing rates (33).

Figure 5 Biomarker testing in advanced non-squamous NSCLC patients in the CRISP Registry



Reference: Griesinger et al. CRISP Registry (33)

2.8.1 KRAS G12C

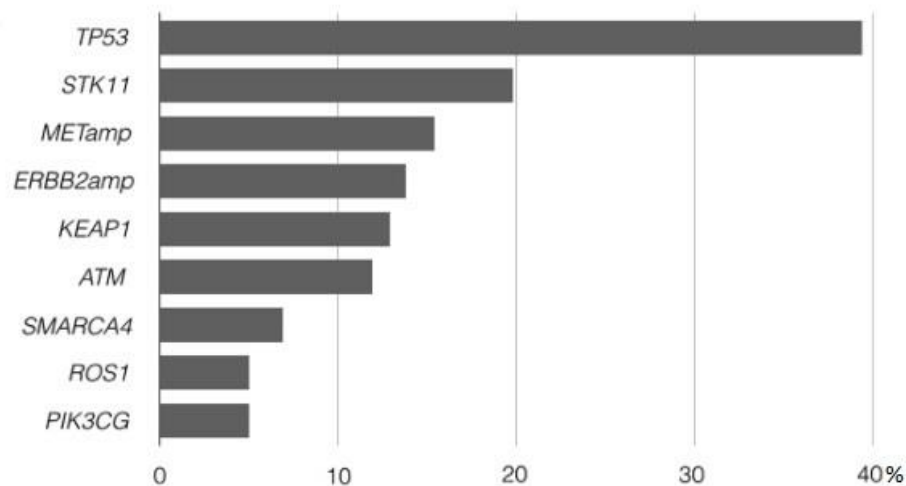
Kirsten rat sarcoma viral oncogene homolog (KRAS) is the most common mutated oncogene in human cancer (34). KRAS mutations lead to activation of various pathways, such as the mitogen-activated protein kinase (MAPK) pathway, promoting tumorigenesis by increasing cell proliferation, while also suppressing apoptosis (35).

Approximately 30% of lung adenocarcinomas exhibit KRAS mutations (36). There are various types of KRAS mutations with KRAS G12C being the most common one, found in roughly 40% of KRAS-mutated patients (37–39). Moreover, a recent study concludes that this specific mutation is associated with female gender and presence of central nervous system (CNS) metastasis (38). According to the current S3 guidelines stage IV KRAS-G12C-mutated NSCLC patients should receive targeted therapy with KRAS inhibitor Sotorasib after failed standard 1st-line therapy (16,40).

In a study performed by Scheffler et al. involving 1078 KRAS-mutated NSCLC patients, approximately half of them had at least one co-mutation. TP53 co-mutation was the most common (39.4%), followed by STK11 (19.8%). MET (4.1%) and ERBB2 (13.8%) amplifications were also observed frequently (37). For further details regarding other co-mutations, refer to figure 6. Another study concluded that the presence of mutations in KRAS G12C, STK11, and KEAP1 together (triple mutation) were associated with a significantly shorter OS and a higher incidence in metastases, especially bone metastases, compared to KRASG12C-only mutated NSCLC. This suggests that this combination of mutations could be considered a

negative prognostic indicator (41). Due to the limited studies available evaluating the presence of co-mutations alongside KRAS G12C, their clinical significance is yet to be fully established (37,42–44).

Figure 6 Frequency of co-mutations in KRAS-mutated NSCLC patients



Reference: Scheffler et al. (37)

2.8.2 EGFR

The Epidermal Growth Factor Receptor (EGFR) belongs to the group of the receptor tyrosine kinase (RTK) and was the first oncogenic target discovered in NSCLC. Dysregulated EGFR signalling can be caused by EGFR overexpression or activating mutations. EGFR overexpression is present in approximately 60% of NSCLCs (45). It is also seen in other malignancies, such as breast cancer, metastatic colorectal cancer, pancreatic cancer, and others. This overactivity triggers cell proliferation, angiogenesis, evasion of apoptosis, and metastasis, through multiple pathways (46).

The prevalence of EGFR mutations in NSCLC patients varies based on genetic ancestry, with rates between 10% to 20% in Caucasian populations and reaching up to 50% in individuals of Asian descent (47). They are more prevalent in women, never smokers, and are associated with adenocarcinoma histology (47,48). Typically located in exons 18-21, EGFR mutations, especially gain-of-function mutations in exon 21, primarily the L858R mutation, or deletions in exon 19, represent the largest subset of EGFR mutations. They account for approximately 70% to 90% of cases, and are commonly referred to as "classic" or "typical" or common EGFR mutations (49,50). The presence of activating EGFR mutations is a favourable prognostic indicator for OS and PFS and also serves as a positive predictive marker for response to tyrosine kinase inhibitor (TKI) therapy (51).

In untreated advanced EGFR-mutated NSCLC, several phase III clinical trials have examined the role of first-generation TKIs (Erlotinib, Gefitinib), and second-generation (Afatinib,

Dacomitinib) EGFR TKIs. These trials demonstrated comparable median response rates of 70% to 75% and improvements in PFS from 10 to 14 months when compared with platinum-based chemotherapy (52–56). In recent years, the third-generation EGFR TKI Osimertinib has shown superior efficacy compared to other standard TKIs by increasing OS (38.6 vs. 31.8 months) and improving PFS (18.9 vs 10.2 months) (57,58). Despite significant treatment advancements and a better understanding of the genetic factors involved, resistance to EGFR TKIs always develops, resulting in disease progression. In patients treated with 1st and 2nd generation TKIs resistance most frequently arises through the development of EGFR T790M mutation (59). Under treatment with the 3rd-generation TKI Osimertinib the most common mechanism of acquired resistance is MET amplification (60,61).

Another mechanism of resistance to EGFR TKIs is a TP53 co-mutation. Ferrara et al.'s 2023 meta-analysis found that TP53 co-mutations in EGFR-mutated NSCLC are linked to shorter PFS and OS, indicating that TP53 co-mutations may represent a distinct subgroup that requires further investigation to understand their role (5).

2.9 Molecular subgroups – Co-Mutations

In NSCLC treatment, understanding the impact of molecular mutations is crucial for developing targeted therapies. Notably, mutations in TP53, STK11, KEAP1, and CDKN2A frequently occur in NSCLC and are known to influence tumorigenesis and treatment resistance. However, their impact as co-mutations on clinical outcomes in driver-mutated NSCLC is not yet fully established, highlighting the need for further research in this area.

2.9.1 TP53

The tumor protein 53 (TP53) gene encodes the tumor-suppressor protein p53 - a powerful regulator of several cellular functions, such as cell proliferation and differentiation, DNA repair, apoptosis, and angiogenesis. The most common genetic mutations found in cancers are TP53 mutations. They are linked to tumorigenesis, tumor growth, metastasis, and treatment resistance (62).

TP53 mutations are the most frequently occurring genetic alterations in NSCLC, seen in ~50% of all cases (63,64). Mutations in TP53 are classified in different ways. According to Poeta et al. there are disruptive and non-disruptive mutations, where disruptive mutations are associated with a negative effect on OS, PFS, and worse response rates in EGFR-mutated NSCLC patients (65). Roeper et al. propose an alternative classification that, based on biophysical parameters and structural analysis, differentiates between pathogenic and non-pathogenic mutations. The pathogenic mutations are associated with a negative effect on OS, PFS, and worse response rates in EGFR-mutated NSCLC patients (4).

TP53 co-mutation in EGFR-mutated NSCLC patients is seen in 17% - 68% of cases (5) and research shows that TP53 mutations in EGFR-mutated NSCLC may play a role in primary resistance to EGFR-TKIs, particularly in first-line therapy. A significant correlation between TP53 co-mutation and reduced OS and PFS has been demonstrated in such patients (4,5,66), regardless of the generation of TKI used (5,67). This indicates the need to consider EGFR/TP53 co-mutated NSCLC as a separate clinical subgroup.

Frost et al. identified that NSCLC patients with KRASG12C/TP53 co-mutations and high programmed death-1 ligand 1 (PD-L1) expression ($\geq 50\%$) show prolonged responsiveness to pembrolizumab monotherapy, a finding further supported by a 2024 study (68,69). While clinical data are available regarding the impact of TP53 as a co-mutation in EGFR-mutated NSCLC, studies investigating its impact on other driver-mutated NSCLC, such as KRASG12C, remain scarce.

2.9.2 STK11

The STK11 (serine/threonine kinase 11) – a tumor suppressor gene first identified in the 1990s in patients with Peutz-Jeghers syndrome, encoding LKB1 (liver kinase B1) (70). LKB1 activates the AMP-activated protein kinase (AMPK) superfamily pathway, which is responsible for the regulation of various metabolic pathways, such as glucose, glutamine, lipid, and serine metabolisms. The loss of LKB1 disrupts these pathways, promoting cell proliferation, tumorigenesis, and altering immune cell functions (71). STK11 mutations are found in 10% - 15% of NSCLC cases and are correlated with unfavourable clinical outcomes (OS, PFS). These mutations serve as negative predictive markers in NSCLC, irrespective of whether patients receive ICI or chemotherapy as first-line treatment (72,73).

In STK11-mutated NSCLC, the most common driver mutation is KRAS, seen in ~50% of cases. KRAS/STK11 co-mutation is associated with a poorer prognosis compared to patients with wild-type KRAS and STK11 genes. However, patients with both mutations exhibit similar clinical outcomes (OS, PFS) compared to those with only a single mutation in either STK11 or KRAS (73).

2.9.3 CDKN2A

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is a tumor suppressor gene encoding the p16INK4a protein in humans which plays an important role in the cell cycle. CDKN2A mutation favours tumorigenesis, cancer metastasis and poor prognosis (74). CDKN2A loss-of-function (LOF) is one of the most prevalent mutations in NSCLC occurring in ~20% of squamous cell carcinoma patients and ~10% of adenocarcinoma cases (37).

Recent clinical data suggests that CDKN2A LOF mutation is associated with a worse response to ICI and shorter OS and PFS compared to CDKN2A wild-type NSCLC (75,76). Additionally, a CDKN2 LOF co-mutation in KRAS G12C-mutated NSCLC seems to be linked to earlier disease progression and poorer clinical outcomes in patients with advanced NSCLC treated with KRAS G12C inhibitor monotherapy (77). This finding highlights the necessity for further research in this area and might prompt the start to consider such patients as a distinct clinical subgroup.

2.9.4 KEAP1

The KEAP1 (Kelch-like erythroid cell-derived protein 1)-NRF2 (nuclear factor erythroid 2-related factor 2) signalling pathway is important for cellular defence and survival in response to oxidative stress (78). When encountering external or internal stressors, this pathway up-regulates synthesis of various cytoprotective proteins, preventing oxidative damage to cells (79). Research in NSCLC patients has shown that inactivation mutations in KEAP1 and gain-of-function mutations in NFE2L2 (the gene encoding NRF2) lead to resistance to several cancer treatments, including chemotherapy, radiation therapy, and targeted therapies (80).

In a 2018 study by Frank et al., next-generation sequencing (NGS) of tumor samples from 1,391 NSCLC patients revealed that 11.3% had KEAP1 mutations, with the majority (72%) diagnosed with adenocarcinoma histology (81). Moreover, Negrao et al. demonstrated that KEAP1 co-mutation in KRAS G12C-mutated NSCLC significantly correlated with a shorter PFS, OS, and ORR (77).

2.10 Treatment

Treatment options for NSCLC include surgery, radiation, and systemic therapies, frequently used in combination as part of a multimodal approach. Curative therapy is available for early-stage disease (I, II) and in some cases in advanced stages (Stage IIIa). However, for most patients with stage IIIB/C and IV NSCLC, there is no curative treatment.

The discovery of ICIs and TKIs alongside the use of predictive biomarkers has vastly improved the prognosis of patients with advanced-stage NSCLC. In a curative setting, surgery is the primary treatment for NSCLC, with lobectomy being the standard resection type (1). According to the German Cancer Society's requirements, every oncological center must hold an interdisciplinary tumor conference at least once a week. All physicians involved in a patient's treatment should participate, and every newly diagnosed or recurrent lung cancer case must be presented before therapy to explore treatment options per current S3 guidelines (16).

Chemotherapy is utilized in NSCLC in neoadjuvant, adjuvant and palliative settings, as well as in combination with radiotherapy. In about 10% of patients with non-squamous NSCLC, EGFR mutations are identified. For these individuals in stage IV, 1st-line treatment with a TKI, such as Osimertinib (FLAURA study, Osimertinib vs. Gefitinib or Erlotinib), or alternatively 2nd and 1st generation TKI such as Afatinib, Erlotinib, or Gefitinib is recommended. Tyrosine kinases initiate a cascade of signals that promote cancer cell division and proliferation. In cases where there is an activating EGFR mutation, the receptor remains constantly active, perpetually signalling for cell division. TKIs are small molecules that penetrate cell membranes and bind to the intracellular domain of the EGFR receptor, disrupting signalling pathways required for cell division, ultimately stopping cancer cell proliferation and inducing programmed cell death (82,83). Recently Osimertinib chemotherapy combination (FLAURA2) and Lazertinib-Amivantamab combinations have been approved based on increased survival vs. the standard of care Osimertinib (84).

In patients without a driver mutation, immune checkpoint inhibitor (ICI) therapy now has become a key component of their treatment. Tumors utilize several immune checkpoints to evade the host immune system, with the programmed PD-1/PD-L1 and cytotoxic T lymphocyte antigen-4 (CTLA-4) pathways being the most researched and advanced in clinical application. Inhibiting these pathways promotes activation and anti-tumor activity of cytotoxic T-cells, essential for mounting an effective immune response against cancer (85).

In NSCLC patients who have PD-L1 expression on at least 50% of tumor cells, Pembrolizumab was correlated with a significantly improved PFS and OS compared to platinum-based chemotherapy. Additionally, it resulted in fewer adverse side effects (86). The KEYNOTE-189 trial demonstrated that adding Pembrolizumab to a platinum-based chemotherapy regimen gives a survival benefit across all PD-L1 expression subgroups, including those

with scores below 1%, with the most pronounced relative benefit observed in patients with a PD-L1 score of 50% or higher (87).

Atezolizumab, another ICI, also showed superior OS and PFS in patients with PD-L1 expression on $\geq 50\%$ of tumor cells or $\geq 10\%$ expression of PD-L1 positive tumor-infiltrating immune cells compared to platinum-based chemotherapy (88). In patients with stage IV non-squamous NSCLC, multiple trials have shown that a combined chemoimmunotherapy with an anti-PD1/PD-L1 antibody results in higher median OS and higher ORR compared to standard-chemotherapy alone (89).

2.11 Research questions

Although substantial data exist on the most prevalent driver and co-mutations in NSCLC, clinical evidence regarding the impact of less common co-mutations in driver-mutated NSCLC remains limited. Recent studies suggest that these less common co-mutations may represent distinct clinical subgroups, warranting deeper investigation. The research questions posed in this study aim to address this critical gap by exploring the effects of these mutations.

- 1) How common are TP53, STK11, CDKN2A, KEAP1 co-mutations in KRAS G12C driver-mutated NSCLC?
- 2) How common are TP53 co-mutations and what are their characteristics (pathogenicity, disruption) in EGFR driver-mutated NSCLC?
- 3) Which impact have co-mutations in TP53, STK11, CDKN2A, and KEAP1 on OS, PFS, and ORR in stage IV KRAS G12C driver-mutated NSCLC compared to only KRAS G12C driver-mutated NSCLC?
- 4) Which impact does a TP53 co-mutation have on stage IV EGFR-mutated NSCLC patients regarding OS, PFS, and ORR?

3 Material & Methods

This chapter outlines the study design, population, data sources, and concludes with a description of the statistical methods used and a summary of the created subgroups.

3.1 Study design, study population, and data sources

In this retrospective, explorative, non-interventional cohort study patients with lung cancer from the Pius Hospital Oldenburg were analysed. The molecular pathological analysis as well as the immune histochemical diagnostics have been performed by the Institute of Hematopathology Hamburg. These institutions are cooperation partners in the North-East-West Lung (NOWEL) network and network center within the national network of genomic medicine (nNGM). The Pius-Hospital is a Deutsche Krebsgesellschaft (DKG)-certified cancer centre. All investigations as part of this study were performed after approval of the study by the responsible ethics committee vote on 21.07.2023 (File number 2023-183).

Initially, patients were examined regarding eligibility for participation in the study. Included were patients which have been diagnosed with NSCLC in the Pius Hospital Oldenburg between 15.06.2016 and 01.02.2024, which had at least 1 driver mutation and were at least 18 years of age. After applying these criteria 190 patients were included in the investigation. Of those patients, 162 were diagnosed with stage IV NSCLC. The patients had agreed that their personal and medical data can be collected and used for scientific purposes.

For the investigation data obtained by routine diagnostics as well as tumor documentation from the cancer centre's databank system ORBIS was used. For evaluation, the data was pseudonymized by removing the personal information and replacing it with a number code.

Included was clinical data, such as patient age at diagnosis, gender, date of diagnosis, last contact between the patient and the hospital, vital status, date of death, and overall survival. Tumor characteristics, such as tumor histology, presence of metastases, location of metastases, stage at diagnosis, and presence of driver and co-mutations were also recorded. PD-L1- and Thyroid Transcription Factor 1 (TTF1)-status were also documented. Additionally, Eastern Cooperative Oncology Group (ECOG) performance status and Charlson Comorbidity Index (CCI) were noted. ECOG is a commonly used scale to assess a cancer patient's ability to perform daily tasks, ranging from fully active (0) to completely disabled (4) (90). CCI is a scoring system used to evaluate the impact of comorbidities on patient mortality

and outcomes, used for risk stratification in clinical settings (91). Smoking status, pack-years, administered treatment and treatment response were also recorded.

Smoking status was classified into ex-heavy smokers, current smokers, light smokers and never smokers. Never smokers are individuals who have smoked less than 100 cigarettes in their life (92), whereas light smokers are individuals, which have less than 10 pack years accumulated in life and have quit smoking for at least 15 years. A pack year is defined as smoking 1 package of cigarettes containing 20 cigarettes per day for a year (93).

3.2 Statistical methods

The data of the 190 cases listed in 3.1 was first embedded into a data matrix in Microsoft Excel and was then transferred and embedded into the statistical analysis software SPSS 29.0.0.0 and a statistical analysis of the data was performed. Initially, a descriptive analysis was carried out to create an overview of patient characteristics, such as the median age at the time of initial diagnosis and smoking status. The tumor characteristics were evaluated, such as cancer histology, cancer stage at diagnosis, presence of metastases, and sites of metastases. Additionally, driver and co-mutation frequencies, and mutation characteristics, such as BRAF mutation classes and TP53-mutation status were being noted. TP53 mutations were classified into disruptive and non-disruptive according to Poeta et. al (65) and pathogenic and non-pathogenic according to Roeper et. al (4) as described in the introduction. Furthermore, PD-L1 testing rate, PD-L1 status and expression of PD-L1 (<1% absent, 1 - 49% low expression, 50 - 100% high expression) were evaluated. TTF1-status was also assessed.

Tables were created to display the descriptive results. After evaluating the results of the descriptive analysis, distinct subgroups were formed based on the available data. The formation of subgroups was limited by the prevalence of the respective mutations among the patients included in the investigation.

The subgroups were systemically assessed in terms of OS, PFS, and ORR. Kaplan-Meier calculations were utilized to evaluate OS and PFS for both 1st- and 2nd-line treatments. Kaplan-Meier curves are a non-parametric statistical tool used to estimate and visualize the time-to-event data, showing survival probabilities over a specified follow-up period in a cohort (94).

Additionally, the long-rank test was employed to assess the null hypothesis that there would be no difference between the driver-mutated and driver- and co-mutated populations, with p-values calculated to investigate whether there is statistical significance.

Furthermore, univariate Cox regression analysis was performed to identify the impact of the mutations on OS and PFS of both 1st- and 2nd-line treatments. The results of this analysis are shown as Hazard Ratio with corresponding 95% confidence intervals and the respective p values.

4 Results

The findings of the investigation are presented in this chapter. Descriptive analysis is used to provide information about the clinical characteristics of the study population. Descriptions of the tumor characteristics and features of driver- and co-mutations follow. After that, the statistical evaluation of subgroups is performed. This encompasses survival time analyses utilizing Kaplan-Meier curves, and univariate Cox regression analyses focussing on clinical endpoints such as OS and PFS.

4.1 Descriptive results

In this subchapter, the descriptive results are being presented. Starting with describing the clinical characteristics of the study population, followed by characteristics of the driver- and co-mutations. At the end of this section, research questions 1 and 2 are being answered by showing how common the studied co-mutations are in driver-mutated NSCLC.

4.1.1 Clinical characteristics of study population

The study population consisted of 190 patients diagnosed with NSCLC, of which 85.3% (n=162/190) were diagnosed with stage IV NSCLC. Age at the time of diagnosis ranged from 26 – 89 years, with a median age of 66. With 53.2% of the participants being male (n=101/190), the study included slightly more males than females.

Of the investigated patients, 58.2% (n=110/189) were heavy smokers in the past, and 12.7% (n=24/189) were current smokers. Nearly 30% of patients were never smokers (24.9%, n=47/189) or light smokers (4.8%, n=8/189). 72.4% patients (n=131/181) were tested positive for PD-L1 and 83.9% of tested patients were positive for TTF-1 (n=151/180). The majority of patients had an ECOG status of 0 or 1 (89.4%, n=169/189). Additionally, 40.7% of patients (n=77/189) had a CCI of 0-2.

In 37.7% of NSCLC IV patients (n=61/162), brain metastases were present at the time of diagnosis. Additionally, 15.4% (n=25/162) of NSCLC IV patients developed CNS metastases during the course of the disease. Table 5 below shows the clinical characteristics in detail.

Table 5 Clinical characteristics of the study population (n=190)

Variable	Total, n=190
Age (years), median (range)	66 (26 – 89)
Sex	
Male	101 (53.2%)
Female	89 (46.8%)
ECOG status*	
0	93 (49.2%)
1	76 (40.2%)
≥2	20 (10.6%)
Histology	
Adenocarcinoma	185 (97.4%)
Squamous cell carcinoma	3 (1.6%)
Other	2 (1.1%)
Stage	
I	3 (1.6%)
II	1 (0.5%)
III	24 (12.6%)
IV	162 (85.3%)
Metastases at first diagnosis**	
Brain	61 (37.7%)
Liver	18 (11.1%)
Other	83 (51%)
Smoking status***	
Never smoker	47 (24.9%)
Light smoker	8 (4.2%)
Current smoker	24 (12.7%)
Ex-heavy smoker	110 (58.2%)
CNS metastasis	
At first diagnosis	61 (37.7%)
Over the course of the disease	25 (15.4%)
CCI, mean (range)	2,61 (0-6)
CCI, groups	
0-2	77 (40%)

3-4	100 (53%)
≥5	13 (7%)
PD-L1 status****	
Negative	50 (28%)
Positive	131 (72%)
TTF-1 status*****	
Negative	29 (16%)
Positive	151 (84%)

*In 1 patient no ECOG status was available

**Metastases at first diagnosis and CNS metastasis n=162

***in 1 patient no smoking status was available

****For 9 patients no PD-L1-status was available

*****For 10 patients no TTF-1 status was available.

Percentages may not total 100 due to rounding

Reference: Own data, own depiction

4.1.2 Driver mutation characteristics of study population

Among the tested individuals, KRAS G12C was the most prevalent driver mutation, observed in 39.1% (n=70/179) of cases, followed by EGFR mutations, detected in 28.2% (n=53/188). BRAF mutations were identified in 7.8% (n=13/166) of tested patients, with class II mutations representing the majority within this category (46.2%, n=6/13). ALK rearrangements were present in 8.1% (n=15/185) of the tested samples. The remaining tested driver mutations each accounted for 5% or less. No NTRK fusions were found in the study cohort (n=0/114). A more detailed overview of the driver mutation characteristics of the study population is shown in table 6.

Table 6 Driver mutation characteristics of the study population

Variable	Mutated	Wild-type	Total
KRAS G12C	70 (39.1%)	109 (60.9%)	179 (100%)
EGFR*	53 (28.2%)	135 (71.8%)	188 (100%)
BRAF	13 (7.8%)	153 (92.2%)	166 (100%)
Class I	3 (23.1%)		13 (100%)
Class II	6 (46.2%)		13 (100%)
Class III	4 (30.8%)		13 (100%)
ALK	15 (8.1%)	170 (91.9%)	185 (100%)
C-MET Amplification	8 (4.9%)	155 (95.1%)	163 (100%)
HER2	7 (4.3%)	156 (95.7%)	163 (100%)

RET	7 (4.3%)	156 (95.7%)	163 (100%)
C-MET Exon 14 Skipping	6 (3.7%)	157 (96.3%)	163 (100%)
ROS1	5 (2.7%)	178 (97.3%)	183 (100%)
NTRK	0 (0%)	114 (100%)	114 (100%)

*EGFR includes druggable mutations on exons 18, 19, 21

Percentages may not total 100 due to rounding

Reference: Own data, own depiction

4.1.3 Co-mutation characteristics of the study population

In 49.4% (n=81/164) of cases, TP53 mutations were present. Of those, 66.7% (n=54/81) were classified as pathogenic mutations, whereas disruptive mutations accounted for 35.8% (n=29/81) of all TP53 mutations. STK11 and CDKN2A mutations were seen in 12% (n=19/158) and 10.8% (n=17/158). 7.6% (n=12/157) of the tested samples showed a mutation in the KEAP1 gene. The remaining co-mutations PIK3CA, RB1, ARID1, and POLE were less common, each occurring in only 2-3% of cases.

Table 7 Co-mutation characteristics of the study population

Variable	Mutated	Wild-type	Total
TP53	81 (49.4%)	83 (50.6%)	164 (100%)
disruptive	29 (35.8%)		81 (100%)
non-disruptive	44 (54.3%)		81 (100%)
disruptive status not available	8 (9.9%)		81 (100%)
pathogenic	54 (66.7%)		81 (100%)
non-pathogenic	27 (33.3%)		81 (100%)
STK11	19 (12%)	139 (88%)	158 (100%)
CDKN2A	17 (10.8%)	141 (89.2%)	158 (100%)
KEAP1	12 (7.6%)	145 (92.4%)	157 (100%)
PIK3CA	5 (3%)	159 (97%)	164 (100%)
RB1	3 (1.9%)	155 (98.1%)	158 (100%)
ARID1A	2 (2.4%)	80 (97.6%)	82 (100%)
POLE	2 (3.4%)	56 (96.6%)	58 (100%)

Percentages may not total 100 due to rounding

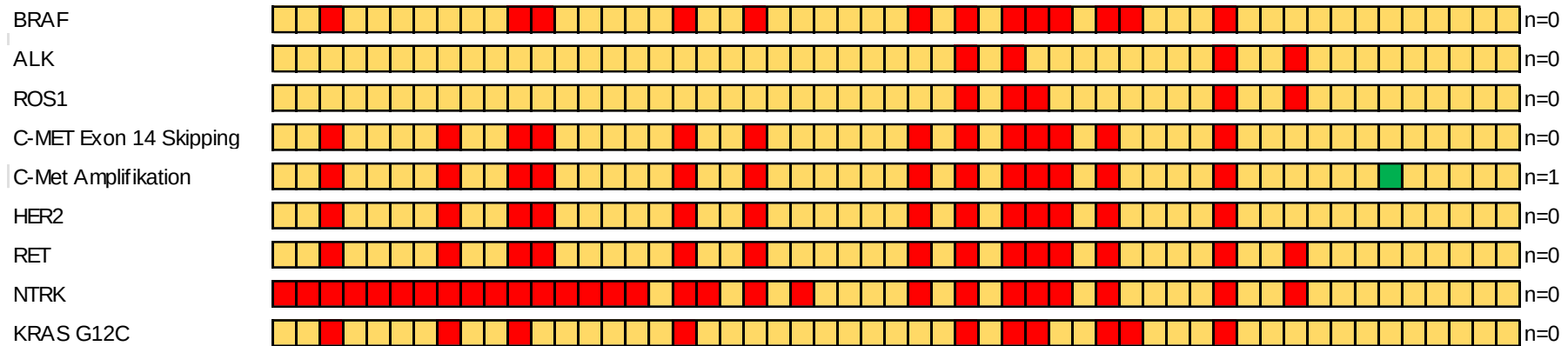
Reference: Own data, own depiction

4.1.4 Mutation status of KRAS G12C & EGFR-mutated NSCLC

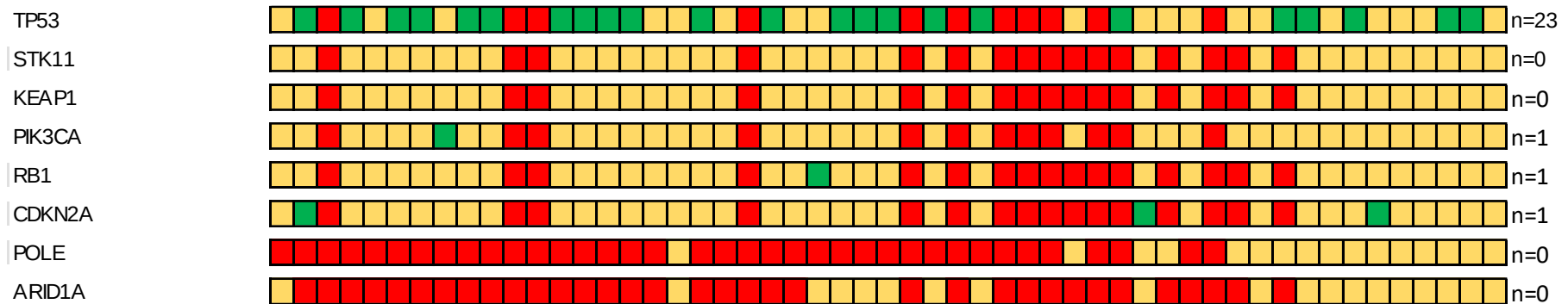


Figure 7 Co-mutation status in KRAS G12C-mt patients (n=70)

Driver-mt



Co-mt



In 12 EGFR-mt Patients the NGS panel wasn't performed

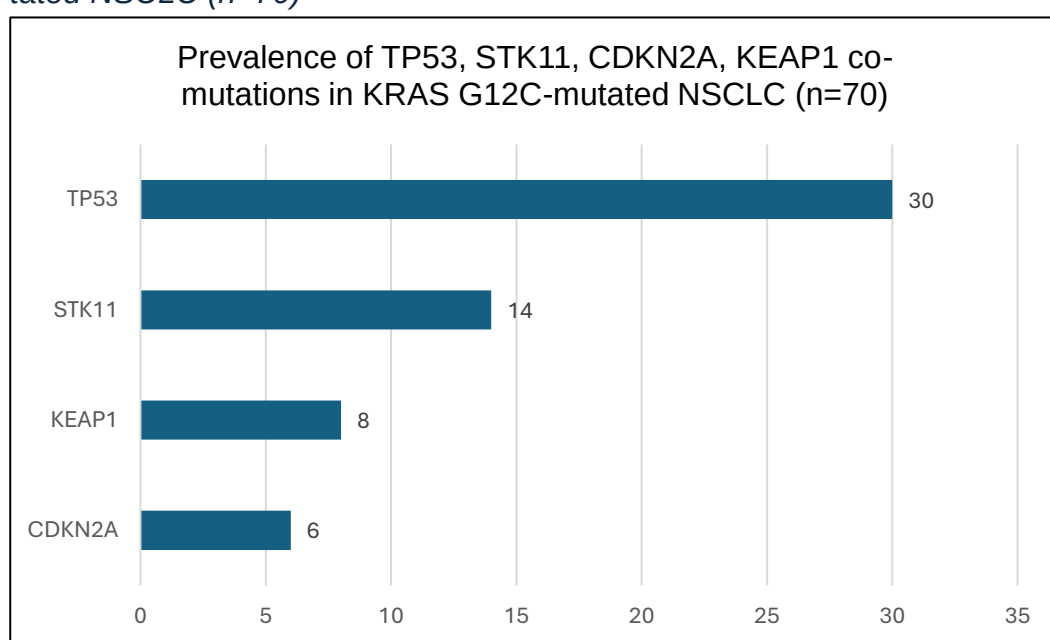
■ not performed
 ■ wild type
 ■ mutation

Figure 8 Co-mutation status in EGFR-mt patients (n=53)

4.1.5 TP53, STK11, CDKN2A, KEAP1 status in KRAS G12C-mutated NSCLC

The cohort of KRAS G12C-mutated NSCLC patients (n=70) presented with a distinct pattern of co-mutations. Specifically, TP53 co-mutation was most prevalent, identified in 42.9% of cases (n=30/70), followed by STK11 co-mutation in 20% of patients (n=14/70), whereas KEAP1 co-mutation was seen in 11.4% (n=8/70) and CDKN2A in 8.6% (n=6/70).

Figure 9 Prevalence of TP53, STK11, CDKN2A, KEAP1 co-mutations in KRAS G12C-mutated NSCLC (n=70)

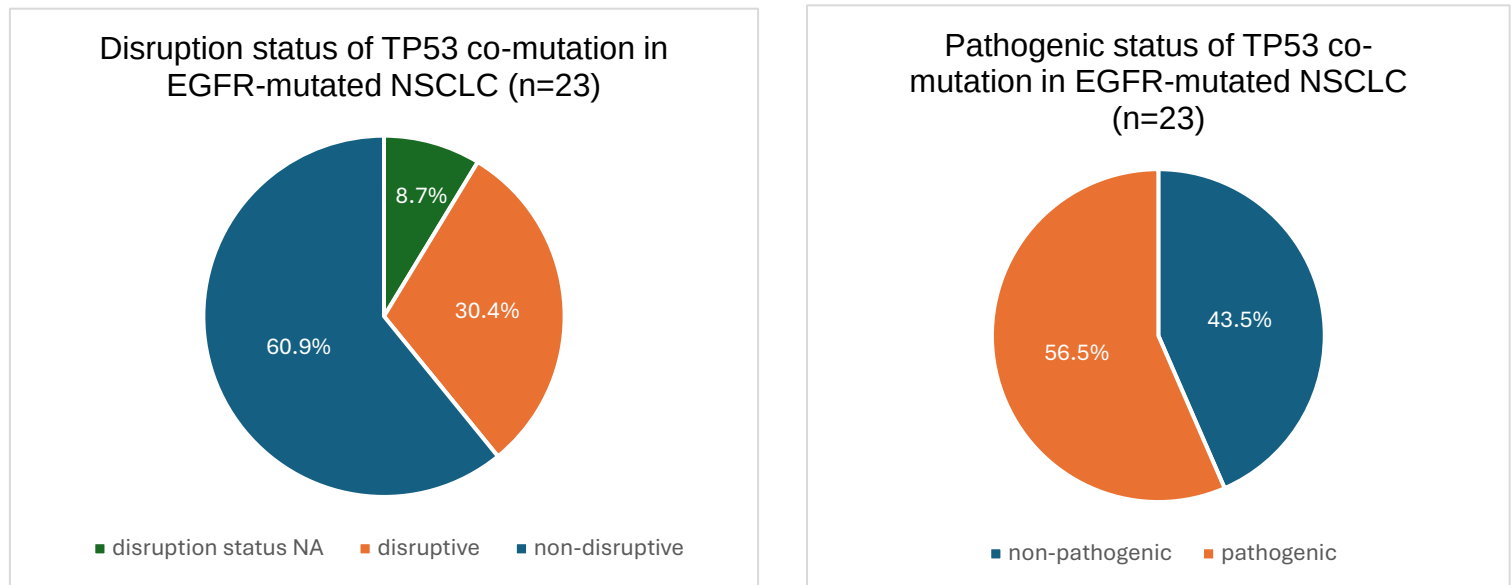


Reference: own data, own depiction

4.1.6 TP53 status in EGFR-mutated NSCLC

43.4% (n=23/53) of the EGFR-mutated patients had a TP53 co-mutation. The distribution of these co-mutations is visualized in figure 8. Notably, 30.4% (n=7/23) of TP53 co-mutations in EGFR-mutated patients are disruptive whereas 56.5% (n=13/23) are pathogenic. In 8.7% (n=2/23) disruptive status was not available.

Figure 10 Disruption & pathogenic status of TP53 co-mutation in EGFR-mutated NSCLC (n=23)



Reference: Own data, own depiction

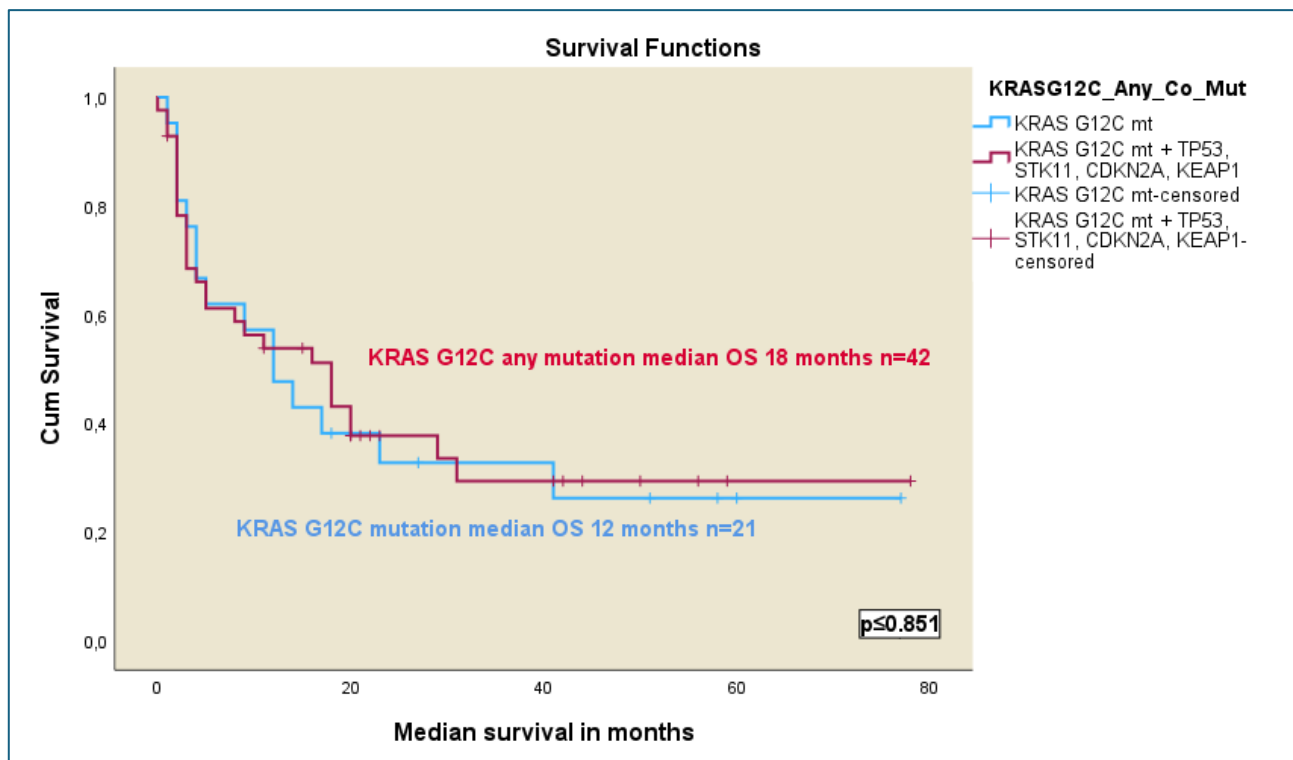
4.2 OS and PFS of Subgroups

To answer research questions 3 and 4, Kaplan-Meier calculations for OS and PFS were performed. Additionally, the impact of co-mutations on OS and PFS was assessed by computing Hazard Ratios using a univariate Cox regression model. The Log-Rank test was employed to calculate p-values.

4.2.1 KRAS G12C-mt vs. KRAS G12C-mt + TP53, STK11, CDKN2A, or KEAP1 co-mutation

Figure 11 shows the OS of NSCLC-patients with a KRAS G12C mutation compared to OS of patients with a KRAS G12C mutation and a TP53, STK11, CDKN2A, or KEAP1 co-mutation. OS with a KRAS G12C mutation was 12 months (n=21), and with a co-mutation, 18 months (n=42). The p-value of 0.851 shows no significant difference between the 2 cohorts. The hazard ratio is 0.943, and with a calculated p-value of 0.855, it is not statistically significant.

Figure 11 OS KRAS G12C-mt vs. KRAS G12C-mt + TP53, STK11, CDKN2A, KEAP1 co-Mutation (n=63)



Reference: Own data, own depiction

The first-line therapy response of patients with only a KRAS G12C mutation (n=18) compared to patients who have a KRAS G12C mutation and a co-mutation (n=39) can be seen in table 8. In patients without co-mutations, chemotherapy achieved a 50% ORR (n= 2/4) with a 75% disease-free survival rate (DFS) (n=3/4), while ICI monotherapy achieved a higher ORR of 60% (n=3/5) and 80% (n=4/5) DFS. ICI + -chemotherapy showed a 44.4% (n=4/9) ORR and DFS.

Among patients with co-mutations, ICI + chemotherapy displayed the highest ORR (56.5%, n=13/23) and DFS (65.2%, n=15/23), followed by ICI monotherapy with 45.5% ORR (n=5/11) and 54.5% DFS (n=6/13).

Table 8 Treatment response 1st-line therapy KRAS G12C-mt vs. KRAS G12C-mt + co-mutation (n=57)

Treatment 1st-line:

KRAS G12C-mt (18 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	4	50%	75%	2	1	0	1
TKI	0	/	/	0	0	0	0
ICI	5	60%	80%	3	1	1	0
ICI + Chemo	9	44.4%	44.4%	4	0	2	3
TKI + Chemo	0	/	/	0	0	0	0

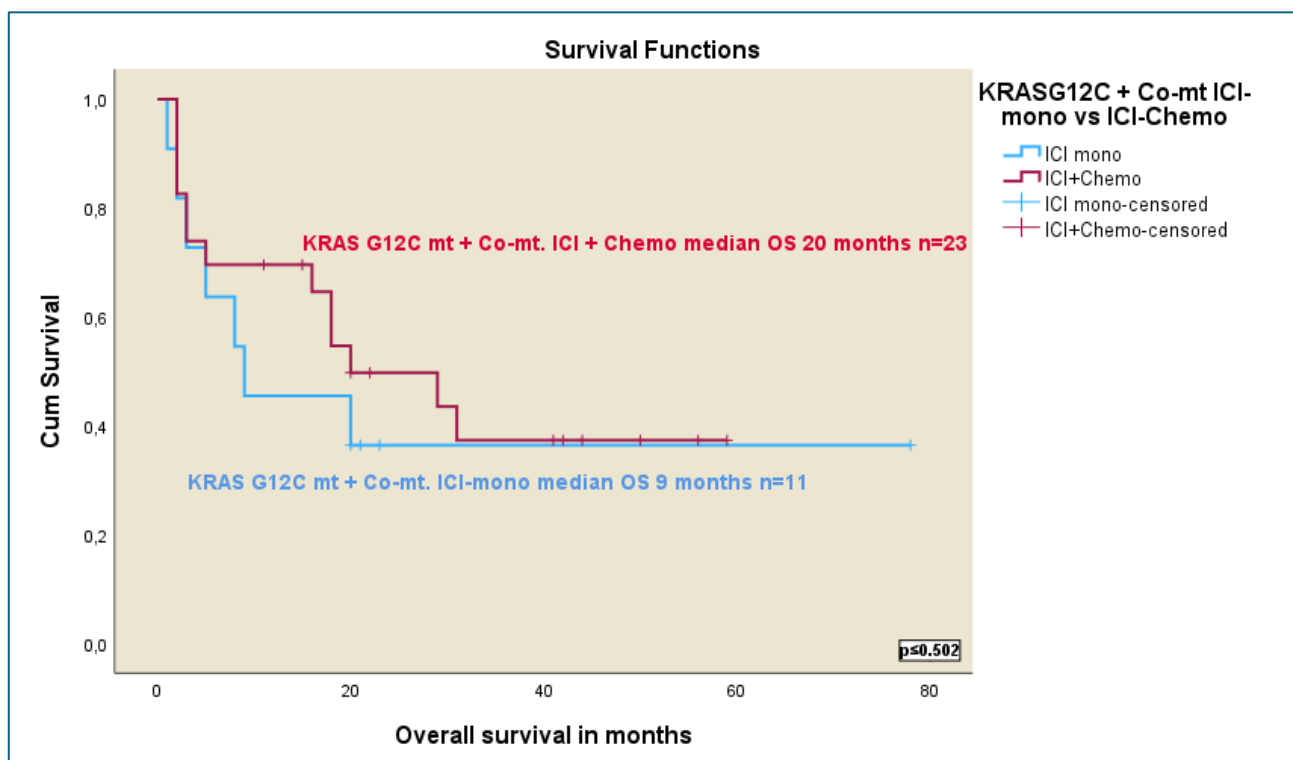
KRAS G12C-mt + co-mutations (39 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	5	40%	40%	2	0	2	1
TKI	0	/	/	0	0	0	0
ICI	11	45.5%	54.5%	5	1	0	5
ICI + Chemo	23	56.5%	65.2%	13	2	5	3
TKI + Chemo	0	/	/	0	0	0	0

Reference: Own data, own depiction

Figure 12 compares the OS of patients treated with ICI monotherapy and ICI combined with chemotherapy.

The cohort receiving ICI monotherapy had an OS of 9 months (n=11), whereas the cohort receiving ICI + chemotherapy had an OS of 20 months (n=23). The p-value of 0.502 shows that the difference is not statistically significant. The corresponding hazard ratio is 0.736 and with a calculated p-value of 0.518, it is not significant.

Figure 12 OS KRAS G12C-mt + co-mt ICI-mono vs. ICI + chemo (n=34)



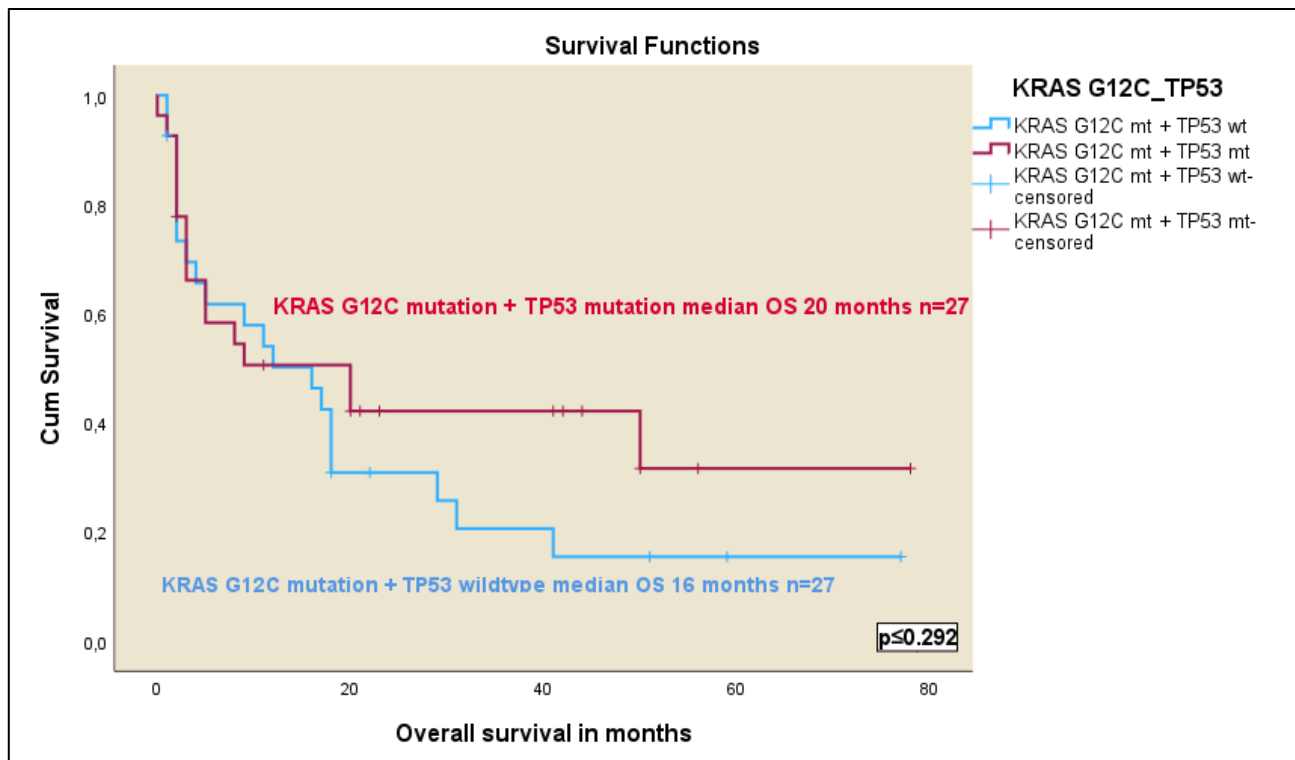
Reference: Own data, own depiction

4.2.2 KRAS G12C + TP53

Figure 13 compares the OS of patients with a KRAS G12C mutation and TP53 wild-type to patients with a KRAS G12C mutation and a TP53 co-mutation.

OS was 16 months (n=27) in the cohort without a TP53 co-mutation and 20 months (n=27) in the cohort with a TP53 co-mutation. The p-value of 0.292 is statistically not significant. The hazard ratio is 0.714 with a p-value of 0.311, which is not significant.

Figure 13 OS KRAS G12C-mt + TP53-wt vs. KRAS G12C-mt + TP53 co-mt (n=54)



Reference: Own data, own depiction

Table 9 shows the response to first-line treatment in patients with a TP53 co-mutation and patients with a KRAS G12C mutation without a TP53 co-mutation. In patients with TP53 wild-type, chemotherapy achieved the highest DFS of 71.4% (n=5/7). ICI therapy showed the highest ORR (66.7%, n=2/3) while ICI + chemotherapy showed the lowest ORR (46.2%, n=6/13) and DFS (53.8%, n=7/13).

In the cohort with a TP53 co-mutation chemotherapy alone achieved 0% ORR (n=0/2) while ICI + chemotherapy led to 57.1% ORR (n=8/14) and 64.3% DFS (n=9/14) demonstrating the highest ORR and DFS in this cohort.

Table 9 Treatment response 1st-line therapy KRAS G12C-mt + TP53-wt vs. KRAS G12C-mt + TP53 Co-Mutation (n=49)

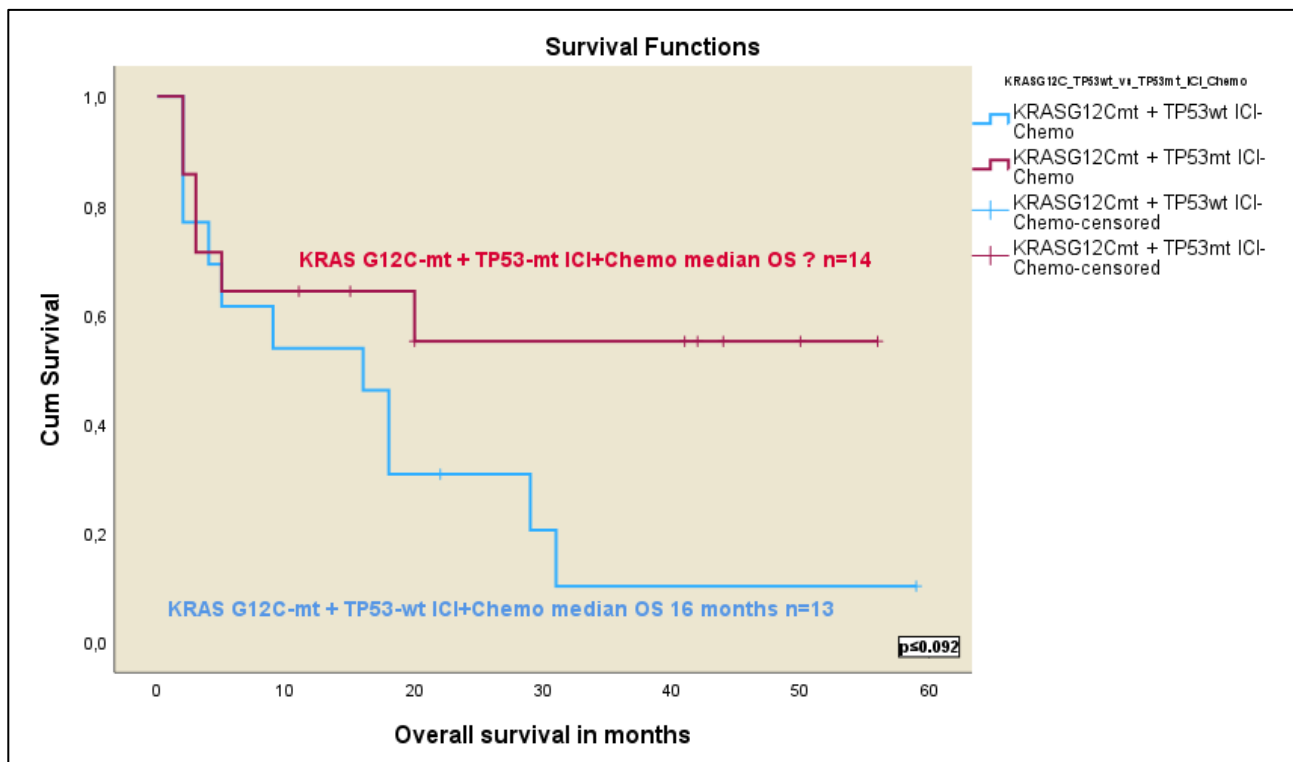
KRAS G12C-mt + TP53-wt (23 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	7	57.1%	71.4%	4	1	1	1
TKI	0	/	/	0	0	0	0
ICI	3	66.7%	66.7%	2	0	0	1
ICI + Chemo	13	46.2%	53.8%	6	1	3	3
TKI + Chemo	0	/	/	0	0	0	0

KRAS G12C-mt + TP53 co-mt (26 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	2	0%	0%	0	0	1	1
TKI	0	/	/	0	0	0	0
ICI	10	50%	60%	5	1	0	3
ICI + Chemo	14	57.1%	64.3%	8	1	3	3
TKI + Chemo	0	/	/	0	0	0	0

Reference: Own data, own depiction

Figure 14 compares the OS of patients who received ICI + chemotherapy as first-line treatment. The OS for the cohort with a TP53 co-mutation was 16 months (n=13), while median OS was not reached for the cohort without a TP53 co-mutation. With a p-value of 0.092 the difference between the two cohorts is not statistically significant. The hazard ratio is 0.445 with a p-value of 0.113. This difference is not statistically significant.

Figure 14 OS KRAS G12C-mt + TP53-wt ICI-Chemo vs. KRAS G12C-mt + TP53-mt ICI-Chemo (n=27)

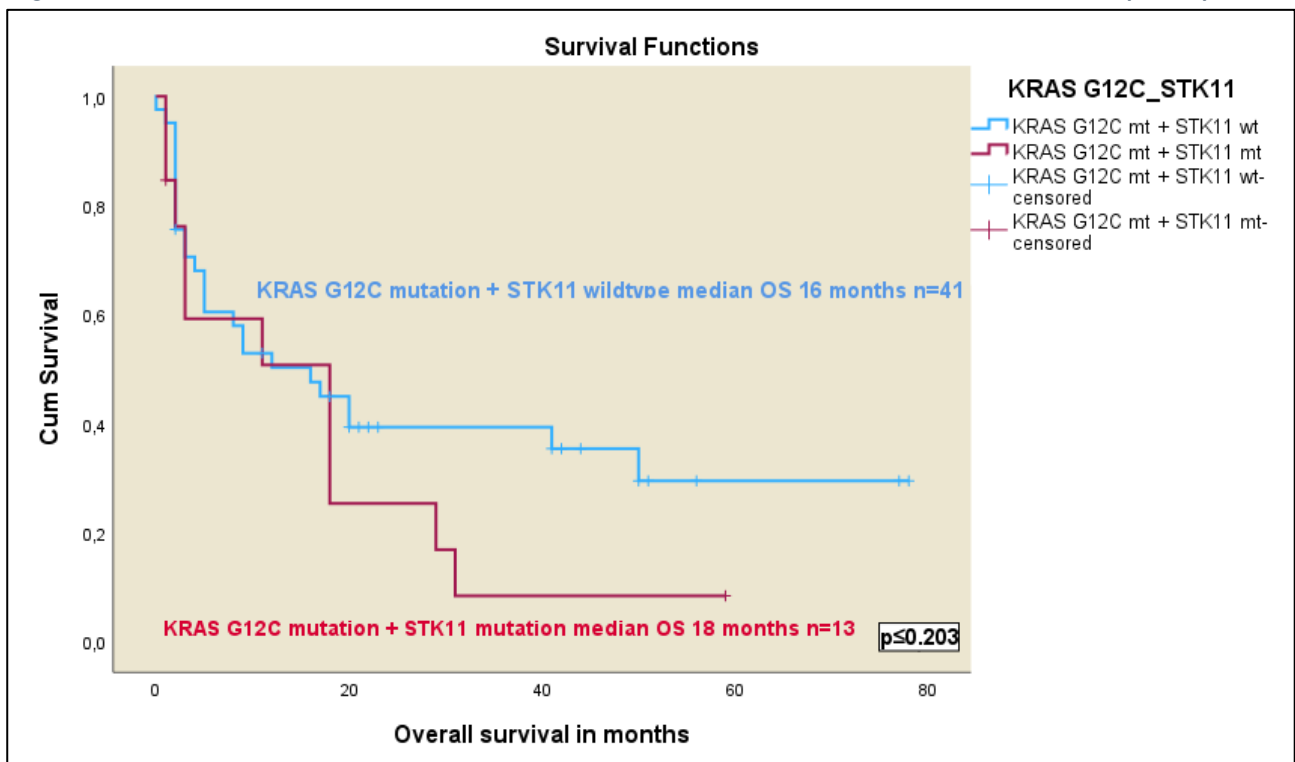


Reference: Own data, own depiction

4.2.3 KRAS G12C + STK11

Figure 15 shows the OS of patients with a KRAS G12C mutation and a STK11 co-mutation compared to patients with a KRAS G12C mutation and STK11 wild-type. Patients with a STK11 co-mutation had an OS of 18 months (n=13) while the cohort with STK11 wild-type had an OS of 16 months (n=41). The p-value of 0.203 is not significant. The determined hazard ratio of 0.644 has a p-value of 0.224, which is also not significant.

Figure 15 OS KRAS G12C-mt + STK11-wt vs. KRAS G12C-mt + STK11 co-mt (n=54)



Reference: Own data, own depiction

Table 10 shows the treatment response to first-line therapy. In the cohort without a STK11 co-mutation ICI monotherapy demonstrated the highest ORR and DFS with 58.3% ORR (n=7/12) and 66.7% DFS (n=8/12). In the cohort with a STK11 co-mutation, chemotherapy displayed a 50% ORR (n=2/4) and DFS (n=2/4), while ICI + chemotherapy achieved the same ORR (n=3/6) and a DFS of 83.3% (n=5/6).

Table 10 Treatment response 1st-line therapy KRAS G12C-mt + STK11-wt vs. KRAS G12C-mt + STK11-co-mt (n=49)

KRAS G12C-mt + STK11-wt (38 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	5	40%	60%	2	1	1	1
TKI	0	/	/	0	0	0	0
ICI	12	58.3%	66.7%	7	1	0	4
ICI + Chemo	21	52.4%	57.1%	11	1	5	5
TKI + Chemo	0	/	/	0	0	0	0

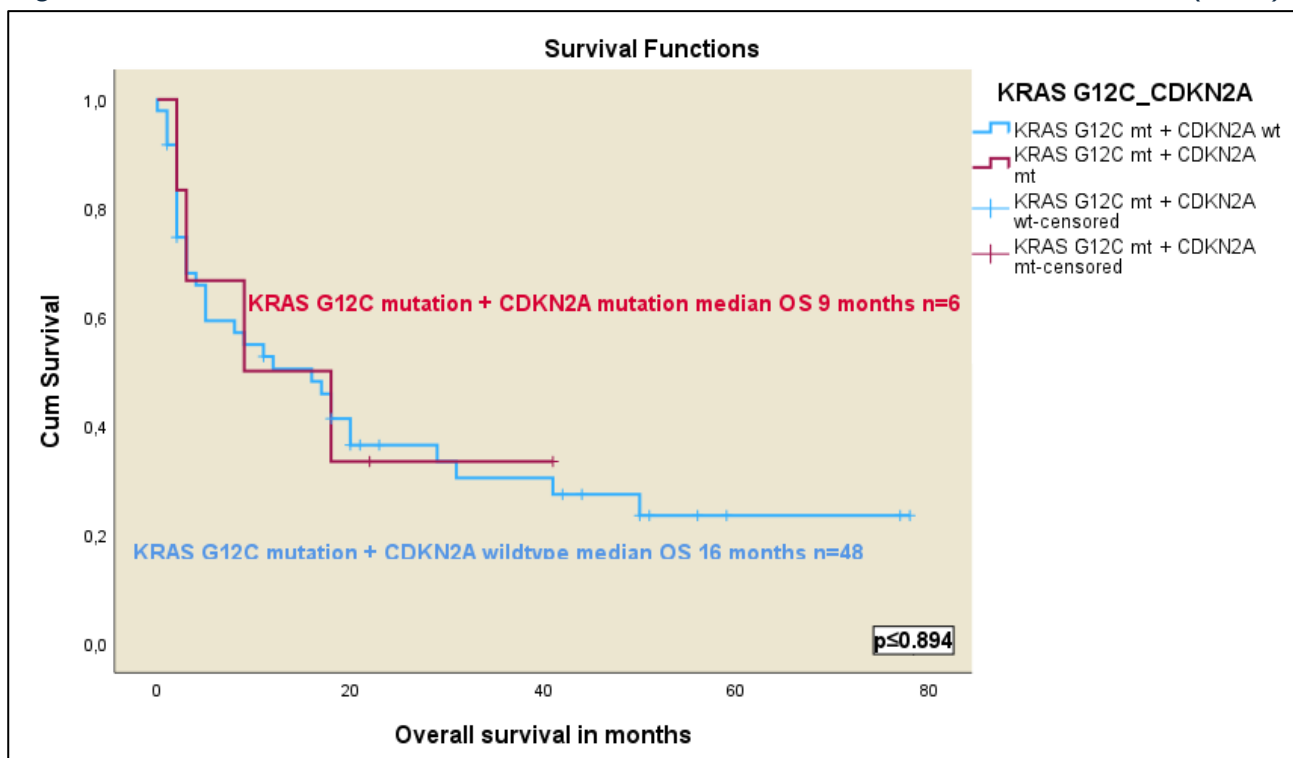
KRAS G12C-mt + STK11 co-mt (11 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	4	50%	50%	2	0	1	1
TKI	0	/	/	0	0	0	0
ICI	1	/	/	0	0	0	1
ICI + Chemo	6	50%	83,3%	3	2	1	0
TKI + Chemo	0	/	/	0	0	0	0

Reference: Own data, own depiction

4.2.4 KRAS G12C + CDKN2A

Figure 16 compares the OS of KRAS G12C-mutated patients with CDKN2A wild-type with KRAS G12C-mutated patients with a CDKN2A co-mutation. The patients without co-mutation had an OS of 16 months (n=48) while the other cohort had an OS of 9 months (n=6). The p-value of 0.894 is non-significant. The corresponding hazard ratio of 0.934 and a p-value of 0.898 is also nonsignificant.

Figure 16 OS KRAS G12C-mt + CDKN2A-wt vs. KRAS G12C-mt + CDKN2A co-mt (n=54)



Reference: Own data, own depiction

Table 11 shows the treatment response to first-line treatment. Among those without a co-mutation, ICI monotherapy and ICI + chemotherapy demonstrated ORRs of 53.8% (n=7/13) and 52.2% (n=12/23) and DFSs of 61.5% (n=4/7) and 56.4% (n=13/23).

In the cohort with CDKN2A co-mutation a combination of ICI + chemotherapy resulted in 50% ORR (n=2/4) and 75% DFS (n=3/4). In both cohorts, chemotherapy alone showed worse ORR and DFS compared with ICI-based treatment regimens.

Table 11 Treatment response 1st-line therapy KRAS G12C-mt + CDKN2A-wt vs. KRAS G12C-mt + CDKN2A co-mt (n=49)

KRAS G12C-mt + CDKN2A-wt (43 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	7	42.9%	57.1%	3	1	1	2
TKI	0	/	/	0	0	0	0
ICI	13	53.8%	61.5%	7	1	0	5
ICI + Chemo	23	52.2%	56.5%	12	1	5	5
TKI + Chemo	0	/	/	0	0	0	0

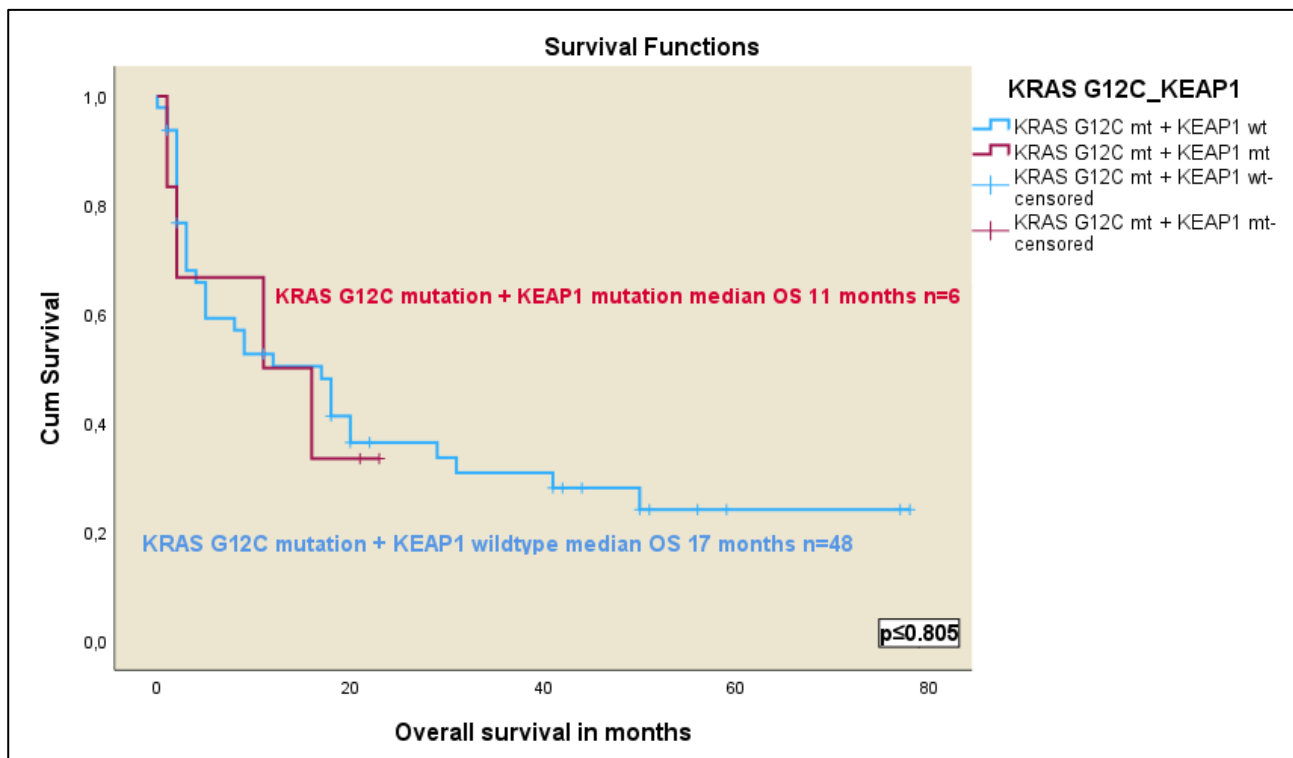
KRAS G12C-mt + CDKN2A co-mt (6 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	2	50%	50%	1	0	1	0
TKI	0	/	/	0	0	0	0
ICI	0	%	%	0	0	0	0
ICI + Chemo	4	50%	75%	2	1	1	0
TKI + Chemo	0	/	/	0	0	0	0

Reference: Own data, own depiction

4.2.5 KRAS G12C + KEAP1

Figure 17 shows the OS of patients with a KRAS G12C mutation and KEAP1 wild-type compared to patients with a KRAS G12C mutation and a KEAP1 co-mutation. In the cohort with a KEAP1 co-mutation, median OS is 11 months (n=6), while in the cohort without co-mutation, it is 17 months (n=48). The p-value of 0.805 indicates that there is no statistically significant difference between the two cohorts. The hazard ratio of 0.881 with a p-value of 0.812 is also not significant.

Figure 17 OS KRAS G12C-mt + KEAP1-wt vs. KRAS G12C-mt + KEAP1 co-mt (n=54)



Reference: Own data, own depiction

Table 12 shows first-line treatment response. In patients with KEAP1 wild-type ICI monotherapy and combined ICI + chemotherapy demonstrated similar ORRs of 50% (n=5/10) and 52% (n=13/25) and a DFS of 60% (n=6/10, n=15/25) and showed superior results compared to chemotherapy alone, resulting in 37.5% ORR (n=3/8) and 50% DFS (n=4/8). In the cohort with a KEAP1 co-mutation ICI monotherapy led to a 66.7% ORR (n=2/3) and DFS (n=2/3).

Table 12 Treatment response 1st-line therapy KRAS G12C-mt + KEAP1-wt vs. KRAS G12C-mt + KEAP1 co-mt (n=49)

KRAS G12C-mt + KEAP1-wt (43 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	8	37,5%	50%	3	1	2	1
TKI	0	/	/	0	0	0	0
ICI	10	50%	60%	5	1	0	4
ICI + Chemo	25	52%	60%	13	2	6	4
TKI + Chemo	0	/	/	0	0	0	0

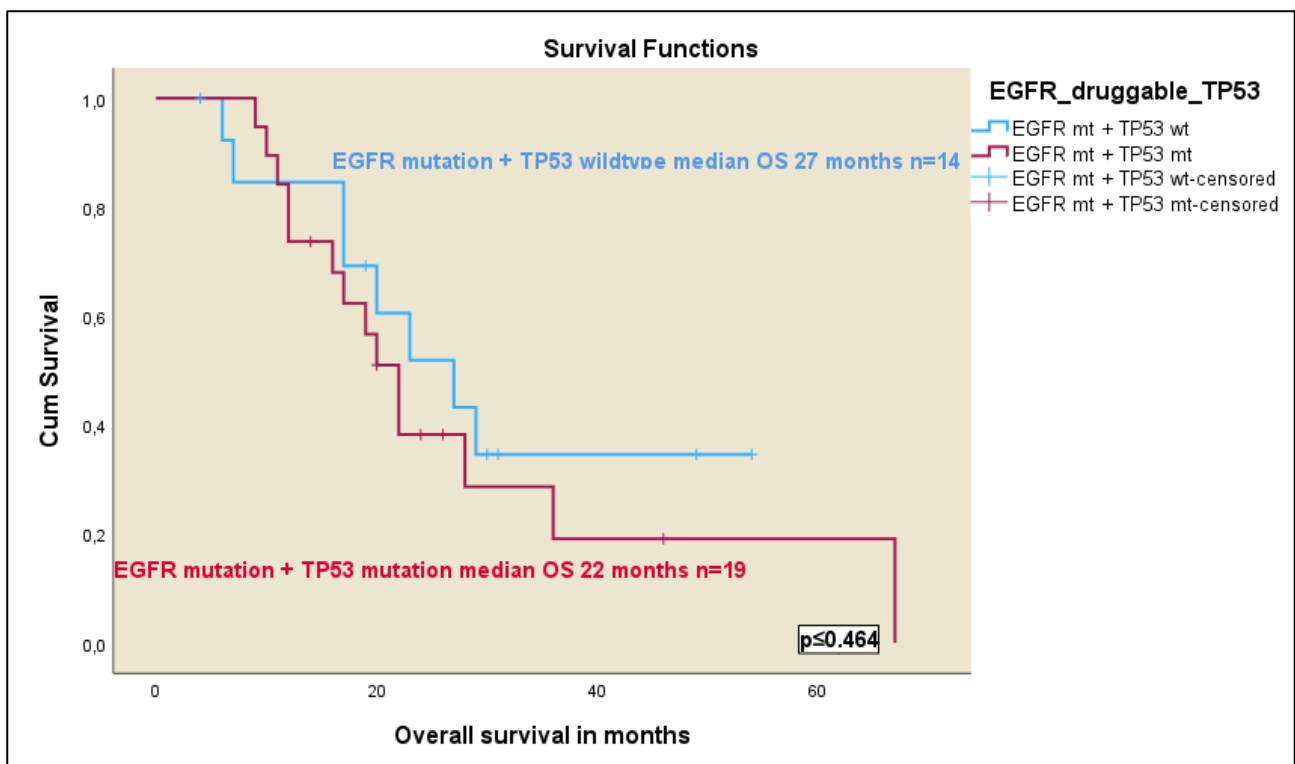
KRAS G12C-mt + KEAP1 co-mt (6 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	1	100%	100%	1	0	0	0
TKI	0	/	/	0	0	0	0
ICI	3	66,7%	66,7%	2	0	0	1
ICI + Chemo	2	50%	50%	1	0	0	1
TKI + Chemo	0	/	/	0	0	0	0

Reference: Own data, own depiction

4.2.6 EGFR druggable + TP53

Figure 18 compares the OS of EGFR-mutated patients without a TP53 co-mutation with patients with an EGFR mutation and a TP53 co-mutation. The patients without TP53 co-mutation had an OS of 27 months (n=14), whereas patients with a TP53 co-mutation had an OS of 22 months (n=19). With a p-value of 0.464, the difference is not statistically significant. With a calculated p-value of 0.472, the hazard ratio of 0.722 is also nonsignificant.

Figure 18 OS EGFR-mt + TP53-wt vs. EGFR-mt + TP53 co-mt (n=33)



Reference: Own data, own depiction

Table 13 shows the treatment response to first-line treatment. In patients without TP53 co-mutation, TKI therapy led to an ORR of 90.9% (n=10/11) and a DFS of 100% (n=11/11). In patients with EGFR mutation and TP53 co-mutation TKI treatment demonstrated an ORR of 88.2% (n=15/17) and a DFS of 94.1% (n=16/17).

Table 13 Treatment response 1st-line therapy EGFR-mt + TP53-wt vs. EGFR-mt + TP53 Co-Mutation (n=31)

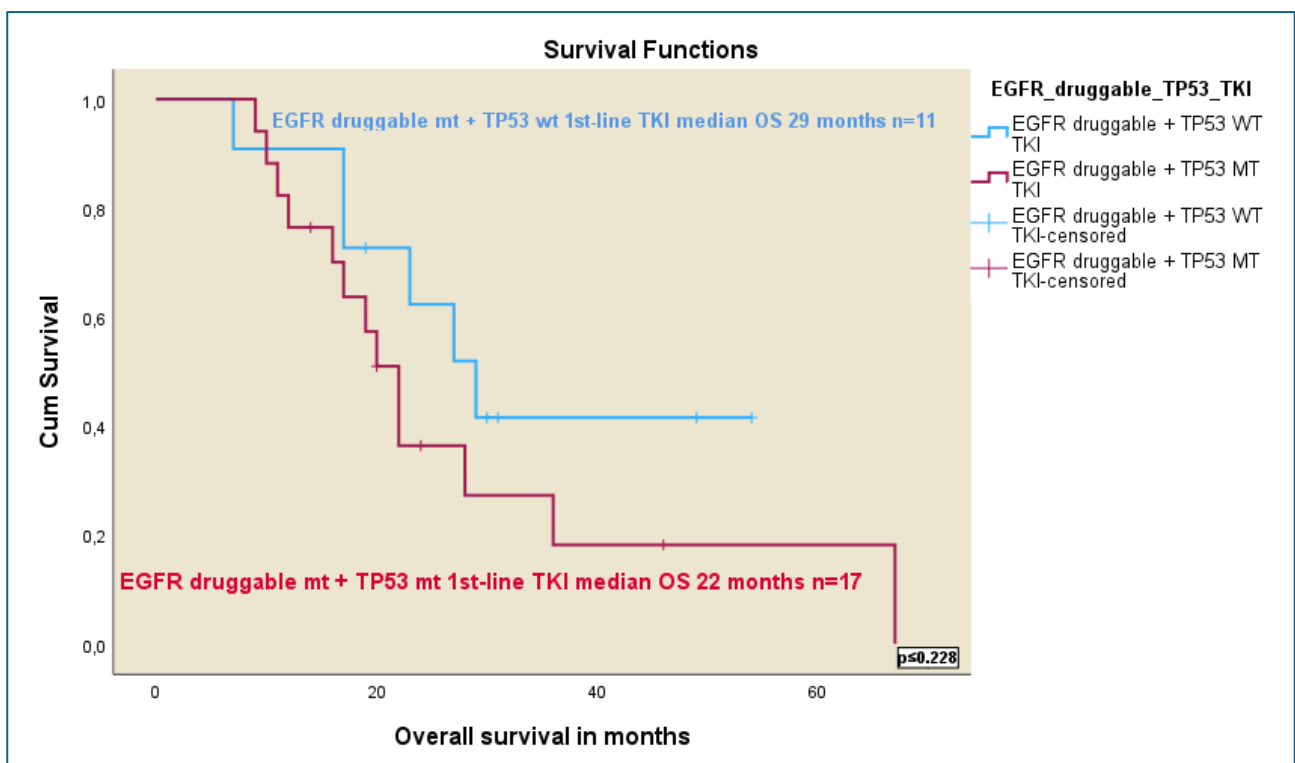
EGFR-mt + TP53-wt (12 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	0	/	/	0	0	0	0
TKI	11	90.9%	100%	10	1	0	0
ICI	0	/	/	0	0	0	0
ICI + Chemo	0	/	/	0	0	0	0
TKI + Chemo	1	100%	100%	1	0	0	0

EGFR-mt + TP53 co-mt (19 Patients)							
Therapy	Number of patients	Best response					
		ORR	DFS	CR/PR	SD	PD	NA
Chemotherapy	0	/	/	0	0	0	0
TKI	17	88.2%	94.1%	15	1	1	0
ICI	0	/	/	0	0	0	0
ICI + Chemo	1	0%	100%	0	1	0	0
TKI + Chemo	1	100%	100%	1	0	0	0

Reference: Own data, own depiction

Figure 19 shows the OS of patients who were treated with a TKI as first-line therapy. Patients without TP53 co-mutation had an OS of 29 months (n=11), whereas patients with a TP53 co-mutation had a median OS of 22 months (n=17). With a p-value of 0.228 this difference is not statistically significant. The hazard ratio is 0.552. With a corresponding p-value of 0.240 it is not statistically significant.

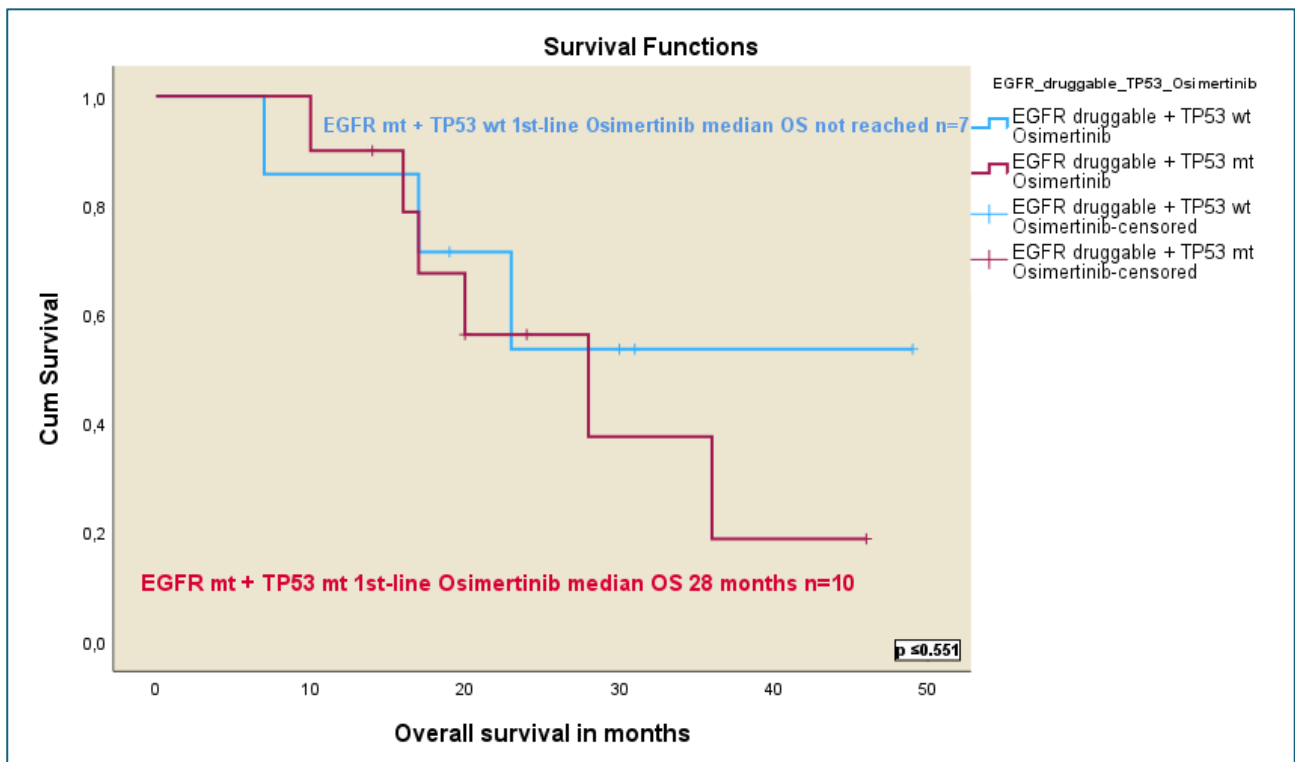
Figure 19 OS EGFR-mt + TP53-wt vs. EGFR-mt + TP53 co-mt 1st-line with TKI (n=28)



Reference: Own data, own depiction

Figure 20 compares two cohorts of patients who received Osimertinib as first-line therapy. For patients without TP53 co-mutation, the median OS has not been reached (n=7), whereas the cohort with the co-mutation had a median OS of 28 months (n=10). With a p-value of 0.551 it is not of statistical significance. The corresponding hazard ratio of 0.659 with a p-value of 0.558 is also not of statistical significance.

Figure 20 OS EGFR-mt + TP53-wt 1st-line Osimertinib vs. EGFR-mt + TP53 co-mt 1st-line Osimertinib (n=17)



Reference: Own data, own depiction

5 Discussion

In this retrospective analysis of 162 Stage IV NSCLC patients treated in the Pius-Hospital Oldenburg, the influence of co-mutations in driver-mutated stage IV NSCLC was investigated. The main emphasis was on EGFR and KRAS G12C-mutated NSCLC patients. Alongside the clinical characteristics of the study population, the clinical endpoints (OS, PFS, ORR) were evaluated.

Clinical characteristics of study population

The median age at diagnosis for the patients in this study was 66 years, slightly younger than the median age of 69 years for NSCLC diagnosis in Germany, with only ~2% diagnosed before age 50 (1). This difference may reflect variations in patient demographics, risk factor exposure, or health care access within our cohort. Lung cancer remains more prevalent in men than in women; however, age-adjusted morbidity and mortality rates are steadily aligning, suggesting shifting epidemiological patterns (6). This trend is reflected in the study cohort, which comprises a nearly balanced gender distribution with a slight male predominance (53.2%; n=101/190). Smoking remains the most significant risk factor for the development of lung carcinoma (7). While decreasing smoking rates, particularly among men in high-income countries, have contributed to a decline in lung cancer cases in this group, the incidence continues to rise among women over 60 (1,95). This likely results from increased tobacco use in this population several decades ago, highlighting the delayed impact of risk factor exposure (95–97).

In Germany, annual lung cancer diagnoses have stabilized at around 40,000 since 2015 after earlier increases (1). These trends highlight the need for gender- and age-specific prevention strategies and ongoing surveillance to assess the long-term impact of tobacco control efforts.

In the study population, 70.9% (n=134/189) of patients were either current or former heavy smokers, reflecting this association. However, in the past decade, increasing evidence highlights the role of additional contributors such as environmental pollutants, occupational exposures, and genetic susceptibility in disease development (98–102).

Most of the patients had a good performance status and were in good physical condition, with the majority of patients having an ECOG status of 0 or 1 (89.4%, n=169/189). ECOG performance status is a well-established prognostic factor in NSCLC, with patients having ECOG ≥ 2 showing significantly poorer outcomes (103).

In our cohort, 89.4% of NSCLC patients had ECOG status 0–1, aligning with a systematic review of 19 studies (n=3600) reporting an average of 21.0% with ECOG >1 and identifying

ECOG ≥ 2 as a significant negative prognostic factor in advanced NSCLC treated with immune checkpoint inhibitors (104).

An even larger meta-analysis, including 60 studies and over 35,000 patients, further confirmed that patients with ECOG ≥ 2 have significantly poorer outcomes under immunotherapy compared to those with ECOG 0–1 (105). These findings support the prognostic relevance of ECOG performance status in NSCLC.

In our cohort, 40.7% of NSCLC patients had a CCI score of 0–2, indicating a moderate comorbidity burden. This is consistent with prior research emphasising the prognostic value of CCI in NSCLC. For example, Zhao et al. showed that higher CCI scores were independently associated with poorer overall survival in patients with stage IIIB–IV NSCLC (106). Additionally, Birim et al. reported that a CCI score ≥ 2 independently predicted worse long-term outcomes in patients undergoing surgical resection for early-stage NSCLC (107). Together, these findings underscore the relevance of CCI as a robust prognostic marker across both early and advanced NSCLC stages.

Influence of co-mutations on driver-mutated NSCLC on clinical endpoints

EGFR/TP53

Studies have shown that in EGFR-mutated NSCLC, TP53 co-mutation occurs in 17–68% of cases and is linked to worse outcomes compared to only EGFR-mutated NSCLC (4,5,66,108,109). Our findings align with this range, as 43.4% ($n=23/53$) of EGFR-mutated patients exhibited a TP53 co-mutation. Compared to only EGFR-mutated NSCLC, these patients also had a non-significantly shorter OS. This aligns with the current state of research as the presence of TP53 co-mutation in EGFR-mutated NSCLC has been linked to primary resistance to EGFR-TKIs, regardless of the generation of TKI used (5,67).

In our investigation, patients without TP53 co-mutations demonstrated a trend toward better clinical outcomes (higher OS, ORR, DFS) when treated with 1st-line TKI therapy. This trend was consistent across different generations of TKIs, although the differences did not reach statistical significance. These observations are in line with previously published data and support current understanding in the field.

Baseline detection of TP53 co-mutations, through both tissue and plasma analyses, even at low allele frequencies, was shown to be associated with poorer OS and may indicate early oncogenic events. Such subclonal mutations can expand during disease progression or histological transformation, being one of multiple mechanisms of possibly driving therapeutic

resistance (110). Additional genomic events like MDM2 amplification further compromise p53 activity, promoting tumor aggressiveness and resistance to EGFR-targeted therapies (111).

EGFR-mutated lung cancers have been found to exhibit a range of concurrent acquired genetic alterations that contribute to therapeutic resistance, including mutations such as EGFR T790M as well as MET, and ERBB2 amplifications (112). An interesting finding in a recent study performed on a small cohort of patients with EGFR-mutated NSCLC suggests that TP53 mutations are not always present at baseline and could even emerge de novo during Osimertinib treatment in selected patients (113).

These findings underscore the importance of re-biopsy and molecular re-evaluation at the time of disease progression to accurately assess resistance mechanisms and guide subsequent treatment decisions.

Similarly, Roeper et al. demonstrated that TP53 mutations are associated with inferior outcomes on Osimertinib therapy (ORR, PFS, and OS) in a T790M-positive EGFR-mutated NSCLC cohort, suggesting that third-generation TKIs may be insufficient to counteract the genomic instability and resistance mechanisms driven by TP53 alterations (67).

These findings underscore TP53 co-mutations as dominant modifiers of treatment response. TP53-targeting agents like APR-246, which restore p53 function (114,115), may enhance outcomes when combined with EGFR TKIs and warrant further investigation in this molecular subgroup of NSCLC(3,116,117).

KRAS-Mutated NSCLC

Overall, KRAS mutations in NSCLC seem to not have a significant impact on overall survival as real-world data from the CRISP registry demonstrate comparable outcomes among patients with KRAS G12C mutations, KRAS non-G12C mutations, and KRAS wild-type (39). A large study of KRAS-mutated NSCLC patients found that 53.5% of the patients had at least one co-mutation (37). TP53, the most common co-mutation, is seen in 21.9 - 39.4% of cases (37,68). STK11 co-mutations were present in 13.0% - 19.8% of cases (37,120). CDKN2A co-mutation was seen in 32.0% of patients (77), while KEAP1 co-mutations were found in 7.0% - 12.9% (37,120).

Our investigation also identified TP53 as the most prevalent co-mutation, occurring in 42.9% (n=30/70) of cases, slightly exceeding the previously reported range (37,68). The prevalence of STK11 (20.0%; n=14/70) and KEAP1 (11.4%; n=8/70) co-mutations in our study is similar to the already established ranges (37,120). CDKN2A co-mutations were observed in

only 8.6% (n=6/70), markedly lower than the reported 32.0% (77). This discrepancy may be attributable to the limited sample size in our study, potentially affecting the statistical significance of our findings.

Given the variability in co-mutation patterns observed across studies and within our cohort, thorough genomic characterization is essential for guiding personalized treatment approaches. Notably, the 2023 National Comprehensive Cancer Network (NCCN) guidelines' endorsement of the KRAS G12C inhibitors marks a pivotal advancement in the therapy for the KRAS-Mutated NSCLC patients; however, the intricate interactions between co-mutations and the tumor immune microenvironment emphasize the urgent need for combination therapies and innovative targeted approaches to improve clinical outcomes in KRAS-driven NSCLC (40,121–123).

KRAS G12C/TP53/STK11/KEAP1/CDKN2A

In this investigation we examined the impact of co-mutations (TP53, STK11, KEAP1, and CDKN2A) in KRAS G12C-mutated, stage IV NSCLC. Contrary to previous reports suggesting that these co-mutations individually contribute to poorer survival outcomes (72,73,75–77,124,125), our analysis did not reveal a significant difference in OS between patients with KRAS G12C mutations alone and those with additional co-mutations. The median OS for patients with only KRAS G12C mutations was 12 months (n=21), whereas those harbouring at least one of the analysed co-mutations had a median OS of 18 months (n=42). While this trend appears unexpected given the known prognostic impact of these mutations, it is likely affected by the limited sample size. To our knowledge, a cohort including all these co-mutations in one analysis has not been studied before, making this a novel contribution to the understanding of KRAS G12C-mutated NSCLC. The result of this investigation is likely explained by the fact that KRAS G12C-mutated patients with TP53 co-mutations compensate the negative predictive effect of other co-mutations.

Notably, the largest portion of this cohort consists of patients with a TP53 co-mutation (n=27/42) and previous studies have demonstrated that TP53 co-mutations in KRAS G12C-mutated NSCLC are associated with worse outcomes (124). However, our analysis did not show any statistically significant difference in OS between patients with and without TP53 co-mutations (20 months vs. 16 months, respectively) and even shows a trend toward a higher OS in patients with TP53 co-mutation. This finding contrasts with the expected negative prognostic effect of this co-mutation and may be explained by the high proportion of

patients in our cohort receiving ICI-based therapies. Evidence in recent years emerged suggesting that KRAS G12C/TP53 mutated NSCLC, especially those with high PD-L1 expression, are more responsive to immunotherapy which could have mitigated the typically expected poorer prognosis (68,69,126).

Recent clinical findings, including our observation of favourable outcomes in TP53 co-mutated patients treated with ICI-based regimens, reinforce this observation, showing that such molecular profiles respond favourably in first-line settings. Notably, the KRYSTAL-7 trial demonstrated a median PFS of over 27 months in patients receiving adagrasib plus pembrolizumab, highlighting the promise of combining targeted therapy with immunotherapy in biomarker-defined NSCLC subgroups (127,128).

KRAS G12C/TP53

Given that TP53 co-mutation has been reported as a negative predictor in KRAS G12C-mutated NSCLC reducing median OS by >30% compared to KRAS-only mutated cases (124), its association with longer OS in our cohort is unexpected. This observation may be linked to treatment differences. The majority of TP53 co-mutated patients (n=24/26) received ICI-based therapy, either as monotherapy (n=10/26) or in combination with chemotherapy (n=14/26). Notably, TP53 co-mutated patients treated with ICI + chemotherapy had better outcomes than those without TP53 mutations (ORR: 57.1% vs 46.2%; DFS: 64.3% vs. 53.8%).

This aligns with studies suggesting that KRAS G12C/TP53 co-mutated tumors with high PD-L1 expression ($\geq 50\%$) are particularly responsive to pembrolizumab monotherapy (68,69). Additionally, recent findings also found that this mutation combination benefits significantly from ICI therapy due to specific molecular characteristics, supporting the use of NGS panel testing to identify these patients(126).

Building on this, the prognostic impact of TP53 co-mutation in KRAS G12C-mutated NSCLC may be context-dependent, depending on PD-L1 status and the treatment regimen employed (68,129).

The enhanced responsiveness to immune checkpoint blockade observed in this subgroup may reflect a more immunogenic tumor microenvironment driven by TP53-associated genomic instability and neoantigen burden, emphasizing the importance of integrating molecular profiling with immune biomarkers to personalize first-line treatment in KRAS-driven NSCLC (129–131) .

In this context, through inhibition of the PD-1/PD-L1 pathway, agents such as pembrolizumab and nivolumab reactivate suppressed T-cell responses and facilitate tumor clearance (132). Beyond this, KRAS G12C inhibitors demonstrate notable immunomodulatory properties, including the ability to reshape the immune microenvironment and enhance anti-tumor responses (133,134). This provides a strong rationale for combining KRAS G12C inhibitors with immune checkpoint inhibitors, and several clinical trials, such as the KRYSTAL-7 trial and the SUNRAY-01 trial, are actively evaluating these combinations to overcome resistance mechanisms and improve long-term outcomes in this molecularly defined patient population (127,135).

KRAS G12C/STK11

STK11 co-mutations in KRAS G12C-mutated NSCLC have been significantly associated with shorter OS and PFS, regardless of whether patients receive ICI or chemotherapy as first-line treatment (72,73,125). Our investigation showed that patients with STK11 co-mutation had a non-significantly longer OS compared to only KRAS G12C-mutated NSCLC patients, with 18 months (n=13) vs. 16 months (n=41). The ORR to ICI plus chemotherapy was similar across both cohorts. However, the limited sample size in our study may restrict definitive conclusions, emphasizing the need for larger cohort studies.

In a very recent study on the KRYSTAL-1 trial cohort, first-line adagrasib achieved an ORR of 30.3% and a median OS of 12.3 months in KRAS G12C/STK11 co-mutated NSCLC, indicating potential benefit despite the historically poor prognosis of this subgroup (136). While our patients were treated with ICI and chemotherapy, the similarly favourable outcomes observed in the KRYSTAL-1 trial with adagrasib suggest that KRAS G12C/STK11 co-mutated NSCLC may derive clinical benefit from multiple therapeutic approaches, challenging the traditionally poor prognostic expectations for this subgroup.

Tailoring treatment to tumor biology, especially in the presence of STK11 or KEAP1 co-mutations, is critical, as these influence immune response and resistance (129,137,138). Combinations with SHP2, SOS1, or immune modulators are under investigation, with promising preclinical data showing durable responses and potential synergy with PD-1 blockade. Notably, SHP2/G12C inhibitor combinations demonstrated antiangiogenic and immune-enhancing effects, and further strategies, such as adding CTLA-4 or OX40-targeted agents, may be needed to achieve lasting remission (139–142). Although our findings did not reach statistical significance, the observed trend toward improved OS in the KRAS G12C/STK11 cohort supports the growing evidence that genomic profile, particularly co-mutations, may

modulate response patterns and should guide future treatment regimen and patient selection in ongoing combination trials.

KRAS G12C/CDKN2A

Previous studies have established that CDKN2A co-mutations are associated with poorer clinical outcomes and worse response to ICI monotherapy compared to only KRAS G12C-mutated NSCLC (75–77). In our cohort, patients harbouring CDKN2A co-mutations had shorter OS compared to those with KRAS G12C mutations alone - 9 months (n=6) vs. 16 months (n=48) - consistent with these prior findings. While the difference did not reach statistical significance, the trend aligns with emerging data indicating that CDKN2A alterations may disrupt cell cycle regulation, foster immune evasion, and diminish therapeutic durability (75,76,143,144).

This underlines the importance of incorporating CDKN2A mutation status into routine molecular profiling to improve risk stratification and treatment selection. Future studies should explore whether therapeutic strategies, such as co-targeting CDK4/6 or incorporating novel immunomodulatory agents, can overcome this negative prognostic influence and improve outcomes in this high-risk subgroup (129,145).

KRAS G12C/KEAP1

It has been demonstrated that KEAP1 co-mutation is significantly associated with worse clinical outcomes in patients with KRAS G12C-mutated NSCLC (77,125). Consistent with these findings, our analysis indicated that the presence of KEAP1 co-mutation was associated with a reduced OS of 11 months (n=6), compared to 17 months in patients without the co-mutation (n=48). Although the observed difference did not reach statistical significance, likely due to the small sample size, our results suggest that KEAP1 mutations may influence survival. These findings emphasize the need for larger, more robust studies to further explore this relationship.

The presence of STK11/KEAP1 co-mutations has been demonstrated to correlate with significantly shorter OS in patients undergoing any type of first-line therapy, regardless of their KRAS G12C mutation status (73,120). Furthermore, co-mutations such as STK11 and KEAP1 not only impact prognosis but also influence therapeutic responses, particularly by driving early resistance to KRAS G12C inhibitors (146–148).

The presence of KEAP1 and STK11 co-mutations has been linked to an immune-suppressed tumor microenvironment and activation of pathways such as NRF2 and PI3K-mTOR, which contribute to resistance against KRAS G12C inhibitors (149,150). Preclinical

evidence of synergy between adagrasib and mTOR inhibitors supports exploring combination therapies to overcome resistance and improve patient outcomes (151,152).

This observation underscores the growing recognition that co-mutation patterns in NSCLC are highly complex and can profoundly influence patient prognosis. While additional research is needed, the findings suggest that integrating molecular profiling into routine clinical practice could help tailor treatment strategies and improve patient outcomes.

Limitations of the study

While this study provides insight into the impact of TP53, STK11, CDKN2A, and KEAP1 co-mutations in KRAS G12C-mutated NSCLC, as well as the effects of TP53 co-mutations on EGFR-mutated NSCLC, several limitations must be acknowledged. The single-center nature of this study (Pius-Hospital Oldenburg) restricts its external validity, as the patient cohort may not be fully representative of the broader NSCLC population. Differences in demographic, environmental, and treatment-related factors may impact the applicability of these findings.

Since lifestyle factors like diet, physical activity, and workplace exposures were not assessed, their potential impact on the findings cannot be ruled out. The sample size was a limiting factor, particularly in certain subgroups. While KRAS G12C and EGFR-mutated NSCLC cases were well represented, subgroups with co-mutations, such as KRAS G12C + CDKN2A or KEAP1, had relatively small sample sizes, potentially affecting statistical robustness. Future multicentre studies with larger cohorts are essential to further explore these associations.

While the study is limited by a single-center design and relatively small sample sizes in some subgroups, the findings highlight clinically relevant trends that warrant further investigation and highlights the importance of molecular testing of NSCLC patients.

6 Conclusion

This study provided valuable insights into the impact of TP53, STK11, CDKN2A, and KEAP1 co-mutations on clinical outcomes in NSCLC patients with KRAS G12C or EGFR mutations. While previous research has suggested that certain co-mutations negatively affect prognosis and treatment responses our findings demonstrate the same tendencies, but with a more nuanced picture. The presence of TP53 co-mutation in EGFR-mutated NSCLC was associated with a trend toward worse survival outcomes, though statistical significance was not reached. Similarly, in KRAS G12C-mutated NSCLC, the influence of co-mutations on OS varied.

A key finding was the improved OS in patients treated with a combination of ICI and chemotherapy, compared to those receiving ICI monotherapy, suggesting a potential synergistic effect. Furthermore, the study highlighted the importance of evaluating PD-L1 expression in patients with KRAS G12C/TP53 co-mutations, as this subgroup may show a more favourable response to immunotherapy.

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8 Declaration of oath

I hereby declare in lieu of oath that I have written this dissertation myself and that I have not used any auxiliary materials or sources other than those indicated. All passages that have been taken literally or figuratively from published or unpublished writings have been marked as such. The thesis has not yet been submitted in the same or similar form to any other examination authority (university).

Oldenburg, 23.10.2025

Massimo Privitelli