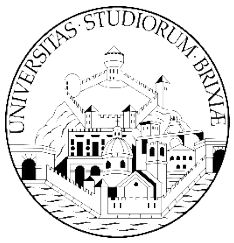

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CICLO XXXVIII

A MULTIMODAL PREDICTIVE APPROACH TO ONCOGENE-ADDICTION IN NON-SMALL CELL LUNG CANCER: INTEGRATING CLINICAL, FUNCTIONAL AND IMAGING BIOMARKERS

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SUMMARY

ABSTRACT

Background: Il carcinoma polmonare non a piccole cellule (NSCLC) oncogene-addicted rappresenta un sottogruppo biologicamente distinto, con importanti implicazioni terapeutiche. Identificare precocemente questi tumori è fondamentale per evitare ritardi terapeutici e ottimizzare l'avvio delle terapie target. Questa tesi esplora l'ipotesi che una combinazione di parametri clinici, funzionali, metabolici e radiomici possa migliorare la stima pre-test dell'oncogene addiction, prima della disponibilità dei risultati molecolari.

Materiali e Metodi: È stata analizzata una coorte osservazionale monocentrica di pazienti con nuova diagnosi di NSCLC, sottoposti a sequenziamento NGS, test di funzionalità respiratoria, PET/TC con ^18F-FDG e radiomica su TC. Sono stati valutati variabili demografiche, DLCO, SUVmax e caratteristiche radiomiche tramite analisi statistiche tradizionali, regressione logistica, modelli CART e Random Forest. I valori mancanti di DLCO sono stati gestiti tramite imputazione multipla. Le prestazioni dei modelli sono state valutate mediante accuratezza, sensibilità, specificità ed errore out-of-bag (OOB).

Risultati: I tumori oncogene-addicted sono risultati più frequenti in donne e non fumatori e mostravano una DLCO più elevata e valori di SUVmax inferiori. La radiomica ha evidenziato maggiore eterogeneità intratumorale nei tumori non oncogene-addicted. I modelli CART hanno identificato abitudine tabagica, DLCO e SUVmax come principali discriminanti, sebbene le loro prestazioni siano risultate limitate dallo sbilanciamento delle classi e dai dati mancanti. Il modello Random Forest ha mostrato prestazioni superiori (errore OOB 21,25%) e ottima sensibilità per i tumori oncogene-addicted, con una specificità più modesta per quelli non oncogene-addicted. Nessun singolo

biomarcatore si è dimostrato sufficiente da solo, ma ciascuno ha contribuito in modo complementare alla descrizione del fenotipo tumorale.

Conclusioni: Questa tesi supporta la fattibilità di un approccio diagnostico integrato e multimodale per anticipare l'oncogene addiction nei pazienti con NSCLC. Parametri funzionali (DLCO), attività metabolica PET (SUVmax) e eterogeneità radiomica forniscono informazioni coerenti con la biologia del tumore e potenzialmente utili nella pratica clinica. Sebbene i modelli debbano essere perfezionati e validati esternamente, questi risultati gettano le basi per percorsi diagnostici più efficienti, volti a ridurre il tempo alla terapia e migliorare la stratificazione dei pazienti.

1. INTRODUCTION: LUNG CANCER

Lung cancer is one of the foremost challenges in contemporary medicine.

Despite substantial progress in diagnostic methodologies and therapeutic strategies over recent years, it continues to account for a considerable proportion of cancer-related mortality worldwide, ranking as the leading cause of oncologic death globally.

This is largely attributable to its frequent detection at advanced stages, when the probability of achieving complete disease remission is markedly reduced [1].

1.1. Epidemiological data

A recent collaborative report by researchers from the International Agency for Research on Cancer (IARC) and the American Cancer Society (ACS) provides an overview of global cancer statistics for the year 2022 [2]. The report was published in CA: A Cancer Journal for Clinicians [3]. These statistics underscore how cancer represents a major public health challenge in the 21st century, associated with substantial social and macroeconomic costs that vary according to tumor type, geographical region, and sex.

In a projection of the global economic burden of cancer from 2020 to 2050 [4], five cancers emerged as the most costly, with tracheal, bronchial, and lung cancers accounting for the highest economic impact.

Cardiovascular diseases and cancer are the leading causes of premature mortality in 127 countries, with cardiovascular diseases ranking first in 70 countries (including Brazil and India) and cancer in 57 countries (including China). These findings reflect an advanced stage of the epidemiological transition that began in the second half of the 20th century and continued into the first half of the current century, during which infectious diseases were progressively supplanted by non-communicable diseases. Based on current trends,

cancer is projected to surpass cardiovascular disease as the leading cause of premature mortality in most countries by the end of this century [5].

According to global cancer statistics [3], nearly 20 million new cases and approximately 10 million deaths were recorded in 2022. By 2050, the annual number of new cases is expected to reach 35 million, representing a 77% increase compared to 2022. Roughly one in five men or women will develop cancer during their lifetime, while one in nine men and one in twelve women will die from it.

Lung cancer was the most frequently diagnosed cancer in 2022 [3], accounting for nearly 2.5 million new cases worldwide, corresponding to one in eight cancers globally (12.4% of all cases). It was followed by female breast cancer (11.6%), colorectal cancer (9.6%), prostate cancer (7.3%), and gastric cancer (4.9%). Lung cancer was also the leading cause of cancer death, with an estimated 1.8 million deaths, representing 18.7% of all cancer-related mortality, followed by colorectal cancer (9.3%), liver cancer (7.8%), female breast cancer (6.9%), and gastric cancer (6.8%).

The disease ranks first among men and second among women for both incidence and mortality. Among men, it is the most frequently diagnosed cancer in 33 countries and the leading cause of cancer death in 89. The highest incidence rates are observed in East Asia, followed by Micronesia/Polynesia and Eastern Europe, with Turkey recording the highest national rate among men worldwide. Among women, lung cancer is the leading cause of cancer mortality in 23 countries, including China and the United States. The highest incidence rates are found in North America, East Asia, and Northern Europe, with Hungary reporting the highest national rate.

Five-year survival rates generally remain below 20% in most countries [6]. A study examining survival differences by stage, histological subtype, and sex in high-income

countries suggested that factors such as treatment, healthcare system organization, and the burden of comorbidities likely play a significant role [7].

1.1.1. Epidemiological data in Italy

The most recent guidelines from the Italian Association of Medical Oncology (AIOM), updated in September 2024 [8], confirm that lung cancer remains the leading cause of cancer-related mortality among men and the second among women, continuing to represent the "Big Killer" of oncology due to its high incidence and mortality.

In 2020 [9], a decline in incidence was observed among men—particularly in the 50–69 age group—whereas an increase was reported among women, especially in those aged 70 years and older. This trend has largely been attributed to the greater prevalence of smoking among women beginning in the late 1980s. With respect to mortality, a reduction compared with 2015 was noted in men, whereas an increase was observed in women, progressively approaching the rates associated with breast cancer.

In Italy, in 2023 [8], approximately 44,000 new cases of lung cancer were estimated (30,000 in men and 14,000 in women), making it the second most frequent malignancy in men (15%) and the third in women (6%). Overall, 117,800 individuals were living with a diagnosis of lung cancer, including 77,200 men and 40,600 women.

In 2022, an estimated 35,700 deaths were attributed to lung cancer (23,600 in men and 12,100 in women); unfortunately, updated mortality estimates for 2023 are not yet available.

The 5-year net survival following diagnosis is 16% in men and 23% in women, with outcomes being negatively affected by the large proportion of patients diagnosed at an advanced stage. This is primarily due to the absence of a nationwide screening program

and the frequent asymptomatic presentation of early-stage disease. Indeed, nearly 70% of patients with stage I non-small cell lung cancer (NSCLC) are asymptomatic; consequently, 44% of patients present with metastatic disease at diagnosis, while only 26% are diagnosed at an early stage [10].

1.2. Screening: current status in Italy and worldwide

Given the significant impact of lung cancer both in Italy and globally, investment in a structured lung cancer screening (LCS) program targeting a high-risk population is more crucial than ever. In Italy, the economic burden of this malignancy is estimated at approximately €2.5 billion per year, including both direct healthcare costs and indirect/social costs related to workdays lost by patients and/or their caregivers, as well as potential early retirements. It has been estimated that the implementation of national lung cancer screening programs in 28 European countries could prevent more than 18,000 deaths among 21 million high-risk individuals at a sustainable cost of roughly €50,000 per life saved.

Over the past two decades, several studies in the United States and Europe have evaluated whether LCS using low-dose computed tomography (LDCT) in at-risk individuals could reduce mortality through earlier diagnosis and treatment [10].

Currently, such screening is not yet part of routine clinical practice in Italy. However, the AIOM guidelines (2021 [9] and the updated 2024 edition [8]) provide a strong positive recommendation: *"In current or former smokers who have smoked at least 15 cigarettes per day for more than 25 years, or at least 10 cigarettes per day for more than 30 years, or who quit less than 10 years ago, annual chest CT screening should be considered as a first-choice option."*

The National Lung Screening Trial (NLST) [11,12], conducted in the United States, was the first large randomized controlled trial (RCT) demonstrating the efficacy of LDCT-based LCS. Participants undergoing three annual LDCT screenings experienced a 20% reduction in mortality compared to the control group receiving standard chest radiography. A subsequent cost-effectiveness analysis of NLST [13] showed that LDCT screening incurred an additional cost of $1,631 per person, providing an incremental 0.0316 life-years per person and 0.0201 QALYs per person, corresponding to an estimated $52,000 per life-year gained and $81,000 per QALY gained.

In 2020, the NELSON study [11,14] further confirmed in Europe the mortality reduction benefit of LDCT screening among high-risk populations, demonstrating results even superior to those of the NLST.

In Italy, several trials have contributed to understanding LCS implementation. The DANTE study (Detection and Screening of Early Lung Cancer by Imaging Technology and Molecular Essays), a prospective RCT initiated in 2001, evaluated the impact of LDCT screening on lung cancer mortality compared with standard clinical practice without screening. After three years of follow-up [15], no significant reduction in cancer-specific or overall mortality was observed, a finding confirmed in a subsequent analysis after a median follow-up of eight years [16].

Similarly, the MILD study (Multicentric Italian Lung Detection) randomized participants into three cohorts: no screening, annual LDCT, and biennial LDCT, with all participants receiving smoking cessation support. The biennial LDCT schedule aimed to reduce costs and radiation exposure without compromising program accuracy. Five-year follow-up results [17] demonstrated equivalence between annual and biennial LDCT in detecting stage I and resectable tumors, although no significant difference in overall or cancer-specific mortality was observed. Extending screening beyond five years [18] enhanced

the benefits of early diagnosis, achieving greater reductions in overall and lung cancer mortality compared with NLST.

The COSMOS study (Continuous Observation of SMOking Subjects), a single-arm prospective study, enrolled high-risk participants aged over 50 with a ≥20 pack-year smoking history or former smokers quitting within 15 years. Participants underwent 10 years of annual LDCT follow-up, revealing only one radiation-induced cancer per 108 lung cancers detected, a negligible figure given the substantial mortality reduction achieved. Most detected cancers were early-stage, with high overall survival [19].

The ITALUNG trial, a randomized controlled study conducted in Tuscany from 2004 to 2013 [20], demonstrated a 30% reduction in lung cancer mortality with LDCT screening. Participants were also encouraged to cease smoking as part of the screening program [21]. LDCT screening additionally showed potential in reducing cardiovascular mortality through the detection of coronary calcifications [22].

At the European level, scientific societies including the European Respiratory Society (ERS), European Society of Radiology (ESR), European Society of Thoracic Surgeons (ESTS), and European Society of Medical Oncology (ESMO) have endorsed the implementation of lung cancer screening [8].

Italian public health authorities have recognized the need for a national lung cancer screening program, similar to established free programs for breast, cervical, and colorectal cancers [10]. In November 2021, the Ministry of Health funded the Italian Lung Screening Network (RISP), a multicenter program led by the National Cancer Institute of Milan (INTM) [8,10]. Launched in 2022, RISP aims to recruit 10,000 high-risk individuals across 18 centers, targeting smokers with ≥1 pack/day for 30 years or former smokers quitting within 15 years, aged 55–75. Participants are enrolled in a centralized national database, which reached 17,294 volunteers by 23 July 2023 [23].

RISP combines secondary prevention via LDCT screening with primary prevention through pharmacologically supported smoking cessation, aiming for synergistic outcomes. The ultimate goal is to integrate both LDCT screening and pharmacological prevention with cytisine into the essential levels of care (LEA), ensuring quality comparable to other national screening programs [10].

In 2022, a Ministry-led strategic program outlined nationwide LDCT screening to include a substantial portion of the population [10]. Key objectives include raising awareness of lung health among smokers and former heavy smokers, engaging primary care physicians for recruitment and follow-up, and centralizing data to ensure program quality and effectiveness [10]. Recent pilot studies coordinated by the Institute for Study, Prevention, and Oncological Network (ISPRO)—ITALUNG-2 (funded by the Tuscany Region) and the CCM 2019 program (funded by the Ministry of Health) [10]—employed two annual rounds of LDCT combined with smoking cessation interventions for 1,170 heavy smokers and former heavy smokers (<10 years since quitting), aged 55–75, with ≥30 pack-year histories across three regions: Tuscany (Florence, Pisa, Massa), Lombardy (Milan), and Piedmont (Turin). Recruitment strategies involved general practitioners and self-enrollment via phone, email, or online registration. Dedicated recruiters facilitated patient participation and care. Participants with negative LDCT results were rescreened after one year, while those with positive findings underwent confirmatory LDCT at 1, 3, or 6 months depending on nodule characteristics or immediate further investigation if highly suspicious. By June 2023, 794 participants had been recruited, primarily heavy smokers (87%), with positive feedback regarding the integration of smoking cessation support [10].

While LDCT screening appears effective in reducing mortality among smokers, its role in non-smokers remains unclear [24]. Non-smokers are defined as individuals with <100 lifetime cigarettes, in contrast to smokers with ≥100 [25].

The prospective TELENT study [26] in Taiwan was the first to assess LDCT screening in non-smokers, enrolling 12,011 participants with additional risk factors, including family history of lung cancer, passive smoke exposure, COPD, or prior tuberculosis. Lung cancer detection exceeded 2%, double the rate observed in NLST [12] and NELSON [14], particularly in participants over 60 with a positive first-degree family history.

Other studies [27,28] corroborated these findings, revealing a high proportion of early-stage lung cancer diagnoses among non-smokers, allowing for more radical, curative treatments with lower recurrence rates compared with palliative systemic therapy for advanced disease [24]. Another study [29] of 28,807 LDCT-screened participants similarly reported predominantly early-stage diagnoses with associated survival benefits.

To optimize LDCT screening among non-smokers, risk prediction models based on sociodemographic and family history variables are needed to identify those most likely to benefit, minimizing overdiagnosis (i.e., detection of indolent tumors). Risk factors may differ between men and women, suggesting that screening eligibility criteria should be sex-specific. Differences in lung cancer epidemiology by sex may relate to genetic variants, hormones, environmental exposures, or oncogenic viruses such as human papillomavirus, though these relationships remain incompletely understood.

With appropriate risk stratification, LDCT screening in non-smokers could achieve effectiveness comparable to that observed in smokers, supporting broader implementation [11,24].

1.3. Risk factors

Tobacco smoking is the primary risk factor for lung cancer, accounting for approximately 80–90% of cases. Other carcinogenic agents, such as asbestos, radon, and heavy metals, also contribute to disease risk, particularly among populations exposed occupationally. Additional risk factors include passive smoking, air pollution and fine particulate matter, genetic polymorphisms, a family history of lung cancer (especially in parents or siblings), prior radiotherapy for other conditions, and chronic pulmonary diseases [1,30].

Bronchopulmonary conditions that trigger local inflammation have been shown to increase lung cancer risk independently of tobacco use, highlighting their importance in individual risk assessment. Specifically, patients with a history of emphysema, chronic bronchitis, pneumonia, or chronic obstructive pulmonary disease (COPD) exhibit an elevated relative risk [31].

An association between asthma and lung cancer has been observed in both smokers and non-smokers [25]. Sarcoidosis, a chronic inflammatory disease affecting the lungs, has long been linked to an increased risk of pulmonary malignancy, although evidence remains inconclusive [32].

Certain infectious diseases also appear to increase lung cancer risk independently of smoking status [25]. These include tuberculosis caused by *Mycobacterium tuberculosis*. Among HIV-positive individuals on antiretroviral therapy, lung cancer has become the leading cause of cancer-related mortality, as other frequent infections may contribute to a chronic inflammatory state that promotes tumor development [33]. Additionally, human papillomavirus (HPV) may represent a further risk factor, particularly in non-smoking women [34].

Conversely, a healthy lifestyle and a balanced diet are important protective factors. Beyond the absence of smoking, a diet rich in fruits, vegetables, whole grains, and dietary fiber, and low in red and processed meats, has been associated with a reduced risk of lung cancer [35].

Conversely, unhealthy dietary habits, insufficient physical activity, and associated obesity can increase the risk of various cancers, including lung cancer, in addition to cardiovascular disease [36]. Diets high in saturated fats and sugars but deficient in essential nutrients may promote a pro-inflammatory environment and oxidative stress, thereby facilitating tumorigenesis.

1.3.1. Tobacco smoking: health effects and primary prevention

According to data from the World Health Organization (WHO) [37], over 8 million people die each year due to tobacco use, with the majority of these deaths occurring in low- and middle-income countries.

Cigarette smoking is not only a major risk factor for cardiovascular and respiratory diseases but remains the leading preventable cause of cancer worldwide. It is associated with at least 20 different types of cancer, most prominently lung cancer, for which it constitutes the principal risk factor, accounting for 85–90% of cases in Italy [9,38]. The relative risk of developing lung cancer is closely related to several factors, including the number of cigarettes smoked per day and the duration of the smoking habit in years [9]. These two parameters are often summarized as “pack-years,” a commonly used clinical measure to quantify a patient’s cumulative exposure to cigarette smoke over time, and, consequently, the associated risk. Pack-years are calculated by multiplying the number of

packs smoked per day by the number of years of smoking. For example, one pack-year corresponds to smoking 20 cigarettes per day (1 pack) for 1 year [39]. Relative risk for smokers compared to non-smokers is approximately 14, while heavy smokers (more than 20 cigarettes per day) have a relative risk of 20.

Passive smoking is also associated with a 20–50% increased risk of lung cancer compared to non-smokers. For individuals who quit smoking, risk declines progressively over 10–15 years, with significant life-year gains for those who stop before age 40. Thus, the earlier the cessation, the greater the risk reduction [9,40]. Moreover, smoking cessation even after a cancer diagnosis, including advanced-stage tumors, has been shown to halve the risk of disease progression or cancer-related mortality [38].

Given the strong correlation between smoking and lung cancer, the challenges in achieving early diagnosis, and the overall poor prognosis of patients, attention has understandably focused on reducing the primary source of risk—smoking cessation—which also yields benefits for many other diseases.

Primary prevention refers to interventions aimed at reducing disease incidence by minimizing exposure to risk factors and promoting a healthy lifestyle. For lung cancer, primary prevention measures include smoking cessation, the promotion of smoke-free environments to reduce passive smoke exposure, management of occupational hazards, reduction of air pollution levels, and implementation of tobacco control policies [1].

Among these strategies, increasing the average tobacco tax has proven to be one of the most effective interventions for reducing demand. A study [41] highlighted the substantial potential of implementing the highest level of tobacco control policies in Europe, estimating that over 1.6 million lung cancer cases could be prevented over a 20-year period.

The overall economic burden of tobacco use is considerable. Direct and indirect costs, arising from healthcare expenditures and productivity losses, are estimated at approximately $1.4 trillion per year, equivalent to 1.8% of global GDP, with nearly 40% of this burden occurring in developing countries. Tobacco taxes are applied in part to counteract this problem and are considered the most cost-effective approach for reducing consumption, particularly among youth and low-income populations [42].

Smoking cessation is often challenging, particularly achieving permanent abstinence. In Italy, the national Telefono Verde contro il Fumo (TVF, Anti-Smoking Helpline) [43] provides support nationwide. Since its inception, the service has handled over 98,000 calls, 40% of which originated from northern regions. In most cases (92%), callers are smokers who have often failed previous cessation attempts. Awareness of the service is predominantly derived from cigarette pack warnings (97%). Calls are also frequently made by family members or friends seeking assistance for loved ones. Among smokers using the service, 65% are male and 35% female. During the call, trained experts provide support, information, and motivation in a personalized, professional manner.

Additionally, Italy hosts specialized Smoking Cessation Centers (Centri Antifumo), annually cataloged by the Istituto Superiore di Sanità [43]. As of May 2022, 223 centers were active, though their distribution is uneven and decreasing over time: approximately 60% are located in the North, and around 20% each in the Central and Southern regions including the islands. These centers involve multidisciplinary teams, including physicians, nurses, and psychologists, and offer a variety of interventions—both free and fee-based—such as individual counseling, pharmacotherapy, group psychotherapy, and individual psychotherapy.

1.3.1.1. Tobacco use in Italy

According to the "Rapporto sul fumo in Italia" [43], presented on World No Tobacco Day 2022 (May 31, 2022), nearly one in four Italians is a smoker, totaling 12.4 million individuals. Among men, 7.5 million (30.2% of the male population) are current smokers and 4.3 million (17.4%) are former smokers; among women, 4.9 million (18.5%) are current smokers and 3.4 million (12.6%) are former smokers.

After a prolonged period of stagnation, the prevalence of smoking increased by 2 percentage points in 2022 compared to 2019, representing approximately 800,000 additional smokers. In 2019, smokers accounted for 22% of the population. The decreasing trend observed among female smokers during 2017–2019 was not confirmed in 2022; on the contrary, the prevalence increased across both sexes.

Additionally, the use of heated tobacco products tripled from 1.1% in 2019 to 3.3% in 2022, although more than one-third of users (36.6%) consider these products less harmful than conventional cigarettes.

Age-specific trends show the highest prevalence of male smokers in the 25–44 age group, whereas among women, the peak prevalence occurs in the 45–64 age group. The lowest prevalence is observed in individuals over 65 years for both sexes. Among male smokers, the proportion consuming more than 20 cigarettes per day is highest (25.6% versus 13.4% of women), while among female smokers, the proportion smoking fewer than 9 cigarettes per day is lowest (36.0% versus 31.4% of men). Nearly half of young smokers aged 15–24 years smoke fewer than 9 cigarettes per day, although 45.5% consume between 10 and 19 cigarettes daily. On average, Italians smoke 11.5 cigarettes per day. While daily consumption has decreased slightly over the past decade (from 13.6 cigarettes/day in 2011), 20.4% of smokers still consume more than 20 cigarettes per day.

Geographically, smoking prevalence is higher in southern Italy for both sexes: 32.6% of men and 21.6% of women. The majority of tobacco consumption involves manufactured cigarettes (84.9%) and hand-rolled cigarettes (14.9%), both of which have declined since 2019 (90.2% and 18.3%, respectively). Hand-rolled cigarettes are more commonly used by young males, particularly in Central Italy.

According to the AIOM 2024 guidelines [8], the average age of smokers in Italy is 45.3 years, and over 70% report having started smoking between the ages of 15 and 20. Of particular concern is the increasing prevalence of smoking among adolescents aged 14–17 years. In 2018, approximately 11% of this population (about 254,000 young people) were classified as "baby smokers," representing one of the highest rates in Europe.

1.3.1.2. Tobacco and tobacco smoke constituents

Over the years, significant progress has been made in understanding the chemistry of tobacco products in relation to their toxicity and carcinogenic potential. In 2012, the U.S. Food and Drug Administration (FDA) compiled a list of harmful and potentially harmful constituents (HPHCs) of unburned tobacco and tobacco smoke, identifying 79 compounds as carcinogenic [44]. In subsequent years, advances in analytical technologies have generated substantial new data, enhancing our understanding of carcinogen levels in tobacco products.

The International Agency for Research on Cancer (IARC) has published 35 monographs since 2012, identifying an increasing number of compounds in unburned tobacco and tobacco smoke as carcinogenic. In total, 83 carcinogens have been recognized: 37 in unburned tobacco and 80 in tobacco smoke, with corresponding levels reported since

2012 [45]. A 2022 review, “Carcinogenic components of tobacco and tobacco smoke: a 2022 update” [46], provides an updated overview of IARC-classified carcinogens in tobacco and tobacco smoke. Today, it is estimated that tobacco and its smoke contain a complex mixture of over 9,500 chemical compounds, many of which have been recognized as hazardous to human health by regulatory agencies. These compounds are associated with an increased risk of a wide range of cancers, most prominently lung cancer, but also cancers of the larynx, esophagus, oral cavity, pharynx, bladder, liver, cervix, kidney, stomach, colorectum, and pancreas [47].

In the same study [46], tobacco and tobacco smoke constituents are generally classified as hydrocarbons, oxygen-containing compounds, nitrogen-containing compounds, and miscellaneous compounds. Furthermore, all tobacco carcinogens can be categorized into ten subgroups: 1) hydrocarbons, 2) amines, 3) N-nitrosamines, 4) ethers, 5) aldehydes, 6) halogenated compounds, 7) nitro compounds, 8) phenolic compounds, 9) miscellaneous compounds, and 10) inorganic compounds. The review provides a detailed list of substances, their characteristics, and IARC classification. Key examples include:

1. **Hydrocarbons** – Among these are 1,3-butadiene (IARC Group 1), isoprene (Group 2B), and styrene (Group 2A). Monocyclic aromatic hydrocarbons include benzene (Group 1), ethylbenzene (Group 2B), and cumene (Group 2B). Sixteen polycyclic aromatic hydrocarbons (PAHs) have been identified, of which only benzo(a)pyrene is classified as Group 1, two are Group 2A, and the remaining (e.g., naphthalene, benzo(a)anthracene, benzo(c)phenanthrene, chrysene) are Group 2B.
2. **N-nitrosamines** – Eleven compounds, including seven acyclic and four cyclic, are classified across IARC Groups 1, 2A, and 2B. Notably, the tobacco-specific nitrosamines NNK (nicotine-derived nitrosamine ketone, acyclic) and NNN (N-

nitrosonornicotine, cyclic) are Group 1 carcinogens. These substances result from the nitrosation of nicotine during tobacco processing and combustion and are present in both smoked products (cigarettes, cigars) and smokeless products (chewing or snuff tobacco) [46,48]. Nicotine, a potent central nervous system stimulant, induces immediate gratification but leads to long-term dependence and tolerance [49]. Nicotine and its metabolite NNK can form DNA adducts, impair DNA repair, and increase susceptibility to mutations and malignant transformation in pulmonary and bladder epithelial cells [50].

3. **Aldehydes** – Aldehydes in tobacco smoke are generally present at higher levels than PAHs and N-nitrosamines. Four compounds are identified as carcinogenic: formaldehyde (Group 1), acrolein (Group 2A), acetaldehyde, and crotonaldehyde (Group 2B). Acrolein, one of the most abundant hazardous components in tobacco smoke, was recently classified as "probably carcinogenic to humans" (Group 2A). Exposure to aldehydes may also arise from exogenous sources such as fried foods, passive smoke, and endogenous biochemical metabolism [46].
4. **Inorganic compounds** – Eight inorganic compounds are recognized, including arsenic, beryllium, cadmium, chromium, nickel, and polonium-210, all classified as Group 1 carcinogens by IARC.

Despite regulatory advances, there have been no clear trends toward reducing the levels of these carcinogens. Regulatory interventions remain essential to control their presence and mitigate the risk of cancer and other tobacco-related diseases [46].

1.3.2. Passive smoking

Exposure to secondhand smoke is also associated with adverse health outcomes, causing approximately 1.2 million deaths annually, including 65,000 child deaths due to smoke-related diseases [37]. Children's exposure to secondhand smoke at home is strongly correlated with the smoking status of household members. Among current smokers, 22.6% report exposing children to secondhand smoke, compared to 5.5% of former smokers and 4.7% of never-smokers. Allowing guests to smoke at home similarly correlates with smoking status: 45.0% of current smokers permit it, versus 24.6% of former smokers and 16.5% of never-smokers. These data indicate lower awareness and sensitivity to secondhand smoke exposure among smokers, and higher attention among former and never-smokers.

More than 15 years have passed since the implementation of Italy's Sirchia anti-smoking law, and compliance with indoor smoking bans has now become widespread. However, with the introduction of alternative nicotine products, such as e-cigarettes and heated tobacco products, the issue of secondhand exposure—or more accurately, exposure to emissions from these products—has recently re-emerged. The health risks associated with these emissions are still under investigation. Notably, 66.8% of e-cigarette users and 74.6% of heated tobacco product smokers report feeling free to use these products in public spaces. These findings represent a clear warning regarding the need for updated legislation that adequately addresses products other than traditional cigarettes [43].

1.3.3. Electronic cigarettes (E-cigarettes): a new trend

Over the past 20 years, the tobacco industry has introduced a wide range of alternatives to traditional cigarettes, including electronic cigarettes (e-cigarettes). First invented in China in 2003, they entered the U.S. market in 2007 and have since experienced a rapid increase in global sales.

In Italy, approximately 2.4% of the population—around 1,200,000 people—use e-cigarettes, either occasionally or regularly. About 80% of e-cigarette users are also conventional smokers, making them dual users. Nearly 3% of habitual or occasional e-cigarette users were never smokers before starting e-cigarettes [43].

E-cigarettes were initially marketed as devices capable of providing smokers with a satisfaction comparable to conventional cigarettes, delivering nicotine in various concentrations while supposedly avoiding harmful effects on health [51]. They were also promoted as a potentially effective smoking cessation tool, although evidence supporting this role remains controversial [52]. Conversely, e-cigarettes may act as a gateway to nicotine use and subsequent dependence, particularly among adolescents who have never smoked, increasing the likelihood of later tobacco consumption by approximately threefold [53], [54]. Fortunately, recent global tobacco regulations now require nicotine to be sold separately, and both e-liquids and nicotine are restricted to consumers aged over 18 years.

According to a World Health Organization (WHO) survey [53], in 2024 approximately 37 million adolescents aged 13–15 use tobacco. In many countries, e-cigarette use among adolescents exceeds that of adults, posing a serious threat to young non-smokers. In 2015, the JUUL—a fourth-generation e-cigarette—gained popularity among youth due to aggressive marketing, an innovative USB-like design, and fruit-flavored options. In 2019,

the U.S. experienced an outbreak of EVALI (E-cigarette or Vaping-Associated Lung Injury) predominantly among 18–34-year-olds [55]. This prompted the U.S. federal government to raise the minimum age for tobacco purchases to 21 and restrict sales of fruit-flavored e-liquids, leaving only tobacco and menthol flavors available. Nevertheless, increased regulatory oversight inadvertently led to the rise of flavored disposable e-cigarettes, with use among high school students increasing elevenfold in 2020, according to the National Youth Tobacco Survey [56]. The rise in youth e-cigarette use appears largely driven by aggressive marketing targeting adolescents and the availability of a wide range of innovative, trendy, and flavored e-cigarettes. As stated by the WHO Director-General on World No Tobacco Day 2024, "These industries actively target schools, children, and young people with new products that are essentially candy-flavored traps" [53].

A 2018 study [57] involving 1,610 students aged 14–18 who had never heard of JUUL or e-cigarettes revealed that risk perception strongly depended on e-liquid flavor. Specifically, fruity flavors were perceived as less harmful and less likely to cause lung cancer than tobacco flavor, both for active use and passive exposure; similar, albeit weaker, trends were observed for candy, menthol, and alcohol flavors. These findings support regulatory efforts to ban flavored e-liquids, which could reduce youth curiosity and usage.

E-cigarettes have rapidly become a growing trend: from 2012 to 2017, the number of users more than doubled in both the EU and the U.S. Curiosity and the perception of being "trendy" are often cited as reasons for experimentation [58].

Studies assessing adult perceptions in the U.S. (2012–2017) [59] and in England (2014–2023) [60] show that an increasing proportion of adults perceive e-cigarettes as harmful or at least as harmful as conventional cigarettes. These results emphasize the need for

accurate public communication regarding e-cigarette risks, particularly targeting young populations. Although e-cigarettes are often marketed as a less harmful and less carcinogenic alternative, evidence suggests they are far from harmless.

Over the years, leading health organizations—including the American Lung Association, the American Academy of Pediatrics, the World Health Organization, and the U.S. Centers for Disease Control and Prevention—have expressed concern regarding the health risks of e-cigarette use. Consequently, in 2016, the U.S. Food and Drug Administration (FDA) amended the Family Smoking Prevention and Tobacco Control Act to enhance oversight and regulation of e-cigarette production and sales [51].

1.3.3.1. Vaping: e-liquids and health effects

E-cigarettes are marketed in various shapes and sizes, with increasingly sophisticated designs evolving across generations. Despite differences in design, all e-cigarettes share a common mechanism: they are battery-powered devices that heat a user-selected liquid contained in a reservoir until it vaporizes, generating an aerosol.

Although e-cigarettes were introduced as a potentially safer alternative to conventional cigarettes—since they do not contain tobacco or involve combustion—evidence has shown that they exhibit a certain degree of toxicity, which still requires further study. This toxicity is related to the aerosol generated by e-cigarettes and is influenced both by the intrinsic toxicity of the e-liquid, which acts as the source of the aerosol, and by chemical byproducts formed when the e-liquid is heated by the coil [51].

E-liquids typically contain nicotine, which may be present at varying concentrations or absent, flavorings, propylene glycol, and vegetable glycerin. These components are

aerosolized and then inhaled [61]. While e-liquids generally contain fewer toxic chemicals than conventional cigarettes, their composition and the design of the devices can vary widely, resulting in greater variability in their health effects [51].

Due to the presence of nicotine, e-liquids may also contain tobacco-related compounds, such as nitrosamines, which are harmful to human health [62]. Flavorings increase product appeal and reduce perceived risk, especially among youth [63]. However, certain flavored e-liquids contain chemicals capable of inducing significant toxicity to the respiratory epithelium upon inhalation [61].

Many e-liquid components, including propylene glycol and vegetable glycerin, are classified as Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration (FDA). Nonetheless, most GRAS studies tested oral ingestion in rodents, not inhalation. Inhalation toxicity can differ substantially from oral toxicity [51,61]. For example, diacetyl, a butter-flavoring chemical listed as GRAS, can cause bronchiolitis obliterans when inhaled [61,64,65].

Currently, thousands of e-liquids exist; some have been evaluated for toxicity in laboratory studies, while others have not. It has been observed that the more chemicals an e-liquid contains, the more likely it is to be toxic. Concentrations of vanillin and cinnamaldehyde, unlike triacetin, correlate with the toxicity of e-liquids [61]. Among flavoring chemicals, benzaldehyde and cinnamaldehyde are aldehydes capable of forming protein adducts [61,66]. Cinnamaldehyde can also impair macrophage phagocytosis [61,67], while vanillin- and chocolate-flavored e-liquids have the greatest impact on the respiratory epithelium [61,68]. A study [69] demonstrated that flavoring chemicals can compromise innate pulmonary immune responses, creating conditions favorable for respiratory diseases. In vitro studies have also shown cytotoxic effects dependent on flavoring chemicals [70], as well as pro-inflammatory responses linked to non-flavoring

components, such as propylene glycol and glycerin, which are present in all e-cigarettes, leading to increased IL-6 and IL-8 secretion [71]. When heated in e-cigarettes, propylene glycol and glycerin undergo thermal degradation, producing aldehydes including formaldehyde, acetaldehyde, and acrolein, which are harmful and even carcinogenic to humans [71].

Another study [72] demonstrated that pulmonary function in mice was impaired by exposure to propylene glycol and vegetable glycerin, although to a lesser extent than when exposed to e-cigarette aerosol containing nicotine and flavorings. To date, only a few in vivo studies have assessed the potential respiratory harm caused by flavorings and other e-liquid components [51]. Thus, continued investigation is essential to allow regulatory agencies, such as the FDA, to better regulate e-liquid composition to protect consumer health.

The heating element itself in e-cigarettes, if exposed to temperatures exceeding 1,000 °C (compared to the usual 300 °C), is a major source of toxic metals, including chromium, nickel, selenium, and aluminum, which are also potentially harmful [51,73].

Studies have found that oxidative DNA damage from e-cigarette aerosol is similar to, or slightly higher than, that caused by conventional cigarette smoke extracts, as the aerosol may also suppress cellular antioxidant responses [74]. In a recent murine study [48], mice exposed to e-cigarette vapor for 12 weeks exhibited extensive pulmonary DNA damage and reduced DNA repair, resulting in lung adenocarcinomas in 9 of 40 mice after 54 weeks of exposure.

Adverse effects of e-cigarette aerosol have been observed throughout the respiratory tract, from the oral cavity to the lungs. These effects include cytotoxicity, impaired immune response, pro-inflammatory action, oxidative damage, and reduced DNA repair capacity.

Current e-cigarette users are more susceptible to viral infections, including COVID-19 [75,76], as well as bacterial infections [77,78], due to compromised immune function. Moreover, e-cigarette users, especially dual users, show increased risk of respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma, as well as overall cardiopulmonary function impairment [79]. E-cigarette aerosol has also been shown to enhance the metabolism of benzo(a)pyrene, a tobacco smoke carcinogen, into genotoxic products [80].

1.3.3.2. Vaping and lung cancer

Current evidence suggests a potential association between e-cigarette use and an increased risk of lung cancer, although unequivocal epidemiological proof is lacking. Given the latency period between exposure to a carcinogen and the development of invasive neoplasia, combined with the recent rapid expansion of e-cigarette use, there has not yet been sufficient time to observe the long-term impact, and it is possible that the full association may not be understood for several years [51,54].

The potential additive (or synergistic) toxicity in dual users—those who consume both traditional tobacco products and e-cigarettes—is another important consideration. The prevalence of dual use is increasing, and the risks associated with this pattern of consumption require further investigation [51]. Already in 2017, a study published in *The New England Journal of Medicine* [81] reported that in the United States, approximately 40% of users, both adults and youth, prefer tobacco-based products, with the most common combination being the simultaneous use of conventional and electronic cigarettes. Clinical research will therefore need to clearly distinguish among different

patient populations: dual users, former smokers, and never-smokers, in order to accurately identify specific effects in each group.

Another limitation in studying this topic is that e-cigarette users can modify the e-liquid and nicotine concentration, introducing additional variables that complicate the analysis. Therefore, further research is necessary, with particular attention to long-term follow-up. It would also be advisable for future smoking cessation campaigns to include education on vaping, with clear messaging on health risks and the potential for nicotine dependence. Given the current scarcity of data regarding the risks associated with these products, consideration should be given to banning vaping in indoor public spaces, due to the potential dangers of secondhand exposure.

1.3.4. Heated Tobacco Products (HTP)

Heated Tobacco Products (HTP) are innovative devices in which the toxicological data are currently limited.

In Europe [82], HTPs are becoming extremely popular, particularly among young people aged 15 to 24, including both smokers and former smokers. The most frequently reported motivations for use are the perception that HTPs are less harmful than conventional cigarettes, the influence of friends and acquaintances that arouses curiosity, and the desire to reduce or quit smoking.

In Italy [43], approximately 3% of the population, nearly 1.7 million people, use these products either regularly or occasionally. Their consumption has tripled from 2019 to 2022. Just over 50% of Italian smokers believe that HTPs are as harmful as traditional

cigarettes, whereas 36.6% believe they are less harmful; unfortunately, this latter perception has become more widespread in recent years.

These products allow users to inhale an aerosol containing nicotine by heating the tobacco to approximately 350 °C, rather than burning it at 900 °C, as occurs in traditional cigarettes. The fundamental operational difference between the two products is therefore the presence of a heating process in HTPs versus combustion in conventional cigarettes [83]. One particularly widespread product is IQOS (I Quit Ordinary Smoking), which operates in a similar way: a battery-powered heating device with a heating blade is inserted into a tobacco stick. HTP sticks are available in various flavors, such as menthol, citrus, chocolate, and many others, similar to e-cigarette liquids; therefore, the composition of the HTP aerosol also varies according to the added flavoring agents. However, HTP devices heat reconstituted tobacco containing nicotine, carrying all the risks associated with nicotine dependence, particularly among adolescents and young adults, in contrast to e-cigarettes, which heat a liquid that may or may not contain nicotine [83].

Given the operational distinction between heating and combustion present in these products and conventional cigarettes, the tobacco industry claims that HTP aerosol contains a lower amount of toxic chemicals and is therefore a safer alternative to traditional smoking. It is worth noting that e-cigarettes were also introduced with similar claims long before modern HTPs. Nevertheless, more than twenty harmful and potentially harmful constituents have been reported at higher concentrations in HTP aerosol than in cigarette smoke. In addition, several toxic compounds not detected in cigarette smoke have been found in HTP aerosol.

For these reasons, in-depth studies on HTP safety, particularly long-term effects, are required. Currently, the health risks of HTP use remain unknown. Most of the available

data on the health effects of HTP aerosol exposure have been generated by the tobacco industry itself. Only a few independent studies have reported short-term physiopathological effects, primarily related to the pulmonary and cardiovascular systems, and no long-term toxicity data currently exist. Therefore, claims by the tobacco industry regarding HTPs as a safer alternative to conventional cigarettes, due to the absence of combustion, are not substantiated [83].

1.3.4.1. Effects of heated tobacco products on the lung

Studies conducted on human bronchial epithelial cells have shown that exposure to aerosol from heated tobacco products induces oxidative stress and a pro-inflammatory cellular response [84], along with elevated levels of reactive oxygen species and DNA damage markers [85]; all of these are mechanisms of toxicity similar to those observed in conventional cigarette smokers.

The European Respiratory Society [86] has advised against the use of any tobacco products, including heated tobacco products, due to the associated risk of lung damage and negative impacts on human health. Even if heated tobacco products may be less harmful for smokers, they remain harmful, addictive, and there is a risk that users may switch to heated tobacco products instead of completely quitting the harmful habit of smoking.

1.3.5. Other risk factors: radon, asbestos and air pollution

Although, as previously emphasized, 80–90% of lung cancer cases are associated with tobacco use, either through active smoking or passive exposure, a significant proportion of new diagnoses occur in never-smokers [3][25]. Lung cancer in these individuals, compared to cases in smokers, is now considered a distinct entity both clinically-pathologically and molecularly; it also appears strongly correlated with the patient's geographic origin, being significantly more common in Asia than in Europe or the United States. This may be due to differences in genetic susceptibility as well as differences in exposure to occupational and/or environmental carcinogens among populations [87][88]. Several environmental and occupational substances (radon, asbestos, chromium, arsenic, beryllium, vinyl chloride, polycyclic aromatic hydrocarbons, chloromethyl ethers, and others) are recognized as lung carcinogens and, like radon and asbestos, can potentiate their harmful effects when combined with tobacco smoke. The World Health Organization (WHO), through the International Agency for Research on Cancer (IARC), has classified radon and all six major forms of asbestos in Group 1, the category of substances known to be carcinogenic to humans [9]. Radon exposure constitutes, after cigarette smoking, the second leading cause of lung cancer among smokers and is the primary risk factor for never-smokers [89].

In a study covering 66 countries [90], radon-attributable lung cancer deaths amounted to 226,057 in 2012, representing 3% of total cancer deaths. Radon is a radioactive gas produced from the decay of uranium in the soil. It is odorless, colorless, and tasteless. When inhaled, radon particles deposit in the respiratory epithelium, potentially damaging DNA and triggering carcinogenic mechanisms; it is also present in water. In a study [91] of 41 never-smoking patients with lung adenocarcinoma, Tumor Mutation Burden (TMB)

was significantly higher in individuals with high radon exposure, approximately double compared to those with low exposure, and their mutational signature was associated with DNA mismatch repair defects. Outdoors, radon quickly dilutes to low concentrations, while higher concentrations are found in poorly ventilated indoor environments, such as homes, schools, and offices—especially basements or lower floors, often unnoticed by occupants [92][93].

The WHO has outlined strategies to reduce residential radon exposure in the "WHO Handbook on Indoor Radon: A Public Health Perspective" [94]:

1. Provide information on indoor radon levels and associated health risks.
2. Implement national programs to reduce risk in areas with high radon concentrations.
3. Develop radon measurement protocols, update building codes, train construction professionals, and provide financial support to remediate existing buildings.
4. Establish a national reference level of 100 Bq/m^3 for annual average indoor radon, with a maximum of 300 Bq/m^3 if the target cannot be achieved, as higher values are associated with increased lung cancer risk.

Regarding occupational exposures, asbestos is known to increase lung cancer risk fivefold [25]. The WHO [95] defines asbestos as a group of naturally occurring mineral fibers historically and commercially used for their chemical-physical properties, such as durability and fire resistance. Due to its use in construction, workers involved in building, maintenance, or demolition of asbestos-containing structures are at risk, even decades after exposure, due to the latency of disease. Asbestos exposure is also strongly associated with mesothelioma, laryngeal and ovarian cancers, and chronic respiratory diseases like asbestosis. It causes over 200,000 deaths annually worldwide, representing more than

70% of work-related cancer deaths. Over 50 WHO member states have banned asbestos to prevent related diseases, and continued protection and monitoring are required due to its historic widespread use.

Air pollution and fine particulate matter (PM) have also been classified by the IARC as Group 1 human carcinogens responsible for lung cancer [96]. Particulate matter includes primary particles, directly emitted from diesel engines, wood combustion, domestic gas heating, and industrial emissions, and secondary particles, formed through chemical reactions in the atmosphere, e.g., from agricultural ammonia emissions. Inhaled particles reach the lungs and systemic circulation, causing cardiovascular, respiratory, and other systemic diseases [10].

Domestic combustion of solid fuels for cooking and heating increases indoor air pollution and lung cancer risk [25]. In China, where high-temperature cooking is common in poorly ventilated kitchens, exposure to cooking fumes containing aldehydes, polycyclic aromatic hydrocarbons, and other known human carcinogens has been studied [97]. The AHSMOG-2 study [98] found a 22% increased lung cancer risk per 10 μg/m³ increase in PM2.5 among never-smokers [9]. The ESCAPE study [99] reported a 55% increased risk of adenocarcinoma—the most common histotype in never-smokers [9]—even in areas with PM2.5 levels below existing guidelines [96].

In Italy, over 72,000 deaths annually (nearly 12% of the total) are attributable to average PM2.5 levels above 5 μg/m³, with more than 39,000 in the Po Valley. Mortality is further exacerbated by interactions between air pollution and heat waves, which are increasingly frequent due to climate change [100]. The WHO updated air quality guidelines in September 2021, lowering PM2.5 limits from 10 to 5 μg/m³ and PM10 from 20 to 15 μg/m³ [10]. In October 2022, the European Commission aligned with WHO guidelines, aiming for “zero pollution” by 2050 [101]. Current Italian regulations set an annual

average PM2.5 limit of 25 μg/m³, and the Po Valley remains among the most polluted areas in Europe [10].

Despite preventive measures, alarming numbers of deaths linked to elevated particulate matter, nitrogen oxides, and ambient temperature continue in Italy, confirming that air pollution is a major public health risk, not only for lung cancer [100].

1.4. Diagnostic and staging workup

1.4.1. Diagnostic suspicion: signs and symptoms of disease

To date, only a small percentage of lung cancers are diagnosed at an early stage, as the disease remains clinically silent for most of its course and tends to manifest only in advanced stages.

Patients may present [102] with symptoms related to local tumor growth, including a new persistent cough or, in smokers with chronic bronchitis, a change in the characteristics and/or timing of a pre-existing cough. Other possible manifestations include chest pain, hemoptysis, recurrent bronchitis or pneumonia that persists over time, often due to tumor-induced obstruction of airway secretions leading to stasis and secondary infection.

Various syndromes may also arise from tumor invasion of adjacent structures. These include:

- **Claude Bernard-Horner syndrome**, characterized by the triad of pupillary miosis, enophthalmos, and ipsilateral eyelid ptosis, typically occurring with apical tumors infiltrating the cervical sympathetic chain.

- **Pancoast-Ciuffini syndrome**, seen when an apical tumor invades the brachial plexus, causing pain, impaired movement, and paresthesia in the ipsilateral neck, shoulder, and arm.
- **Superior vena cava syndrome**, where tumor expansion along the mediastinum compresses the superior vena cava, impeding venous return to the heart and causing edema of the face and shoulders ("cape-like" distribution).

Tumor involvement of the recurrent or phrenic nerve may lead to voice changes or diaphragmatic paralysis, respectively. Other systemic symptoms include shortness of breath, persistent fatigue, anorexia, unexplained weight loss, cachexia, and occasionally fever. When metastases are present, patients may also experience bone pain, jaundice, and cervical lymphadenopathy.

Paraneoplastic syndromes [103], particularly in **small-cell lung cancer (SCLC)**, may also prompt medical evaluation. These syndromes can occur at any disease stage and their severity is not necessarily correlated with the size of the primary tumor. In undiagnosed SCLC, the presence of paraneoplastic syndromes provides an opportunity for earlier diagnosis and treatment, potentially improving survival. The most common paraneoplastic manifestations involve the endocrine and nervous systems (Paraneoplastic Neurologic Syndromes, PNS), occurring in an estimated 3–5% of SCLC patients.

Endocrine syndromes result from ectopic production of biologically active hormones or peptides by the primary tumor, including:

- **Syndrome of inappropriate antidiuretic hormone secretion (SIADH)**, which may present with falls, headaches, nausea, fatigue, muscle cramps, seizures, tremors, and depressed mood.

- **Ectopic Cushing syndrome**, characterized by weight gain, hypertension, peripheral edema, and muscle weakness.

Neurologic syndromes usually have a subacute, progressive course, likely triggered by autoantibody production causing autoimmune phenomena. In SCLC, the most frequently diagnosed neurologic paraneoplastic syndrome is **Lambert-Eaton Myasthenic Syndrome (LEMS)**, presenting with lower-limb weakness, reduced tendon reflexes, and autonomic involvement such as constipation and xerostomia. Severe forms may also cause respiratory muscle weakness.

1.4.2. Instrumental investigations

According to the **AIOM guidelines** [8] and the **European Society for Medical Oncology (ESMO) guidelines** [104, 105], a structured radiologic workup is recommended following the suspicion of lung carcinoma.

A thorough **clinical history** is essential and should include:

- Smoking habits and/or exposure to passive smoke.
- Medical comorbidities.
- Occupational history, especially potential exposure to carcinogens.
- Family history of lung cancer or other respiratory diseases.
- Previous cancers or prior thoracic radiotherapy.
- History of tuberculosis or recurrent pneumonia.
- Weight loss, dietary habits, and alcohol consumption.

A complete **physical examination** is mandatory.

The **diagnostic and clinical staging phase** requires targeted use of imaging techniques and tissue sampling to obtain cytological or histological confirmation of the suspected lesion.

1. **Computed Tomography (CT)**
    - When a chest X-ray raises suspicion, a **contrast-enhanced CT of the thorax, upper abdomen, and lower cervical region** is recommended to assess potential involvement of other organs such as the liver, adrenal glands, and supraclavicular lymph nodes.
    - **Contrast-enhanced CT** is the imaging modality of choice for staging, providing information to define the T (tumor), N (node), and M (metastasis) parameters.
    - Features to evaluate include lesion size, shape, density, and growth over time. Larger lesions, irregular margins, and progressive growth are more likely to be malignant. Density patterns can be solid, ground-glass, or part-solid.
    - CT may produce **false positives**, e.g., due to bronchopneumonic processes or non-neoplastic lymphadenopathy (granulomatous reactions), requiring further assessment with **ultrasound, magnetic resonance imaging (MRI)**, or histological confirmation.

2. **Magnetic Resonance Imaging (MRI)**
    - Contrast-enhanced MRI is preferred when brain metastasis is suspected, either following a CT scan or in patients with neurological symptoms.

3. **PET-CT with 18F-FDG**
    - PET-CT has become pivotal for the **diagnostic workup of solitary pulmonary nodules**, preoperative staging, and post-treatment monitoring.
    - It identifies areas of high glucose metabolism, allowing biopsy targeting and improving diagnostic yield.
    - PET-CT improves staging accuracy over CT alone, particularly for **extra-thoracic and bone metastases**, and is useful for mediastinal lymph node assessment (N parameter).
    - PET-CT can yield **false positives**, so mediastinal lymph nodes that appear positive should be further evaluated with **invasive echo-endoscopic techniques** to avoid erroneously excluding patients from potentially curative surgery.
4. **Invasive Mediastinal Staging**
    - Techniques such as **endobronchial ultrasound (EBUS) with transbronchial needle aspiration** and, if negative, **mediastinoscopy**, are recommended even when CT and PET-CT are negative, particularly in:
        - Tumors > 3 cm.
        - Central tumors.
        - Tumors with ipsilateral hilar lymph node involvement.
    - Recent studies [106, 107] suggest that the prevalence of occult mediastinal metastases in patients with central primary tumors may be lower than previously assumed, potentially reducing the need for invasive mediastinal staging in selected cases with negative CT and PET-CT findings.

1.4.3. Histological typing of lung cancer

Over the past 15 years, the therapeutic landscape of lung cancer has undergone profound changes, increasingly highlighting the importance of a **multidisciplinary approach**. Currently, most treatment decisions depend not only on the definition of histological subtype but also, and especially, on the **molecular profile** of the tumor. This underscores the need, following a clinical diagnosis, for **adequate tissue sampling** to enable precise pathological and molecular characterization.

The **fifth edition of the World Health Organization (WHO) classification of thoracic tumors (2021)** [108] represents the reference standard for histopathological diagnosis. More than 95% of lung cancers fall into four main histological categories:

- **Non-small cell lung cancer (NSCLC)**: squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma.
- **Small cell lung cancer (SCLC)**: pulmonary small cell carcinoma.

In Western countries, **adenocarcinoma** has become the most frequent subtype, whereas **squamous cell carcinoma** and, particularly, **SCLC** have shown a progressive decline. The accurate distinction between **NSCLC** and **SCLC** is crucial, as it underpins the development and clinical application of **histology-driven therapeutic strategies** [8, 10]. According to **ESMO** [104, 105] and **AIOM** [8] guidelines, the choice of invasive procedure for histological typing depends on:

- the central vs peripheral location of the primary tumor,
- its growth pattern (endobronchial vs peribronchial),
- the presence of mediastinal and/or distant metastases.

Central Tumors

- Most central tumors can be sampled via **bronchoscopy**.
- **Endobronchial ultrasound (EBUS)** improves diagnostic yield by combining direct visualization of endobronchial lesions with guided sampling. It is particularly effective in large, centrally located tumors.

Peribronchial Tumors

- Tumors located near medium- to large-caliber airways but not directly involving the lumen can be sampled by:
    - **Transbronchial biopsy (TBB)**
    - **Transbronchial needle aspiration (TBNA)**
- EBUS, with its ultrasound probe, enhances the precision of biopsy and aspiration by allowing real-time needle guidance into the lesion.

Peripheral Tumors

- Diagnostic management is more complex. Options include:
    - **CT-guided transthoracic needle aspiration (TTNA)**
    - **Transthoracic biopsy**
- TTNA provides high diagnostic accuracy but carries an increased risk of pneumothorax, depending on needle size and lesion characteristics.

Sampling of Metastatic Lesions

In advanced disease, **CT and/or PET-CT** can reveal "superficial" metastases (e.g., chest wall lesions, supraclavicular lymph nodes, pleural effusions), which may be safely and effectively sampled under **external ultrasound guidance**.

Lymph Node Assessment

- **TBNA/TBB**, introduced in the 1980s, allows sampling of hilar and mediastinal lymph nodes adherent to the airways. Targetable stations include **4R, 4L, 7, 10R/L, and 11R/L**.
- This technique shows **high specificity** and **good sensitivity**, offering a less invasive but accurate alternative to mediastinoscopy.
- Sensitivity is influenced by lymph node size (lower for ≤1 cm), station involved (higher for 4R and 7), and operator expertise.
- **EBUS-TBNA** improves yield by allowing real-time ultrasound-guided sampling, with high sensitivity even for small nodes. However, it cannot access non-adjacent stations such as **8 (paraesophageal)** and **9 (pulmonary ligament)**, which require **endoscopic ultrasound (EUS)**.

In potentially operable patients with **cN0/N1 disease**, systematic exploration of mediastinal stations with **combined EBUS-TBNA and EUS** is recommended, reaching a sensitivity of 93% and a negative predictive value of 97% [109, 110].

Mediastinoscopy

- Allows direct evaluation of paratracheal, pretracheal, and precarinal nodes.
- Requires general anesthesia and short hospitalization.
- Its use has declined following the widespread adoption of EBUS-TBNA.
- Today, it is mainly indicated in **NSCLC with suspected N2–N3 involvement** when endoscopic procedures are unavailable or non-diagnostic [111].

Video-Assisted Thoracoscopic Surgery (VATS)

- VATS can be used for biopsies of mediastinal stations inaccessible by endoscopic means and for pleural biopsies when pleural metastases are suspected.

Pleural Effusion

- **Thoracentesis** may serve both diagnostic purposes—through cytological analysis of pleural fluid—and palliative purposes for symptom relief.

1.4.4. TNM classification

Lung cancer staging, based on the TNM system, is currently defined by the 9th Edition, as reported in the ESMO [104], [105] and AIOM [8] guidelines. This universally accepted system relies on three parameters: the anatomical extent of the primary tumor (T), the degree of lymph node involvement (N), and the presence of distant metastases (M).

The TNM system is routinely applied in clinical practice as it provides essential prognostic information and guides the selection of the most appropriate therapeutic strategy for each individual patient.

The TNM classification of lung cancer undergoes periodic revisions. In a very recent study published in March 2024 [112], the International Association for the Study of Lung Cancer (IASLC) compiled and analyzed a new database to update the forthcoming 9th Edition, which will become effective in January 2025 [8]. The main modifications concern the N and M descriptors, resulting in further expansion of clinical staging.

Specifically, new subcategories have been introduced for N2 (N2a: involvement of a single ipsilateral mediastinal or subcarinal lymph node station; N2b: involvement of multiple ipsilateral mediastinal lymph node stations, with or without subcarinal involvement) and for M1c (M1c1: multiple extrathoracic metastases confined to a single organ system; M1c2: multiple extrathoracic metastases affecting more than one organ system).

These refinements in the 9th Edition TNM classification aim to improve the granularity of disease extent nomenclature, thereby providing greater benefit in light of the increasing differentiation and complexity of modern therapeutic approaches [8], [112].

Figure 1: 9th TNM Lung Cancer edition (copied by DOI: 10.1016/j.chest.2024.05.026)

TABLE 3] Definitions for the T, N, and M Descriptors

T: Primary tumor	
T0	No evidence of primary tumor
Tis	Carcinoma in situ (squamous cell carcinoma or adenocarcinoma)
T1	Tumor surrounded by lung or visceral pleura, or in a lobar or more peripheral bronchus
T1mi	Minimally invasive adenocarcinoma[a]
T1a	Tumor ≤ 1 cm in greatest dimension[b]
T1b	Tumor > 1 cm but ≤ 2 cm in greatest dimension
T1c	Tumor > 2 cm but ≤ 3 cm in greatest dimension
T2	Tumor with any of the following features:
T2a	- Tumor > 3 cm but ≤ 4 cm in greatest dimension - Invades visceral pleura or invades an adjacent lobe - Involves main bronchus (not carina) or atelectasis/obstructive pneumonitis extending to the hilum[c]
T2b	Tumor > 4 cm but ≤ 5 cm in greatest dimension
T3	Tumor with any of the following features: - Tumor > 5 cm but ≤ 7 cm in greatest dimension - Invades parietal pleura or chest wall, thoracic nerve roots (eg, T1, T2), or stellate ganglion - Invades pericardium, phrenic nerve, or azygous vein - Separate tumor nodule(s) in the same lobe as the primary
T4	Tumor with any of the following features: - Tumor > 7 cm in greatest dimension - Invades vertebra, lamina, spinal canal, subclavian vessels, brachial plexus, or cervical nerve roots - Invades thymus, trachea, carina, recurrent laryngeal nerve, esophagus, or diaphragm - Invades heart or great vessels (aorta, superior/inferior vena cava, intrapericardial vessels) - Separate tumor nodule(s) in a different ipsilateral lobe than that of the primary
N: Regional lymph node involvement	
N0	No regional lymph node metastasis
N1	Metastasis(es) in ipsilateral pulmonary or hilar lymph nodes
N2	Metastasis(es) in ipsilateral mediastinal and/or subcarinal lymph node(s)
N2a	...involving a single ipsilateral mediastinal/subcarinal nodal station
N2b	...involving multiple ipsilateral/subcarinal mediastinal nodal stations
N3	Metastasis in supraclavicular or scalene node(s) or contralateral mediastinal/hilar node(s)
M: Distant metastasis	
M0	No distant metastasis
M1	Distant metastasis
M1a	Malignant pleural or pericardial effusion[d] or pleural/pericardial nodules Separate tumor nodule(s) in a contralateral lobe
M1b	Single extrathoracic metastasis[e]
M1c	Multiple extrathoracic metastases
M1c1	...involving a single organ system[f]
M1c2	...involving multiple organ systems

TX, NX = T or N status unable to be assessed. TX includes tumors proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy. (Reprinted with permission from Rami-Porta et al.[7])

[a]Solitary adenocarcinoma (≤ 3 cm), predominantly lepidic, and ≤ 5 mm invasion in any one focus.

[b]A superficial spreading tumor of any size with its invasive component limited to the bronchial wall, which may extend proximal to the main bronchus, is classified as T1a.

[c]Atelectasis/obstructive pneumonitis may involve part of or the entire lung.

[d]Pleural effusions are excluded that are cytologically negative and clinically judged not to be due to cancer (eg, transudative, nonbloody).

[e]This includes involvement of a single nonregional node.

[f]A diffuse organ system, such as the skeleton, is considered one organ (ie, metastases limited to several bones are classified as M1c1).[7]

Figure 2: Comparison od 8th and 9th stage groups for lung cancer (copied by DOI: 10.1016/j.chest.2024.05.026)

8th Edition TNM Categories

T/M	Label	N0	N1	N2	N3
T1	T1a	IA1	IIB	IIIA	IIIB
	T1b	IA2	IIB	IIIA	IIIB
	T1c	IA3	IIB	IIIA	IIIB
T2	T2a Inv	IB	IIB	IIIA	IIIB
	T2a >3-4	IB	IIB	IIIA	IIIB
	T2b >4-5	IIA	IIB	IIIA	IIIB
T3	T3 >5-7	IIB	IIIA	IIIB	IIIC
	T3 Inv	IIB	IIIA	IIIB	IIIC
	T3 Same Lobe Nod	IIB	IIIA	IIIB	IIIC
T4	T4 >7	IIIA	IIIA	IIIB	IIIC
	T4 Inv	IIIA	IIIA	IIIB	IIIC
	T4 Ipsi Nod	IIIA	IIIA	IIIB	IIIC
M1	M1a Pl Dissem	IVA	IVA	IVA	IVA
	M1a Contr Nod	IVA	IVA	IVA	IVA
	M1b Single Les	IVA	IVA	IVA	IVA
	M1c Mult Les	IVB	IVB	IVB	IVB

9th Edition TNM Categories

T/M	Description	N0	N1	N2		N3
				N2a	**N2b**	
T1	T1a ≤1 cm	IA1	IIA	IIB	IIIA	IIIB
	T1b >1 to ≤2 cm	IA2	IIA	IIB	IIIA	IIIB
	T1c >2 to ≤3 cm	IA3	IIA	IIB	IIIA	IIIB
T2	T2a Visceral pleura / central invasion	IB	IIB	IIIA	IIIB	IIIB
	T2a >3 to ≤4 cm	IB	IIB	IIIA	IIIB	IIIB
	T2b >4 to ≤5 cm	IIA	IIB	IIIA	IIIB	IIIB
T3	T3 >5 to ≤7 cm	IIB	IIIA	IIIA	IIIB	IIIC
	T3 Invasion	IIB	IIIA	IIIA	IIIB	IIIC
	T3 Same lobe tumor nodule	IIB	IIIA	IIIA	IIIB	IIIC
T4	T4 >7 cm	IIIA	IIIA	IIIB	IIIB	IIIC
	T4 Invasion	IIIA	IIIA	IIIB	IIIB	IIIC
	T4 Ipsilateral tumor nodule	IIIA	IIIA	IIIB	IIIB	IIIC
M1	M1a Pleural/pericardial dissemination	IVA	IVA	IVA	IVA	IVA
	M1a Contralateral tumor nodule	IVA	IVA	IVA	IVA	IVA
	M1b Single extrathoracic lesion	IVA	IVA	IVA	IVA	IVA
	M1c1 Multiple lesions, 1 organ system	IVB	IVB	IVB	IVB	IVB
	M1c2 Multiple lesions, >1 organ system	IVB	IVB	IVB	IVB	IVB

Figure 2 – *Comparison of eighth and ninth edition stage groups for lung cancer. New N and M categories are indicated in bold font; red outlines highlight the TNM combinations that are reassigned. Contr Nod = contralateral separate tumor nodule; Inv = invasion; Ipsi Nod = ipsilateral separate tumor nodule; Les = lesion (extrathoracic metastatic lesion); Mult = multiple; Pl Dissem = pleural or pericardial involvement; Same Lobe Nod = same lobe separate tumor nodule. (Reprinted with permission from Rami-Porta et al.[7])*

In light of the focus of this study, the discussion will henceforth concentrate on non–small cell lung cancer (NSCLC).

Small cell lung cancer (SCLC) warrants separate consideration. This histological subtype accounts for a minority of cases, its onset is strongly associated with tobacco exposure, and it is characterized by an extremely aggressive biological and clinical behavior.

For decades, no significant advances were achieved in its management; however, more recently, promising results have been reported, although the likelihood of achieving durable disease control remains limited [10].

1.5. NSCLC: Therapeutic Approaches for Localized and Locally Advanced Disease

1.5.1. Treatment of early-stage disease

According to the 2024 AIOM guidelines [8], the treatment of choice for patients with early-stage non-small cell lung cancer (NSCLC)—namely stage I, stage II, and selected cases of stage IIIA/IIIB—is radical surgery (Figure 1). This approach offers the potential for complete cure or, at minimum, a significant improvement in prognosis.

The radical intent is essential, since non-radical resections result in survival rates comparable to those of non-operated patients. However, surgery is not a viable option for individuals who, despite having limited lesions, present signs of advanced disease. Such procedures should be performed in high-volume thoracic surgery centers, where greater surgical expertise is associated with reduced postoperative mortality, both in the immediate postoperative period and in the long term [113–115].

Surgical eligibility is assessed according to three criteria:

1. **Biological operability** – the likelihood of achieving radicality based on disease stage.
2. **Anatomical operability** – the ability to perform the least extensive resection while still ensuring radicality.
3. **Functional operability** – the predicted postoperative respiratory capacity sufficient to maintain adequate lung function.

Based on these parameters, the following definitions apply:

- **Operable:** a patient with resectable disease and adequate physiological reserve to tolerate surgery;
- **Resectable:** disease that can be completely removed surgically;
- **Curative:** a procedure capable of achieving disease eradication.

Only resections with curative intent should be undertaken, characterized by:

1. Removal of the tumor with histologically confirmed negative margins;
2. Absence of residual neoplastic tissue at the resection edge;
3. Systematic dissection of loco-regional lymph nodes.

Perioperative complications are relatively frequent and may include atrial fibrillation or other arrhythmias, atelectasis, pulmonary infections, ARDS, respiratory failure, bronchopleural fistula, empyema, and pulmonary embolism. The postoperative mortality rate following lobectomy ranges from 2–5%, depending on the extent of resection, the patient's cardiopulmonary condition, and prior induction chemotherapy. Mortality is higher for pneumonectomy, particularly right pneumonectomy, which is associated with increased early and late morbidity and mortality compared to left pneumonectomy. Patient age appears to influence morbidity rather than mortality.

Prognosis for patients with early-stage disease undergoing radical resection is primarily determined by pathological staging, where tumor size (T factor) and involvement of hilar or mediastinal lymph nodes (N factor) are the most significant prognostic variables. Five-year overall survival (OS) ranges from 60–80% in stage I, 40–60% in stage II, and 20–40% in stage III patients [116]. Disease recurrence, when it occurs, is more frequently

local within the first two years post-surgery, while the incidence of distant relapse increases after the third year [117].

To mitigate the risk of recurrence, either local or distant, adjuvant therapy may be indicated in radically resected NSCLC. The AIOM 2024 guidelines [8] recommend cisplatin-based adjuvant chemotherapy for stage II–III patients younger than 75 years. For stage I, active surveillance is preferred, while evidence for patients over 75 years remains insufficient.

The role of tyrosine kinase inhibitors (TKIs) in the adjuvant setting has also been investigated. Notably, EGFR-targeted therapies may improve outcomes in early-stage resected NSCLC. In 2023, the *New England Journal of Medicine* [118] reported results from the phase III ADAURA trial [119], demonstrating a significant overall survival benefit with adjuvant osimertinib in patients with stage IB–IIIA EGFR-mutant NSCLC who had undergone resection, with or without prior adjuvant chemotherapy. Based on these findings, AIOM guidelines [8] recommend: *"In patients with resected stage IB–IIIA NSCLC harboring classic EGFR mutations (Del19/L858R), a 3-year course of osimertinib should be considered following platinum-based adjuvant chemotherapy (if indicated)."*

Similarly, regarding PD-1/PD-L1 inhibitors, given the improvement in both recurrence-free and overall survival compared with supportive care, the 2024 AIOM guidelines [8] state: *"In patients with resected stage II–IIIA NSCLC without EGFR/ALK alterations and with PD-L1 expression ≥50%, a 1-year course of atezolizumab should be considered following platinum-based adjuvant chemotherapy."* Conversely, adjuvant radiotherapy has been shown to worsen outcomes in radically resected stage I–II NSCLC, and therefore should not be considered postoperatively [8].

For patients with inoperable T1-2N0 NSCLC—most often due to significant comorbidities, advanced age, medical contraindications, or refusal of surgery—an alternative to surgical resection is radiotherapy, particularly stereotactic body radiation therapy (SBRT). According to the AIOM guidelines [8], SBRT should be considered the first-line treatment in this population. SBRT not only provides a therapeutic option for inoperable patients but also demonstrates superior outcomes compared to conventional radiotherapy, with high rates of local control and minimal toxicity [120–122].

1.5.2. Treatment of locally-advanced disease

According to the 2024 AIOM guidelines [8], locally advanced NSCLC should be categorized as resectable or unresectable (Figure 2). This classification depends on the extent of the primary tumor, the degree of nodal involvement, and the response to any induction therapy. All therapeutic strategies must be assessed within the context of an experienced multidisciplinary team, with the goal of identifying the most appropriate approach for each patient.

With regard to **resectable disease**, the guidelines [8] state that:

- In patients with resectable stage II–III NSCLC, platinum-based neoadjuvant chemotherapy followed by surgery may be considered as a first-line treatment option, depending on individual risk–benefit assessment.
- In patients with stage IIIA–IIIB disease, combined induction chemo-radiotherapy at radical doses followed by surgery is a valid therapeutic option and should always be evaluated within a multidisciplinary setting.

The rationale for neoadjuvant therapy lies in its potential to render unresectable tumors operable, or to reduce perioperative and postoperative risks in patients with resectable tumors. In particular, neoadjuvant chemo-radiotherapy aims to achieve clinical downstaging prior to surgery and, more importantly, to increase the likelihood of pathological response, including nodal clearance and pathological complete response.

If mediastinal lymph node involvement (pN2) is identified postoperatively, adjuvant radiotherapy (post-operative radiotherapy, PORT) should be considered, as it appears to improve prognosis in this subgroup of patients.

In contrast, for **unresectable disease**, the guidelines [8] specify that:

- In patients with unresectable stage IIIA/IIIB or stage IIIC NSCLC, multimodal therapy is indicated and must be assessed and agreed upon within a multidisciplinary context. Furthermore, in stage IIIB (N3) or stage IIIC disease, molecular characterization of the tumor is recommended as an initial step—analogous to stage IV—in order to assess eligibility for targeted therapies.
- In patients with unresectable stage IIIA/IIIB or IIIC NSCLC and good performance status, concurrent chemo-radiotherapy at radical doses should be considered the preferred first-line option over sequential treatment, given its demonstrated survival benefit. Specifically, platinum-based chemotherapy in combination with radiotherapy is recommended.
- In patients with unresectable stage III NSCLC who achieve response or stable disease following radical-dose concurrent chemo-radiotherapy, and whose tumors exhibit PD-L1 expression ≥1%, consolidation therapy with durvalumab for 12 months should be considered as the standard of care.

1.6. NSCLC: Advanced Stage Disease – Oncogene-Addicted and Non-Oncogene-Addicted

A significant proportion of patients with non–small cell lung cancer (NSCLC) are diagnosed at an advanced stage, defined as stage IIIB/IIIC not amenable to locoregional treatment, or stage IV.

These patients are not eligible for surgical intervention; consequently, the diagnostic process relies predominantly on small biopsies and/or cytological specimens, which enable a more detailed characterization of the neoplasm and allow for the adoption of specific therapeutic strategies.

Advances in imaging techniques, as previously discussed, now facilitate sample acquisition by guiding biopsy procedures, thereby providing tissue of sufficient quality and quantity for a comprehensive diagnosis, including both morphological/histological assessment and biomarker analysis [123].

Molecular investigations on tumor tissue aim to identify potential therapeutic targets for biologically tailored treatments—particularly in the setting of non-squamous histology—or for immunotherapy, thereby classifying the disease as **oncogene-addicted** or **non-oncogene-addicted** [8], [104]. This distinction is based on the presence or absence of oncogenic drivers, i.e., molecular alterations that, unlike so-called "passenger" mutations, are essential for tumor development and maintenance [124].

At present, treatment selection for patients with advanced-stage NSCLC relies on an integrated evaluation of multiple factors. These include tumor histology (squamous vs. non-squamous), the presence of actionable molecular alterations, PD-L1 expression levels, the number and distribution of metastatic sites (when present), as well as patient-related features such as age, performance status (PS), and comorbidities [8].

Accordingly, molecular testing should include evaluation of **EGFR, ALK, ROS1, BRAF, RET, HER2, KRAS, MET, NTRK**, and **PD-L1**. In the case of PD-L1, results not only indicate positivity or negativity but also quantify the percentage of expression, which is crucial for determining the most appropriate therapeutic strategy [125], [126]. Test results should then be discussed within a multidisciplinary tumor board to ensure comprehensive patient management, optimize the diagnostic–therapeutic pathway, and improve clinical outcomes [125].

Nonetheless, in real-world clinical practice, many patients with NSCLC do not undergo adequate molecular testing, leading to gaps in both diagnosis and treatment. Globally, the adoption of molecular testing for lung cancer remains limited, mainly due to high costs, turnaround times, and limited awareness [127]. Moreover, lower socioeconomic status appears to be associated with reduced access to molecular testing for targetable mutations, thereby restricting patient access to precision therapies [128].

1.6.1. Non-oncogene-addicted disease

In patients with advanced non-small cell lung cancer (NSCLC) lacking actionable molecular alterations suitable for targeted therapies, treatment selection is currently based primarily on tumor histology, patient clinical status and comorbidities, and the level of PD-L1 expression (Figure 3, [8]). PD-L1 is a biomarker assessed by immunohistochemistry, and its expression level predicts the likelihood of response to immune checkpoint inhibitors. These agents, targeting the PD-1/PD-L1 axis, enhance the patient's immune response against tumor cells by restoring T-cell effector function.

Specifically, they are monoclonal antibodies—such as nivolumab, atezolizumab, pembrolizumab, and durvalumab—that prevent inhibitory signaling in effector T cells, thereby sustaining their cytotoxic activity against tumor cells [129].

Higher levels of PD-L1 expression have been correlated with improved therapeutic outcomes; in particular, PD-L1 expression in at least 50% of tumor cells is considered the most clinically relevant predictor of efficacy for anti-PD-(L)1 antibodies [130], [131]. At present, immunotherapy is supported as a standard treatment for patients with advanced NSCLC, either as monotherapy or in combination with chemotherapy. This approach has demonstrated the potential to significantly improve outcomes, providing long-term survival in approximately one out of five treated patients [129]. Notably, patients with metastatic NSCLC without targetable mutations and with PD-L1 expression are those most likely to receive PD-1/PD-L1–directed therapy in the first-line setting [131].

The randomized, open-label, phase III KEYNOTE-024 trial [132] enrolled 305 patients with metastatic NSCLC, PD-L1 expression ≥ 50%, and no EGFR mutations or ALK rearrangements. Participants were randomized to pembrolizumab or platinum-based chemotherapy, with crossover to pembrolizumab permitted upon disease progression. At 5-year follow-up [133], pembrolizumab significantly prolonged median overall survival, with the greatest benefit observed in tumors exhibiting high PD-L1 expression. Another multicenter study [134] demonstrated a substantial survival benefit in NSCLC patients with PD-L1 expression ≥ 90% treated with first-line pembrolizumab. Following these and similar results, pembrolizumab received FDA approval for first-line treatment of tumors with PD-L1 ≥ 1%, and EMA approval for tumors with PD-L1 ≥ 50% [131].

Atezolizumab, based on the positive results of the phase III Impower110 trial [135], has also been approved by both the FDA and EMA as first-line monotherapy in metastatic NSCLC with high PD-L1 expression [131]. According to the 2024 AIOM guidelines [8],

for patients with metastatic NSCLC, PD-L1 ≥ 50%, absence of EGFR mutations or ALK rearrangements, and good performance status (0–1), first-line treatment with pembrolizumab or atezolizumab should be considered a preferred option over chemotherapy. Similarly, the phase III EMPOWER-Lung 1 trial [136] compared cemiplimab, a PD-1 inhibitor, with platinum-based chemotherapy in the first-line setting for metastatic NSCLC with PD-L1 ≥ 50% and no EGFR, ALK, or ROS1 alterations. Cemiplimab significantly improved both progression-free survival (PFS) and overall survival (OS) [131]. The drug has recently been approved by both the FDA and EMA for first-line use in patients with metastatic NSCLC and PD-L1 ≥ 50%, a recommendation also endorsed in the 2024 AIOM guidelines [8].

Importantly, anti-PD-(L)1 therapies can be safely and effectively combined with cytotoxic chemotherapy, a strategy that has now become standard practice for most patients with metastatic NSCLC (mNSCLC) [131]. The phase III KEYNOTE-189 trial [137] demonstrated that the addition of pembrolizumab to platinum-based chemotherapy improved both OS and PFS compared with chemotherapy alone [131]. The 2024 AIOM guidelines recommend that in patients with advanced squamous and non-squamous NSCLC, no EGFR or ALK alterations, PD-L1 < 50%, and good performance status (0–1), first-line chemo-immunotherapy followed by maintenance treatment upon response or stable disease should be considered a preferred option. Beyond chemo-immunotherapy, combinations of different immune checkpoint inhibitors, such as anti–PD-1/PD-L1 with anti–CTLA-4 antibodies—with or without short-term chemotherapy—have also shown meaningful improvements in both PFS and OS in mNSCLC [131].

More recently, immunotherapy has demonstrated efficacy in both neoadjuvant and adjuvant settings for patients with early-stage disease [125], [138]. Finally, according to Italian guidelines [8], in patients not selected by molecular characteristics or PD-L1

expression, cisplatin-based chemotherapy remains effective in prolonging OS compared with best supportive care. These findings highlight the critical importance of tailoring treatment strategies to the specific clinical and molecular profile of each patient.

1.6.2. Oncogene-addicted disease

Nowadays, the study of the molecular profile of disease is an integral part of the diagnostic and therapeutic pathway for a specific type of lung cancer, namely non-small cell lung cancer (NSCLC) [126]. As previously described (paragraph 1.4.3), lung cancer is histologically classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter is significantly more common and is further subdivided into squamous and non-squamous histotypes.

Squamous NSCLC is strongly associated with high-tar tobacco smoking and is rarely considered in the context of targeted therapy. Non-squamous NSCLC is the predominant histological subtype in Western and many Asian countries. This category primarily includes adenocarcinomas, which account for the majority of cases, and large cell carcinomas, which are relatively rare [126]. Indeed, it can be stated that approximately 85% of lung cancers are NSCLC, with adenocarcinoma as the predominant histological subtype [139]. Around 50–70% of Asian patients and 30–40% of non-Asian patients with non-squamous NSCLC harbor actionable mutations, and the use of appropriate targeted therapies significantly improves overall survival [126], [140]. Other studies [124], [139], [141] estimate that up to two-thirds of patients with advanced non-squamous NSCLC carry driver mutations, most of which (approximately 50%) currently have therapeutic relevance.

In recent years, significant advances have been made in molecular diagnostic techniques [142]. These developments have enabled the identification of essential biomarkers to be assessed in clinical practice and have defined the most appropriate diagnostic methodologies for their detection, thus allowing the adoption of specific targeted therapies recommended by national and international guidelines [8], [104]. A deeper understanding of the genomic landscape of NSCLC has revolutionized clinical management and led to the introduction of highly effective targeted treatment strategies for patients with advanced or metastatic disease, expanding therapeutic options and improving survival and safety compared to conventional chemotherapy [141].

EGFR-directed tyrosine kinase inhibitors (TKIs) were the first targeted therapies approved by the EMA in 2005 for NSCLC patients, followed by ALK-directed therapies in 2012, ROS1 in 2016, and BRAF p.V600E in 2017. These developments have dramatically shifted the lung cancer treatment paradigm toward rational selection of targeted therapies as standard of care [142].

According to the European Society for Medical Oncology (ESMO) guidelines updated in January 2023 [104], several important recommendations for clinical practice are highlighted, two of which are summarized below concerning the diagnosis and treatment of oncogene-addicted metastatic NSCLC:

1. An adequate quantity and quality of tissue should be obtained from the disease site to allow a correct histological and molecular diagnosis, enabling precise therapeutic decision-making.
2. Molecular testing is indicated for patients with advanced non-squamous NSCLC, with exceptions for particular cases such as young patients (<50 years), never-smokers (<100 cigarettes in their lifetime), light former smokers (≤15 pack-years), or long-term former smokers who quit more than 15 years ago.

The following section provides a detailed overview of each biomarker, including their frequency in the NSCLC population, the recommended molecular investigations for their identification, the epidemiological and clinical characteristics of patients associated with specific biomarkers, and a brief reference to currently approved targeted therapies.

a) **EGFR mutation:** The reported frequency of EGFR mutations in patients with pulmonary adenocarcinoma varies considerably, from 10–20% in European and North American populations to over 50% in Asian populations [126, 143–145]. These mutations are much more frequent in patients with adenocarcinoma, in non-smokers compared to smokers, and in women compared to men [125, 126, 146]. There are two main types of EGFR mutations, defined as common or "classical": in-frame deletions in exon 19 (ex19del) and the L858R substitution in exon 21, which account for 85–95% of all detectable and treatable mutations [8, 126]. Currently, there are first-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs), including gefitinib and erlotinib, second-generation TKIs such as afatinib, and third-generation TKIs such as osimertinib, which improve progression-free survival (PFS) compared to chemotherapy [147]. As is well known, tumors can acquire resistance mechanisms over time. In EGFR-mutated patients, the most common molecular mechanism of resistance to first- and second-generation TKIs is the EGFR T790M mutation, present in 50–60% of cases [125, 147]. Italian guidelines [8] recommend that:

    1. For patients with advanced NSCLC with classical EGFR mutations (Ex19dels, L858R) and confirmed T790M mutation (via tissue or liquid biopsy) at progression after gefitinib, erlotinib,

dacomitinib, or afatinib, osimertinib should be considered as the first option (over chemotherapy).

2. For patients with locally advanced or metastatic NSCLC with classical activating EGFR mutations (Ex19dels, L858R), first-line treatment with the EGFR-TKI osimertinib should be considered as the preferred option over gefitinib or erlotinib. Thus, osimertinib is recommended both in the case of resistance and progression after first- or second-generation TKIs and as first-line therapy for classical activating EGFR mutations. ESMO guidelines updated in January 2023 [104] suggest determining EGFR mutational status in advanced NSCLC, covering exons 18–21. If material is limited, identifying at least the most common mutations—exon 19 deletions and L858R in exon 21—is recommended. Since no PCR kits cover all known EGFR mutations responsive to TKIs, some potentially targetable mutations could be missed. To overcome this limitation, next-generation sequencing (NGS) panels are used, which are highly sensitive and capable of detecting all EGFR mutation types [126].

b) **ALK and ROS1 rearrangements:** ALK and ROS1 rearrangements occur in 5–8% and 1–2% of non-squamous NSCLC, respectively [126]. Like EGFR mutations, they are characteristic of non-smokers and females with adenocarcinoma; unlike EGFR, no pronounced racial differences exist. They tend to occur in young-onset NSCLC, whereas the incidence of L858R increases gradually with age [126, 139, 148]. Survival in advanced-stage patients with ALK or ROS1 rearrangements has

significantly improved thanks to specific TKIs [139, 148]. For ALK, crizotinib was the first approved TKI, followed by second-generation TKIs (ceritinib, alectinib, brigatinib) and third-generation lorlatinib [148]. For ROS1, crizotinib was the first approved TKI, followed by entrectinib (FDA 2019, EMA 2020) and repotrectinib (FDA-approved for naive and previously treated patients). In Italy [8], only crizotinib and entrectinib are approved and reimbursed for ROS1-positive NSCLC. Guidelines recommend specific molecular investigations for ALK and ROS1. FISH is complex and costly; thus, IHC can substitute FISH for ALK, while ROS1 IHC requires confirmation with an alternative method. PCR and NGS panels are also applicable [126].

c) **BRAF mutations:** Activating BRAF mutations are generally mutually exclusive with EGFR, ALK, and ROS1 alterations [104, 149]. They occur in ~2% of NSCLC, mainly adenocarcinomas [149, 150]. The BRAF V600E substitution, present in 50–60% of adenocarcinomas, predominates in female non-smokers and may confer higher tumor aggressiveness; other BRAF mutations are more common in males and smokers [125, 150]. ESMO guidelines recommend first-line dabrafenib-trametinib for advanced or metastatic NSCLC with BRAF V600 mutations [151]. NGS is the preferred method for detection [125].

d) **NTRK1/2/3 fusions:** These occur in <1% of NSCLC, mainly adenocarcinomas, with NTRK1 most commonly involved [125]. Despite no specific clinical or pathological predictors, testing is crucial as EMA- and FDA-approved therapies (larotrectinib, entrectinib) exist and can be

considered as first-line options in Italy, with NGS or IHC recommended [8, 125].

e) **MET alterations:** Oncogenic MET activation occurs via exon 14 skipping (2–4% of non-squamous NSCLC) or gene amplification (1–5%). Patients with MET exon 14 skipping are typically older, female (60–70%), and have a smoking history (54–64%) [8]. MET amplification is also a resistance mechanism in ~20% of EGFR-mutated cases [125, 126, 152]. Capmatinib and tepotinib are effective and safe in advanced NSCLC with MET exon 14 skipping after prior platinum-based chemotherapy or immunotherapy. FISH is preferred for amplification detection, while NGS panels with broad coverage are recommended for exon 14 mutations [125].

f) **RET fusions:** RET rearrangements occur in 1–2% of NSCLC, mainly adenocarcinomas [125]. They are more common in young, non-smoking females [126]. Selective RET inhibitors (selpercatinib, pralsetinib) show high response rates and are recommended by ESMO for RET-positive NSCLC, though EMA indications are limited to patients naïve to RET inhibitors. Italian guidelines recommend platinum-based chemotherapy as first-line; selpercatinib can be considered upon progression after chemotherapy, with NGS preferred, though FISH or PCR are also feasible [8, 125].

g) **KRAS mutations:** KRAS is the most frequently mutated oncogene, found in ~25% of NSCLC [125, 104], mainly in Caucasians, without sex preference, and primarily in former or current smokers [153]. Prevalence in Asian populations is ~10% [143]. Mutations occur across

adenocarcinoma subtypes and 5% of squamous tumors [125]. Codons 12, 13, and 61 are typically affected, with codon 12 mutations accounting for 80% of cases, including KRAS G12C (10–13%), G12V (5%), and G12D (4%) [154]. G12C and G12V are smoking-associated; G12D is more frequent in non-smokers [153]. KRAS testing is usually by PCR or NGS panels, with sotorasib recommended for KRAS G12C-positive patients after first-line therapy failure [8, 104].

h) **HER2 alterations:** HER2 overexpression, amplification, and exon 20 insertions occur in 3–38%, 3%, and 1–4% of NSCLC, respectively, mainly in adenocarcinomas of female non-smokers [125, 155]. NGS (DNA or RNA-based) is preferred over IHC or FISH [125]. Trastuzumab deruxtecan has shown efficacy post-first-line therapy but is FDA-approved only, not EMA [104]. Italian guidelines do not define a standard treatment [8].

Over the last 20 years, multiple studies have evaluated associations between smoking, sex, age, ethnicity, and clinical-radiological features with mutational profiles, enabling rapid selection of appropriate targeted therapy. Swiss cohort studies [146, 156] found that non-smokers with targetable mutations had significantly longer overall survival compared to patients without actionable mutations. EGFR mutations are more frequent in non-smoking females; KRAS in smokers; and ALK rearrangements in young non-smokers, often with mediastinal lymph node involvement even in small primary tumors [161].

Overall, NSCLC in never-smokers (LCNS) displays distinct epidemiologic, clinical, and molecular profiles compared to smoking-related NSCLC. Despite advances, there is still

no standardized protocol for predicting mutational status, causing variability and delays in diagnostics and treatment. Multidisciplinary research and evidence-based approaches are essential to ensure timely, precise, and targeted therapy, improving outcomes for these patients.

2. Materials and Methods

2.1 Study Design and Population

This observational prospective study included consecutive patients with newly diagnosed non–small cell lung cancer (NSCLC) who underwent diagnostic evaluation at our institution between January 2023 and June 2024. All patients provided written informed consent for the use of anonymized clinical and imaging data for research purposes. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Eligible patients were required to have a histologically confirmed diagnosis of NSCLC and to have undergone next-generation sequencing (NGS) of tumor tissue. Patients were excluded if tissue was insufficient for molecular profiling or if imaging studies were incomplete or non-retrievable. Clinical and demographic variables, including age, sex, smoking status, pack-years, and time-to-treatment (TTT), were recorded at baseline. Smoking status was categorized as never-smoker, former smoker, or current smoker according to standardized definitions.

2.2 Molecular Analysis

Tumor molecular profiling was performed on formalin-fixed, paraffin-embedded samples using a validated NGS panel covering the main actionable alterations in NSCLC, including *EGFR*, *KRAS*, *ALK*, *ROS1*, *RET*, *MET*, *HER2*, and *BRAF*. Mutations and gene rearrangements were identified following manufacturer instructions and reported according to international standards. Patients were classified as oncogene-addicted if at least one known actionable driver alteration was detected.

2.3 Pulmonary Function Testing

Pulmonary function tests (PFTs) were performed using standardized equipment and according to international guidelines. Forced expiratory volume in 1st second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and diffusion capacity for carbon monoxide (DLCO) were recorded as percent predicted values adjusted for age, sex, and height.

Because DLCO values were missing for a subset of patients, two approaches were implemented:

1. **Complete-case analysis**, excluding patients without DLCO values.
2. **Multiple imputation**, using a chained-equations strategy to estimate missing DLCO values based on available demographic, functional and imaging variables. The imputed dataset was used for sensitivity analyses and in the CART model incorporating DLCO.

2.4 Imaging Acquisition and PET/CT Analysis

All patients underwent diagnostic 18F-FDG PET/CT according to institutional protocols. Patients fasted for at least 6 hours prior to FDG injection, and serum glucose levels were checked before tracer administration. Scanning was performed approximately 60 minutes after injection on a dedicated PET/CT scanner.

SUVmax was measured by placing a standardized three-dimensional volume of interest (VOI) over the primary tumor. PET/CT images were reviewed by two experienced nuclear medicine physicians, and SUV values were extracted from the institutional PACS system.

CT imaging used for radiomics extraction was acquired during routine diagnostic work-up, using standard thoracic protocols with ≤1.5 mm slice thickness.

2.5 Radiomic Feature Extraction

Radiomic analysis was performed on the primary tumor using a semi-automated segmentation workflow. Tumors were manually delineated on contrast-enhanced CT scans by an experienced thoracic radiologist, with segmentation subsequently reviewed by a second reader to ensure consistency.

Images were resampled to isotropic voxels, and intensity values were normalized prior to feature extraction. Extracted features included first-order statistics, gray-level co-occurrence matrix (GLCM) features, gray-level run-length matrix (GLRLM) features, and Laplacian of Gaussian (LoG)-filtered texture features. After extraction, features were inspected for multicollinearity and reduced through variance and correlation filters. Logistic regression was used for univariate assessment, and selected features were incorporated into subsequent machine learning analyses.

2.6 Statistical Analysis

Continuous variables were compared using Student's t-test or Mann–Whitney U test depending on data distribution. Categorical variables were compared using χ^2 or Fisher's exact tests. Diagnostic performance metrics, including area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated for key variables such as DLCO and SUVmax.

2.7 Classification and Regression Tree (CART) Models

Two CART models were developed to explore the hierarchical interactions among clinical, functional and imaging predictors of oncogene addiction:

1. **CART Model 1 (Complete-case):** Built using only patients with complete DLCO, radiomic and PET data.
2. **CART Model 2 (DLCO-imputed):** Built using the imputed dataset in which all patients contributed a DLCO value.

Both trees were constructed using the Gini impurity index as the splitting criterion, with pruning optimized through 10-fold cross-validation to prevent overfitting. Performance metrics included overall accuracy, sensitivity, specificity, balanced accuracy and Cohen's Kappa.

2.8 Random Forest Analysis

A Random Forest classifier was developed to assess the combined predictive value of clinical, functional, metabolic and radiomic variables for identifying oncogene-addicted tumors. The model included the following predictors**:** SUVmax**,** DLCO (% predicted), smoking status**,** and two radiomic features **(**Uniformity and Entropy**)**. The Random Forest was implemented in R using the *randomForest* package, with 500 trees (ntree = 500) and mtry = 3, meaning three variables were randomly sampled as candidates at each split. Class imbalance was addressed implicitly through bootstrap aggregation (bagging), which preserves proportional representation in each bootstrap sample.

The dataset used for the model employed imputed DLCO values (train_imp), enabling all patients to contribute to the analysis. The classifier was trained on this dataset using default Gini impurity for split selection. Model performance was evaluated using the out-of-bag (OOB) error, confusion matrix, class-specific error rates, and variable importance metrics. The OOB error provides an internal cross-validation estimate, as approximately one-third of the samples are excluded from each bootstrap iteration and used to test each tree.

2.9 Statistical Framework

A multistep statistical strategy was used to explore the association between clinical, functional, metabolic and radiomic parameters and the presence of oncogene-addicted tumors. All analyses were performed in R using the *stats*, *MASS*, *pROC*, and *caret* packages.

2.9.1 Preliminary Analyses

To assess differences in diffusion capacity across smoking categories, a Kruskal–Wallis test was applied because DLCO values did not follow a normal distribution. Pairwise comparisons were explored using post-hoc nonparametric procedures when global significance was detected.

The association between radiomic features was evaluated through Pearson's product–moment correlation, specifically examining the relationship between Uniformity and Entropy, two key texture measures known to present conceptual interdependence. Correlation strength and confidence intervals were computed to assess collinearity prior to regression modelling.

2.9.2 Logistic Regression Modelling

A logistic regression model was constructed to identify independent predictors of oncogene addiction. The initial multivariable model included:

- **Smoking status** (categorical with never-smoker as reference; fumo1 and fumo2 indicating former and current smokers),
- **SUVmax**, and
- **Uniformity** (radiomic feature).

The model was estimated using maximum likelihood, and coefficients, standard errors, Wald z-statistics and p-values were obtained. The exponentiated coefficients were interpreted as odds ratios (ORs), and 95% confidence intervals were calculated.

2.9.3 Stepwise Model Selection

To obtain a parsimonious model, backward stepwise selection was applied using the *MASS* package (*stepAIC* function). Variables were sequentially removed based on Akaike Information Criterion (AIC), retaining only predictors that significantly contributed to model fit. The final selected model included:

- **Smoking status**, and
- **Uniformity**.

2.9.4 Model Performance Evaluation

Model performance was assessed through ROC curve analysis using *pROC*. The optimal probability threshold was derived from Youden's index (best_threshold = 0.5415201).

Using this threshold, model predictions were compared with molecular results to obtain a confusion matrix and performance indices:

- Accuracy and its 95% CI
- Sensitivity and specificity
- Positive predictive value (PPV) and negative predictive value (NPV)
- Cohen's Kappa
- Balanced accuracy
- McNemar's test for marginal homogeneity

These metrics provided an evaluation of the diagnostic utility of the final logistic regression model.

2.10 Software

All analyses were conducted in GraphPad Prism (GraphPad Software, Inc., Boston, MA, U.S.A.), R (version 4.5.2) and Rstudio.

3. Results

A total of 375 patients with newly diagnosed non–small cell lung cancer (NSCLC) were included in the analysis. All patients underwent next-generation sequencing (NGS) of tumor tissue, and complementary clinical, functional, metabolic and radiomic parameters were collected depending on availability. The dataset allowed a comprehensive evaluation of demographic factors, pulmonary function, tumor imaging features and PET-derived metabolic metrics in relation to oncogene-addicted versus non–oncogene-addicted disease.

3.1 Patient Characteristics

The distribution of key demographic variables differed substantially between the two molecular groups. Oncogene-addicted tumors occurred more frequently in women and in never-smokers, whereas non–oncogene-addicted tumors were predominantly found in men with significant smoking exposure. Although cumulative pack-years were markedly higher among non-oncogene–addicted patients, the proportion of current smokers and recent ex-smokers did not differ significantly. No significant differences in age, staging or time-to-treatment (TTT) were observed.

Table 1. Patients' demographics

	Oncogene addicted (N=267)	**Non oncogene addicted (N=108)**	**OR**	**IC (95%)**	**p**
Male (n, %)	155 (58,1%)	77 (71,3%)	0,557	0,343 – 0,910	**0,019**
Age (years)	68,5 ± 9,1	68,3 ± 8,3	-	-	0,809
Smoking history (n, %)	97 (36,3%)	71 (65,7%)	0,036	0,004 – 0,217	**<0,001**
Active smokers (n, %)	44 (45,4%)	40 (56,3%)	0,643	0,352 – 1,215	0,211
Former smokers <10 years (n, %)	14 (26,4%)	11 (35,5%)	1,532	0,564 – 3,979	0,460
P/Y (n)	39,0 ± 21,1	45,1 ± 23,7	-	-	0,153
Time to treatment (days)	22.8 ± 33.5	20.9 ± 15.1	-	-	0.333

Table 2. TNM stage distribution in oncogene- and non–oncogene-addicted NSCLC

	Oncogene addicted (N=267)	**Non oncogene addicted (N=108)**	**OR**	**CI (95%)**	**p**
Stage (n, %) **Stage I** **Stage II** **Stage III** **Stage IV**	 7 (2,6%) 14 (5,2%) 31 (11,6%) 202 (75,7%)	 5 (4,6%) 5 (4,6%) 20 (18,5%) 76 (70,4%)	-	-	0,194
T staging (n, %) **T1** **T2** **T3** **T4**	 44 (16,5%) 59 (22,1%) 43 (16,1%) 34 (12,7%)	 22 (20,4%) 19 (17,6%) 14 (13,0%) 20 (18,5%)	-	-	0,307
N staging (n, %) **N0** **N1** **N2** **N3**	 28 (10,5%) 9 (3,4%) 46 (17,2%) 74 (27,7%)	 13 (12,0%) 2 (1,9%) 15 (13,9%) 33 (30,6%)	-	-	0,916
M staging (n, %) **M0** **M1**	 54 (20,2%) 202 (75,7%)	 30 (27,8%) 76 (70,4%)	0,677	0,407 – 1,131	0,171

3.2 Molecular Findings

Among oncogene-addicted cases, KRAS mutations were the most common driver (48%), followed by EGFR mutations (23%). Together, these accounted for approximately 70% of all oncogene-driven tumors. The remaining 30% comprised less frequent alterations such as ALK, ROS1, RET, MET, HER2, and BRAF.

Table 3. Oncogene-addicted mutations distribution.

Non-Oncogene-Addicted	**108**	**29%**
Oncogene-Addicted	**267**	**71%**
KRAS	128	48%
EGFR	62	23%
ALK	17	6%
MET	15	6%
BRAF	14	5%
HER2	10	4%
RET	9	3%
ROS1	4	1%
Other	8	3%

3.3 Pulmonary Function Tests

Pulmonary function testing was available in 87 patients. DLCO (% predicted) was higher in oncogene-addicted patients (76% vs 66%; p = 0.009), suggesting better preserved alveolar–capillary membrane integrity. No significant differences were observed in FEV_1, FVC, or FEV_1/FVC.

In diagnostic analyses, DLCO <80% exhibited an AUC of 0.69, with sensitivity 93%, specificity 36%, and a negative predictive value of 88%, indicating that preserved DLCO strongly argued against non-oncogene–addicted disease. Because DLCO was missing for a subset of patients, imputation was performed to allow inclusion of DLCO in the multivariable tree-based models.

Table 4. Pulmonary Function Test results

	Oncogene addicted (N=45)	**Non oncogene addicted (N=34)**	**p**
Weight (kg)	67,8 ± 13,6	73,3 ±13,4	**0,017**
Height (cm)	167,1 ± 8,4	169,1 ± 9,3	0,190
BMI (kg/m²)	24,5 ± 4,1	25,7 ± 4,1	0,103
FVC (% pred.)	102,9 ± 25,1	100,0 ± 19,9	0,581
FEV1 (% pred.)	92,0 ± 28,5	86,7 ± 23,4	0,377
FEV1/FVC (%)	68,5 ± 10,9	66,2 ± 9,7	0,317
FEF 25-75 (% pred.)	73,0 ± 38,2	66,0 ± 32,8	0,397
DLCO (% pred.)	76,0 ± 15,6	66,0 ± 14,4	**0,009**

Figure 1. ROC curve for DLCO

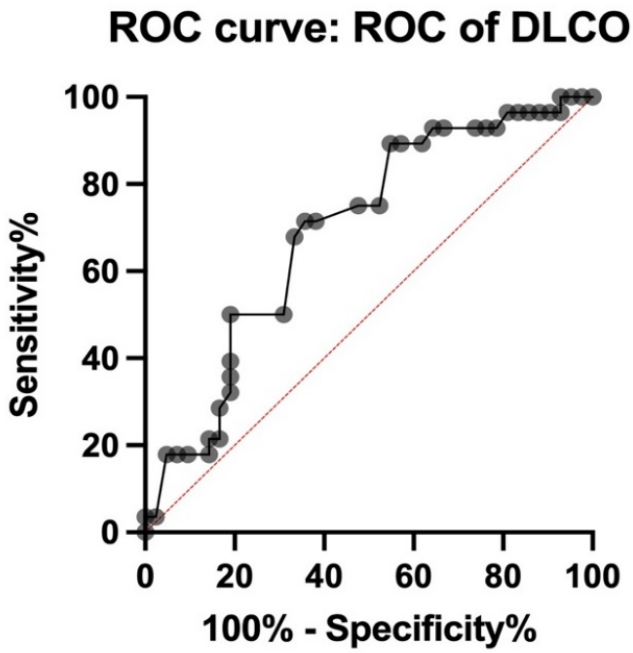


Table 5. ROC curve analysis.

ROC curve, cut-off DLCO <80%		
Area	0,692	
Standard error	0,064	
95% IC	0,567 - 0,817	
p	0,007	
	Value	**95% IC**
Sensitivity	0,929	0,774 - 0,987
Specificity	0,357	0,230 - 0,508
Positive predictive value	0,491	0,361 - 0,621
Negative predictive value	0,882	0,657 - 0,979
Likelihood ratio	1,444	-

3.4 Radiomic Features

Radiomics was available for 273 patients (197 oncogene-addicted, 76 non oncogene-addicted). Logistic regression identified three features significantly associated with oncogene addiction:

- Entropy, higher in non–oncogene-addicted tumors, indicating greater intratumoral heterogeneity.
- Uniformity, higher in oncogene-addicted tumors, consistent with more homogeneous growth patterns.
- LoG6_Range, lower in oncogene-addicted cases, again reflecting reduced textural variability.

These markers support known biological differences between smoking-related cancers and molecularly driven adenocarcinomas.

Table 6. Radiomic features.

Variable	**OR (IC95)**	**P value**
Mean density	1.003 (0.991 - 1.014)	0,6450
Entropy	2.022 (1.005 - 4.065)	0,0483
Interquartile Range	1.022 (0.999 - 1.046)	0,0625
Mean Absolute Deviation	1.034 (0.994 - 1.075)	0,0975
Robust Mean Absolute Deviation	1.053 (0.997 - 1.113)	0,0653
Uniformity	0.021 (0.001 - 0.626)	0,0259
Variance	1 (1 - 1.001)	0,2900
LoG6_Range	0.923 (0.853 - 0.999)	0,0464
LoG6_Variance	0.921 (0.841 - 1.01)	0,0799

3.5 PET/CT Metabolic Activity

PET/CT scan was available in 236 patients (160 oncogene-addicted, 76 non oncogene-addicted). PET-derived SUVmax also differed between groups. Non–oncogene-addicted tumors exhibited higher metabolic activity (SUVmax 13.2 vs 11.7; p = 0.027). Although SUVmax <3.98 demonstrated very high specificity (99%) and a PPV of 89% for oncogene addiction, sensitivity remained extremely low (8%), limiting its use as a screening tool. SUVmax therefore emerged as a potent but highly selective confirmatory indicator.

Figure 2. SUVmax distribution; 0: non oncogene-addicted, 1: oncogene-addicted

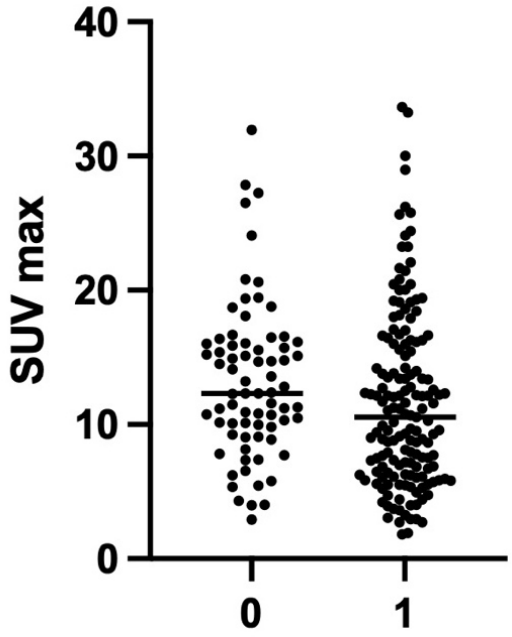


Figure 3. ROC curve for SUVmax

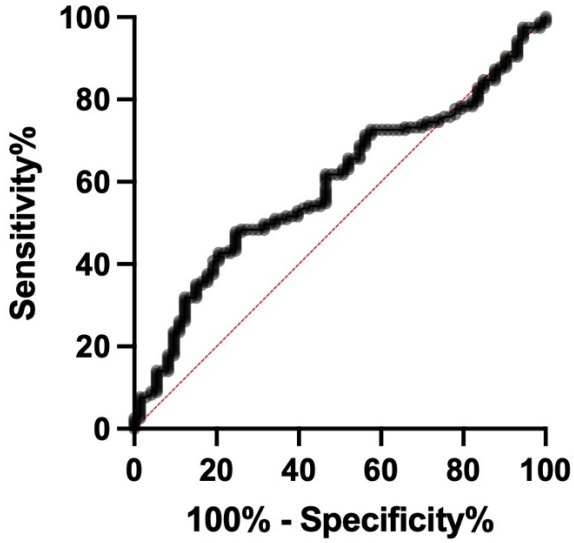


Table 7. ROC curve analysis

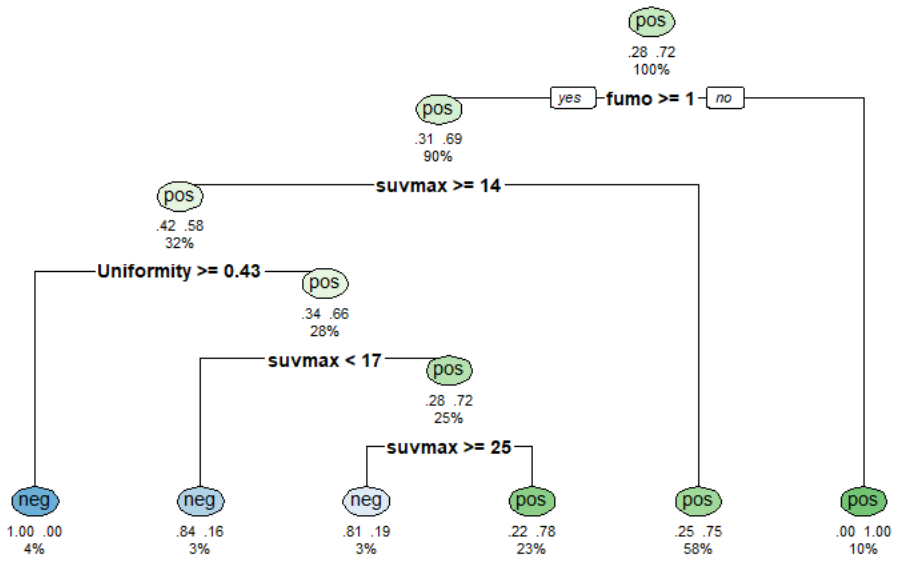
ROC curve, SUV max <3.98		
Area	0,591	
Standard error	0,038	
95% IC	0,516 - 0,666	
p	0,027	
	Value	**95% IC**
Sensitivity	0,076	0.044 – 0.128
Specificity	0.986	0.926 – 0.999
Positive predictive value	0.889	0.684 – 1.000
Negative predictive value	0.518	0.447 – 0.589
Likelihood ratio	5.580	

3.6 CART Model Without DLCO Imputation

The first CART model was generated using only complete-case data. The tree identified smoking status as the primary discriminant. Never-smokers were overwhelmingly classified as oncogene-addicted, reflecting strong epidemiological associations. Among smokers, sequential splits in SUVmax (14, 17, and 25) and Uniformity were used to refine classification, although the structure revealed substantial complexity in this subgroup.

Model performance was limited: accuracy was 65.9%, with very low sensitivity (16.7%) for non-oncogene–addicted disease and an overall balanced accuracy close to chance (51%). Cohen's Kappa was close to zero, indicating minimal agreement beyond chance. The model predominantly classified tumors as oncogene-addicted, reflecting class imbalance.

Figure 4. CART model without DLCO implementation

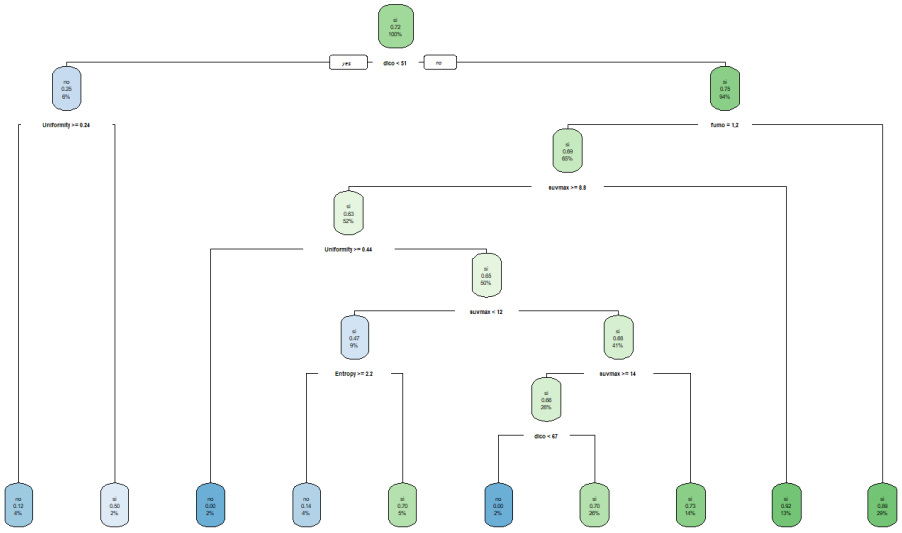


3.7 CART Model With Imputed DLCO

After imputation of missing DLCO values, a second CART model was developed. The inclusion of DLCO markedly altered the model structure: DLCO <51% became the root split, highlighting DLCO as the most informative variable when available for all patients. Among patients with DLCO ≥51%, smoking history again emerged as a major discriminator. In smokers, multiple imaging predictors shaped the decision process, beginning with SUVmax ≥8.8, and followed by a series of radiomic (Uniformity, Entropy) and metabolic thresholds (SUVmax 12 and 14), as well as an additional DLCO node (<67%).

Model performance showed modest improvement in classification of oncogene-addicted cases, with sensitivity rising to 86.4%, but specificity for non-oncogene–addicted tumors remained low (18.2%). Accuracy was 67.9% and balanced accuracy approximately 52%. The model remained biased toward oncogene-addicted predictions, though less severely than the non-imputed version.

Figure 5. CART model with imputed DLCO



3.8 Random Forest Model

The Random Forest classifier demonstrated improved performance relative to the decision tree models. The overall out-of-bag (OOB) error rate was 21.25%, corresponding to an approximate accuracy of 78.75%, indicating that the ensemble approach substantially increased predictive stability and reduced misclassification.

The confusion matrix showed that the model correctly identified the majority of oncogene-addicted tumors (172 correctly classified vs 25 misclassified), with a class error of 12.7% for the positive class. Performance for the non–oncogene-addicted class was more modest: 43 cases were correctly classified, while 33 were incorrectly labeled as oncogene-addicted, yielding a class error of 43.4% for the negative class.

These results indicate strong discriminative performance for oncogene-addicted tumors, but persistent difficulty in identifying non-oncogene–addicted disease, reflecting the underlying class imbalance of the dataset. Nevertheless, the Random Forest model achieved superior global performance and substantially more balanced predictive behavior compared with single-tree approaches.

Variable importance values (not shown in the summary but inherent to the model structure) further indicated that SUVmax, DLCO and smoking status were among the most influential predictors, with radiomic Uniformity and Entropy contributing additional but comparatively smaller information gains.

3.9 Preliminary Analyses

The Kruskal–Wallis test revealed a significant difference in DLCO across smoking groups ($\chi^2 = 7.59$, df = 2, $p = 0.022$). This indicates that gas transfer efficiency is not evenly distributed across smoking categories, supporting its biological relevance in the characterization of tumor phenotypes.

Correlation analysis demonstrated a very strong negative correlation between Uniformity and Entropy ($r = -0.963$, 95% CI: −0.971 to −0.953, $p < 2.2 \times 10^{-16}$). This high degree of collinearity suggests that these radiomic features capture highly overlapping information, an important consideration for multivariate modelling.

3.10 Logistic Regression Model

In the initial logistic regression including smoking status, SUVmax and Uniformity, smoking status emerged as a strong predictor of oncogene addiction. Former smokers (fumo1) and current smokers (fumo2) had significantly lower odds of harboring oncogene-addicted tumors compared with never-smokers:

- **fumo1:** OR = 0.049 (p = 0.0046)
- **fumo2:** OR = 0.085 (p = 0.0186)

Uniformity was also significantly associated with oncogene status (OR = 0.0007, p = 0.047), with lower values predicting a higher probability of non-oncogene-driven tumors. SUVmax did not reach statistical significance in this multivariable model (p = 0.17).

3.10.1 Stepwise-Selected Model

After backward elimination, the final model retained smoking status and Uniformity, both of which remained significant predictors:

- **fumo1:** OR = 0.049 (95% CI 0.0025–0.300, p = 0.0066)
- **fumo2:** OR = 0.085 (95% CI 0.0044–0.477, p = 0.0219)
- **Uniformity:** OR = 0.000676 (95% CI 6.4e-07–0.348, p = 0.0284)

The direction and magnitude of these associations indicate that both smoking exposure and radiomic texture homogeneity exert substantial influence on the probability of oncogene addiction.

3.10.2 Model Performance

Using the ROC-derived optimal threshold of 0.5415, the logistic regression model achieved:

- **Accuracy:** 0.717 (95% CI 0.614–0.806)
- **Sensitivity:** 0.7586
- **Specificity:** 0.6471
- **PPV:** 0.786
- **NPV:** 0.611
- **Balanced Accuracy:** 0.703
- **Kappa:** 0.4008 (moderate agreement)
- **Mcnemar's test:** p = 0.844 (no significant asymmetry)

These results demonstrate that the selected logistic regression model provides moderately strong discriminative ability, particularly for identifying oncogene-addicted tumors, with performance superior to chance and clinically meaningful levels of agreement.

4. Discussion

This study investigated whether a multimodal combination of demographic, functional, metabolic, and radiomic features could support a personalized diagnostic approach for predicting oncogene addiction in newly diagnosed non–small cell lung cancer (NSCLC). Across the full set of analyses (including classical statistics, logistic regression, radiomic modelling, CART decision trees, and Random Forest classification), a coherent narrative emerged: specific clinical and imaging-derived variables reflect underlying tumor biology and are informative for molecular stratification. However, their predictive capacity varies considerably by method, and no single feature offered sufficient diagnostic accuracy on its own. Instead, meaningful insights emerged when variables were analyzed in combination, emphasizing the value of multimodal integration.

4.1 Clinical and Functional Determinants

The epidemiological profile of oncogene-addicted NSCLC in the cohort closely mirrors prior literature: oncogene-driven tumors occurred more frequently in younger patients, females, and never-smokers, whereas non–oncogene-addicted tumors clustered among former and current smokers. The significance of smoking history was confirmed statistically and remained one of the strongest predictors across all models. In logistic regression, both former and current smokers showed markedly reduced odds of oncogene addiction compared with never-smokers, with effect sizes exceeding a tenfold difference. This strong clinical signal provides a robust contextual foundation on which imaging

biomarkers can build but also highlights the inherent limitations of epidemiological predictors, which lack the precision required for individual prognostication.

Pulmonary function data contributed further biological insight. DLCO values varied significantly across smoking categories, and reduced diffusion capacity was associated with non–oncogene-addicted tumors. Given that smoking-related emphysema and microvascular damage impair gas exchange and are strongly linked to complex mutational patterns, this association is physiologically consistent. In univariate analysis, DLCO <80% demonstrated moderate discrimination, and after imputation it became the principal discriminator in the CART model. This suggests that DLCO is a promising functional biomarker whose full potential could not be realized in the present study due to missing data. The strong influence of DLCO in the imputed dataset underscores the need for complete functional assessment in future studies.

4.2 Metabolic and Radiomic Biomarkers

PET-derived SUVmax values differed between molecular subgroups, with oncogene-addicted tumors showing lower metabolic activity on average. This finding aligns with published evidence demonstrating reduced FDG uptake in *EGFR*-mutated adenocarcinomas and other driver-positive lesions, possibly reflecting lower glycolytic dependency or distinct metabolic programming. SUVmax contributed significantly to both CART models and was consistently ranked among the most informative predictors in the Random Forest analysis.

Radiomic features provided complementary information by capturing spatial heterogeneity within the tumor. Uniformity and Entropy, two texture descriptors with strong inverse correlation, were both associated with oncogene status. Lower Uniformity

(reflecting greater heterogeneity) was associated with non–oncogene-addicted tumors, consistent with the greater genomic instability and architectural complexity typically observed in smoking-related carcinogenesis. Notably, Uniformity remained a significant predictor in the stepwise logistic regression model, highlighting its independent contribution beyond smoking status and SUVmax.

4.3 Performance of Predictive Models

The logistic regression model achieved moderate discriminative performance, with an accuracy of 0.72, balanced accuracy of 0.70, and a Kappa of 0.40. These values indicate a clinically meaningful but not definitive ability to distinguish oncogene-addicted from non–oncogene-addicted NSCLC. Smoking status and Uniformity were retained in the final model, confirming their relevance and suggesting a potential minimal predictive panel.

The CART models provided intuitive, interpretable visualizations of variable interactions, but exhibited limited predictive accuracy and poor class balance. The tree built without DLCO overpredicted oncogene addiction, whereas the imputed-DLCO tree overemphasized DLCO at the root node. These findings reflect the intrinsic limitations of single-tree classifiers, which are sensitive to data imbalance, missing values, and local optimization.

In contrast, the Random Forest classifier demonstrated substantially improved performance, with an out-of-bag error of 21.25% and an estimated accuracy approaching 79%. The model showed strong sensitivity for oncogene-addicted tumors, but continued difficulty in correctly identifying non–oncogene-addicted cases. This pattern is expected in imbalanced datasets and highlights the need for dedicated rebalancing techniques in

future modelling. Importantly, Random Forest results validated the relative importance of smoking status, DLCO, and SUVmax, with radiomic features contributing additional, though smaller, gains in predictive power.

4.4 Biological Interpretation and Multimodal Insight

Taken together, these findings support a biological model in which:

- **Smoking status** reflects long-term exposure driving mutational complexity and non-targetable tumorigenesis.
- **DLCO** captures the functional consequences of smoking-related parenchymal injury and thus serves as a proxy for the host–lung environment shaping tumor biology.
- **SUVmax** provides metabolic insight, with lower glycolytic activity suggesting oncogene addiction.
- **Radiomic texture** captures intratumoral heterogeneity, distinguishing oncogene-driven tumors from more chaotic, smoking-associated lesions.

Each modality provides a distinct but complementary lens on oncogene addiction. Their combined interpretation (rather than reliance on any single metric) appears most promising for future diagnostic frameworks.

4.5 Study Limitations

This study has several important limitations that must be acknowledged when interpreting the findings. First, the analysis was conducted in a single-center observational setting, which inherently restricts external validity. The diagnostic pathways, imaging protocols, patient demographics and referral patterns at our institution may not fully reflect those of other centers. As a result, both the distribution of molecular subtypes and the behavior of the predictive models may differ in more heterogeneous or geographically diverse populations. Multicenter validation would be necessary to confirm the generalizability of the identified associations.

A second major limitation is the disproportionately high prevalence of oncogene-addicted tumors within our cohort. This characteristic likely reflects a combination of institutional factors. Our center maintains strong expertise in the molecular characterization of thoracic malignancies and adheres to comprehensive NGS testing for all newly diagnosed NSCLC cases, which can increase the detection rate of actionable alterations compared with centers using more selective or stepwise testing strategies. In addition, the population served by our institution includes a substantial proportion of never-smokers and individuals with lower cumulative tobacco exposure, groups in which oncogene-driven tumors are known to be more frequent. These epidemiologic dynamics likely contributed to an overrepresentation of molecularly driven cancers relative to global NSCLC epidemiology.

This imbalance had methodological consequences: machine-learning models trained on datasets with a dominant class tend to develop a classification bias toward the majority category, as observed in the CART and Random Forest analyses. The models consistently showed strong sensitivity for oncogene-addicted tumors but struggled to correctly

identify non-oncogene–addicted cases, resulting in reduced specificity. Although ensemble methods partially mitigated this issue, the imbalance limits the discriminative performance and real-world applicability of the models. Future work should incorporate explicit class-balancing strategies, such as oversampling, synthetic data generation or cost-sensitive learning.

Additional limitations include the presence of missing DLCO values, which required multiple imputation. While imputation allows a more complete use of the dataset, it introduces assumptions that may not fully capture the underlying physiology, and it influenced the structure of the predictive models. Radiomic analysis was limited to a relatively small subgroup of patients and remains sensitive to variations in CT acquisition parameters and segmentation methods, despite efforts to maintain consistency. Finally, the study's observational design prevents causal inference; associations between biomarkers and molecular status should therefore be interpreted as indicative rather than determinative.

Taken together, these limitations highlight the need for larger, prospectively collected, multicenter datasets to validate the multimodal predictive approach proposed in this work. Such studies will be essential to evaluate the reproducibility, stability and clinical usefulness of the models before real-world implementation.

4.6 Overall Interpretation and Implications

Despite these limitations, the results present a compelling case for the integration of functional, metabolic, and radiomic biomarkers into a personalized pre-test probability approach for oncogene addiction. While current performance metrics do not yet reach clinical applicability, the Random Forest model demonstrates that multimodal integration yields meaningful improvements over traditional clinical assessment alone. Future research should focus on larger multicenter datasets, standardized imaging protocols, explicit class-balancing strategies, and advanced machine-learning methods. Such efforts could ultimately lead to predictive tools capable of guiding molecular testing strategies and reducing delays in initiating targeted therapy for NSCLC.

5. Conclusions

This thesis explored the potential of a multimodal diagnostic strategy, integrating clinical, functional, metabolic and radiomic features to predict oncogene addiction in non–small cell lung cancer (NSCLC). Across multiple analytical approaches, a consistent finding emerged: while no single biomarker provides sufficient discriminative accuracy on its own, the combined interpretation of these complementary domains offers meaningful insight into the underlying tumor biology and enriches the pre-test probability of actionable molecular alterations.

From a clinical standpoint, smoking status proved to be the strongest epidemiological predictor, with never-smokers showing a markedly higher likelihood of harboring oncogene-addicted tumors. Functional assessment of gas exchange through DLCO added significant biological depth, reflecting the impact of smoking-related parenchymal damage and reinforcing its potential role as an indirect biomarker of tumor phenotype. Metabolic imaging through SUVmax provided further differentiation, with oncogene-driven tumors displaying lower glycolytic activity. Radiomic texture features, particularly Uniformity and Entropy, contributed additional microarchitectural information, capturing the intratumoral heterogeneity that distinguishes smoking-associated carcinogenesis from more homogeneous oncogene-driven lesions.

Analytically, the results demonstrated that classical statistical modelling, decision trees and ensemble learning each contribute unique strengths. Logistic regression delivered transparent, interpretable associations between predictors and oncogene addiction; CART models offered intuitive visual pathways for understanding variable interactions; and Random Forests—by aggregating hundreds of decorrelated trees—achieved the strongest overall predictive performance. The consistency with which smoking status, DLCO,

SUVmax and radiomic uniformity appeared as influential variables across these methods underscores their collective potential for integrated predictive modelling.

However, the findings also highlight limitations that currently preclude clinical application. Sample size constraints, missing DLCO values, class imbalance and radiomic acquisition variability all influenced model performance. While the Random Forest achieved promising accuracy, specificity for the non–oncogene-addicted class remained suboptimal, and no model underwent external validation. These limitations emphasize that although multimodal prediction is a promising field, further methodological refinement and larger datasets are essential before translation into clinical workflows.

Future research should focus on expanding the dataset to include larger, multicenter cohorts with standardized imaging and comprehensive functional testing. Increasing cohort diversity will not only improve statistical power but also allow the development of more generalizable predictive models. Addressing class imbalance through oversampling, cost-sensitive algorithms or synthetic minority techniques (e.g., SMOTE) is critical to improving specificity for non–oncogene-addicted tumors.

Radiomics, while promising, requires further methodological rigor. Harmonization of CT acquisition parameters, adherence to IBSI standards, and use of repeatability studies will strengthen feature robustness. Integration of advanced radiomic techniques, such as deep learning–based embeddings or wavelet decomposition, may reveal patterns inaccessible through conventional handcrafted features.

A key translational step will be the prospective validation of predictive models within real-world diagnostic pathways. Embedding multimodal prediction into molecular testing algorithms could optimize turnaround times, prioritize patients for urgent NGS, and potentially reduce delays in initiating targeted therapy. Ultimately, the integration of

functional, metabolic and radiomic biomarkers into a clinically usable pre-test probability tool may enhance patient stratification and streamline precision oncology workflows.

In conclusion, this thesis demonstrates the feasibility and potential value of a multimodal approach for predicting oncogene addiction in NSCLC. While the current models are not yet ready for implementation, the conceptual framework laid here forms a strong foundation for future innovations. With larger harmonized datasets, refined machine-learning techniques and prospective validation, multimodal prediction has the potential to become an essential component of personalized lung cancer diagnostics, bridging the gap between radiology, functional assessment and molecular oncology.

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