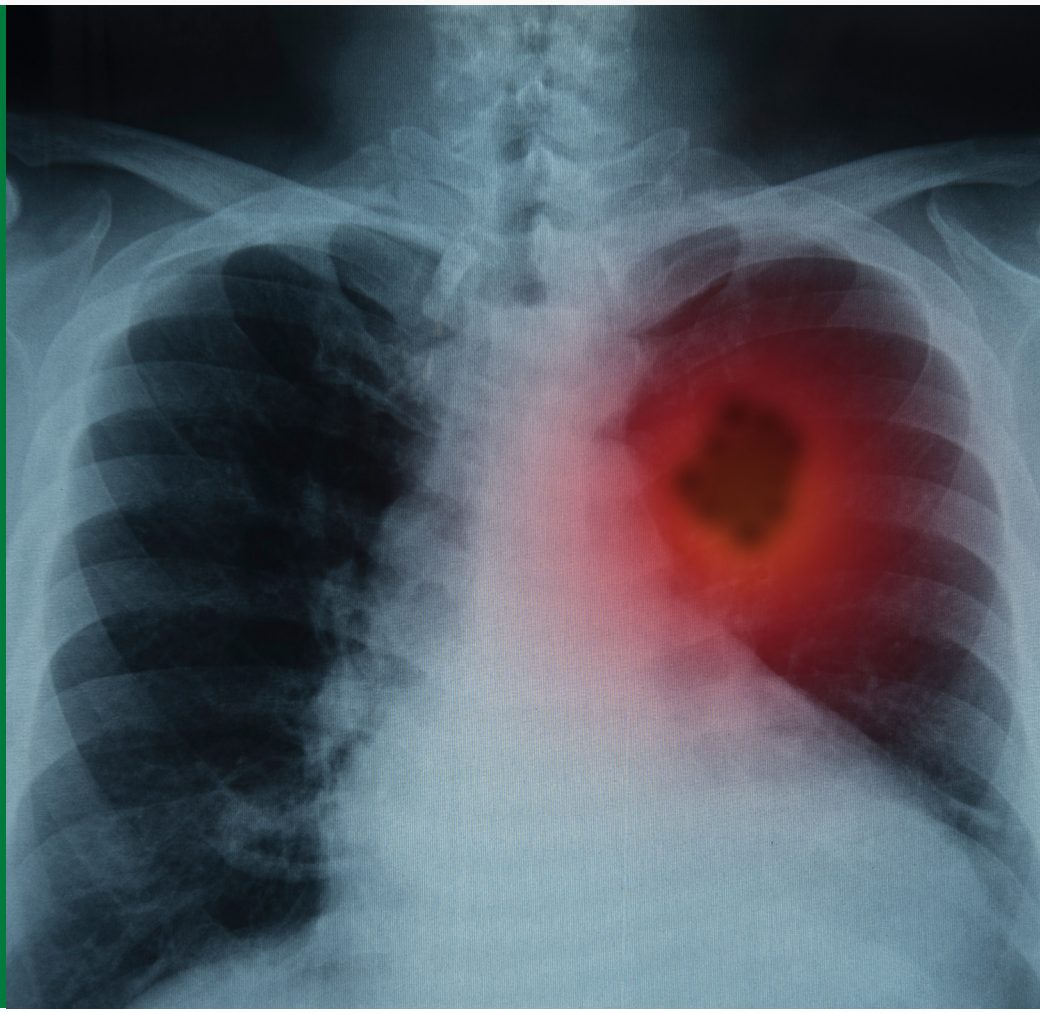
Pathology & Oncology Research

New Diagnostic and Therapeutic Possibilities in Lung Cancer



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Editorial: New diagnostic and therapeutic possibilities in lung cancer

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KEYWORDS

lung cancer, molecular pathology, targeted agenst, immunotherapy, irradiaditon

Editorial on the Special Issue
New diagnostic and therapeutic possibilities in lung cancer

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Traditionally, oncotherapy, which is a complex treatment option decided upon by multidisciplinary oncoteams, were a mix of surgery, systematic drugs, and irradiation. Over the last decades, systematic treatment has only involved cytotoxic drugs. In systematic treatment we used cytotoxic agents in different combinations (Cisplatin, Carboplatin, Paclitaxel, Docetaxel, Gemcitabine, and Vinorelbine). From 2000 there has been a paradigm shift in Oncology, with the results of clinical trials on VEGFR inhibitors (bevacizumab) influencing treatment choices. Later new agents, such as EGFR inhibitors, were identified as a possible treatment for Her-2 positive Breast Cancer. A big step in the treatment of Lung adenocarcinoma occurred in 2004 when EGFR mutations were identified in around 20% of Lung adenocarcinoma patients. Since then, an increasing number of first-, second-, and third-generation EGFR inhibitors have been identified with different inhibitions. Nowadays, we can treat Lung adenocarcinoma patients with the *de novo* or resistant T790 M mutation. Molecular pathology results are also rapidly changing the field of predictive biomarkers of lung adenocarcinomas. Many rare mutations have been identified in adenocarcinomas (ALK, KRAS, ROS-1, BRAF, MEK, and so on). We refer to these are rare mutations because the presence in the histology is not more than 1%–6%. The result of these targeted agents are very effective, with positive results found in clinical trials, and this is the reason why the involvement of driver mutations are referred to the Oncoteam before deciding upon first treatment recommendations.

As is known from previous oncotherapy guidelines, all cytotoxic combinations can be used in daily practice. This is why in this Lung Cancer Special Issue we are talking

about new possibilities. In Hungary we are very proud of the new HUNCEST screening program, which involves low-dose computerized tomography (CT), Molecular pathology results (driver mutations and liquid biopsy), and new irradiation possibilities (Chemo-irradiation, stereotaxic radiosurgery, and stereotaxic radiotherapy). Targeted therapies are used not only in metastatic settings but in early settings as well. The new stars in the field of systematic therapies are immunotherapies (PDL-1, PD1, PDL-1,2 and CTLA-4 inhibitors) in mono or in combinations. The clinical trial results are very promising, however for longer survival we should keep in mind the different side effects. Neuroendocrine carcinoma in the Lung is rare, so the article gives a new perspective for treating it.

We can explain the decreasing incidence and mortality of lung cancer is Hungary between 2011 and 2021, and we will present the results in this topic as well.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The authors declare that no Generative AI was used in the creation of this manuscript.

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The role of immunotherapy in early-stage and metastatic NSCLC

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In the past decade we have seen new advances and thus remarkable progress in the therapeutic options for non-small cell lung cancer (NSCLC). Among cytostatic therapies with new approaches in molecularly targeted therapies, we see new developments in a wide range of applications for immunotherapies. In this review we discuss the new potential modalities for the use of immune checkpoint inhibitors (ICIs) in the frontlines, including in early-stage (perioperative) and metastatic settings. The perioperative use of ICIs in both neoadjuvant and adjuvant settings may show benefits for patients. In early-stage NSCLC (from stage IIB and above) a multimodality approach is recommended as the gold standard for the treatment. After surgical resection platinum-based adjuvant chemotherapy has been the standard of care for many years. Based on the benefit of disease-free survival, the approval of adjuvant atezolizumab and adjuvant pembrolizumab was a significant breakthrough. In the metastatic setting, the use of immune checkpoint inhibitors with chemotherapy, regardless of PD-L1 expression or ICI alone (PD-L1 expression equal to or greater than 50%) also improves overall survival and progression-free survival.

KEYWORDS

NSCLC, PD-(L)1, ICI, perioperative, metastatic

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide, with more than 40% of patients diagnosed at stage IV [1]. The management approach for NSCLC primarily relies on the stage of the disease. However, advances in molecular pathology diagnostics, targeted therapy, and immunotherapy are expanding the range of treatment options and enhancing the prospects for improved survival rates. Immunotherapy has altered the treatment approach for several malignancies over the past 6–8 years, with NSCLC being among the most impacted. Tumor cells frequently reduce the expression of immune surveillance-related proteins, shielding them from the host's protective immune response [2]. Numerous approaches have been developed to boost the body's immune system in its fight against cancer cells by targeting pathways that suppress immune responses. In typical circumstances, activated T cells carry a receptor

called the programmed cell death-1 (PD-1) protein. PD-1 helps regulate immune responses to prevent them from becoming overly aggressive. Its counterpart, PD-L1, is found in both immune and tumor cells. The interplay between the PD-1/PD-L1 pathways plays a critical role in allowing tumors to evade the immune system. However, when this interaction is blocked, it reactivates T cell-mediated antitumor immunity, offering a survival advantage in various advanced and treatment-resistant cancers [3]. In total, seven immune checkpoint inhibitors (ICIs) have been approved in the United States (US) and Europe (EU) for the initial treatment of advanced or metastatic NSCLC. These ICIs include pembrolizumab, atezolizumab, cemiplimab, nivolumab + ipilimumab, and durvalumab + tremelimumab. Available treatment options include ICI monotherapy, combined ICI therapy, and ICI in conjunction with chemotherapy [4–16].

Perioperative treatment

Immunotherapy has become a very important part of the perioperative treatment of NSCLC. As shown in the following studies, the addition of ICI to perioperative chemotherapy treatment is very promising. Some studies tried the ICI + ICI combination, but because of its higher toxicity, this study was canceled [17]. Immunotherapy has become an increasingly important component of the perioperative treatment of NSCLC. The *National Comprehensive Cancer Network* (NCCN) has made its recommendations regarding the treatment with atezolizumab or pembrolizumab in the adjuvant setting, and nivolumab + chemotherapy or pembrolizumab + cisplatin doublet therapy and postoperative therapy in the neoadjuvant-adjuvant setting for specific patient populations with NSCLC [18].

Biomarkers

PD-L1 overexpression means a worse prognosis for the patients, namely, decreased disease-free survival (DFS) and overall survival (OS), which is clearly true in the cases of resected NSCLC tumors, so higher levels of PD-L1 indicate worse survival data. In the case of tumor mutation burden (TMB), the results are controversial as to whether they have a prognostic value for resected stage I-II NSCLC. Perioperative ctDNA analysis appears to be very useful in predicting event-free survival (EFS). Low preoperative and undetectable postoperative ctDNA levels mean better EFS [19]. From the perspective of response to therapy, in the IMpower010 trial in patients whose NSCLC tumor expressed PD-L1 more than 1%, better tumor regression was seen, but after deeper statistical analysis, it seems the high PD-L1 expressing (≥50%) group benefits the most in DFS. In contrast to the Keynote-091 study this benefit could not be demonstrated. A relatively new approach in the determination of minimal residual disease (MRD) is to use ctDNA analysis as another biomarker of perioperative ICI. After publications such as the results of the Mermaid studies we will see the place of this approach. Other potential future biomarkers such as blood TMB level, the ratio of lymphocytes that are infiltrating the tumor, and, e.g., KEAP1, STK11, and TP53 gene mutations may predict the benefit of ICI therapy [20].

Neoadjuvant treatment

Some trials have examined the efficacy of neoadjuvant cisplatin-based chemotherapy, showing an increase in overall survival (HR 0.84) [21]. Objectivizing the efficacy of neoadjuvant therapy is easier compared to adjuvant treatment because the pathologic response can be seen directly in the surgical specimen. A complete pathologic response (pCR) which means that no living tumor cells can be seen in the surgical specimen was found to be more beneficial for survival [22]. Major pathologic response (MPR) is an important parameter, which is defined as 10% or less of living tumor cells compared to necrosis and stromal cells in resected tissue [23]. This definition of MPR has been set to predict OS in prospective treatment [24]. In cases where neoadjuvant therapy was chosen, the proposed risk of delay or cancellation of surgery due to treatment-induced adverse events (AE) or disease progression was also considered. A few trials of neoadjuvant immunotherapy treatment are presented that may provide clarity in some of these situations (Table 1).

In the **Checkmate 816** trial the examined patients had stage IB-IIIA NSCLC (TNM 7th edition), no previous anticancer therapy, PD-L1 expression determined, ECOG 0-1, while EGFR and ALK alterations were excluded. In the experimental arm they received nivolumab + chemoterapy (3 cycles every 3 weeks) or chemotherapy alone in the control arm, equally distributed. In total, 83.2% of the experimental arm and 77.8% of the control group had R0 resection. Surgery was canceled only in 1.1% and 0.6% of patients due to adverse events. The follow-up period was not less than 21 months in this study. The median EFS was 31.6 months (statistically not reached) in the experimental arm and 20.8 months in the control arm. HR for recurrence, death or progression was 0.63. The greatest benefit in EFS was observed in stage IIIA, with PD-L1 expression of more than 1%, a non-squamous histological type and a carboplatin component in the treatment. PCR was 24% in the experimental arm and 2.2% in the control arm. The MPR was 36.9% in the experimental arm and 8.9% in the control arm. The statistically calculated median OS was not reached in either group (HR 0.57). After exploratory analysis, it became obvious that in patients with a complete pathologic response, the median EFS was significantly better in the experimental arm (26.6 vs. 18.4 months). The ctDNA clearance was higher in the experimental arm (56%) than in the control arm (35%), which correlated with the differences in

TABLE 1 Clinical trials of perioperative immunotherapy for NSCLC.

Trial	ICI agent	Stage	pCR (ICI arm)	MPR (ICI arm) (%)	G3 or higher TRAE (ICI arm) (%)	Rate of surgery (ICI arm)
Checkmate 816	nivolumab	IB-IIIA (TNM 7)	24%	36.9	33.5	83.2%
NADIM	nivolumab	IIIA	63.4%	82.9	13.5	90.2%
NADIM II	nivolumab	IIIA and IIIB	37%	54	19	93%
Checkmate77T	nivolumab	II-IIIB	25.3%	35.4	78	32%
Impower030	atezolizumab	II, IIIA, or select IIIB (T3N2)	only the study design was available			
Neotorch	toripalimab	II-III	24.8%	48.5	64.3	not known
Keynote-671	pembrolizumab	II, IIIA or IIIB (N2)	18.1%	30.2	44.9	81.2%
AEGEAN	durvalumab	IIA-IIIB (N2)	only the study design was available			

TRAE: treatment-related adverse event, ICI: immune checkpoint inhibitor, pCR: pathologic complete response, MPR: major pathologic response.

EFS between the two groups. The investigators also found a positive correlation between pCR and ctDNA clearance. Grade 3 or higher side effects were almost equal in the two groups (33.5% and 36.9%) [25, 26].

Neoadjuvant + adjuvant treatment

NADIM is a single-arm study that is now in phase II. Its aim was to measure the role of the ctDNA level in prognosis. The researchers enrolled stage IIIA NSCLC patients who were likely to have the potential for surgical removal of the tumor. The treatment was carboplatin doublet with paclitaxel plus nivolumab in the neoadjuvant setting, followed after surgery by nivolumab for 1 year (at a known dose). In total, 90.2% of the planned population underwent surgery. OS was 81.9% in the overall treated group and 91% in the nivolumab group at 36 months follow-up. A total of 63.4% of patients had pCR, including 82.9% of patients with MPR. The researchers found that neither TMB nor PD-L1 were independent predictors of long-term survival. Before treatment, low ctDNA levels were associated with longer progression-free survival (PFS) and OS (HR: 0.20) and zero ctDNA levels after the adjuvant treatment were associated with improved PFS and OS (HR: 0.26). Treatment-related adverse events (TRAEs) of grade 3 or higher were observed in 13.5% of patients [27].

NADIM II is a phase II trial that enrolled stage IIIA and IIIB NSCLC patients. They were randomly assigned in a 2:1 ratio to receive 3 cycles of nivolumab with paclitaxel + carboplatin (experimental arm), and after surgery (R0 resections) mono nivolumab 4 weekly for 6 months. The chemotherapy alone (3 cycles of paclitaxel + carboplatin) control group, before and after surgery received paclitaxel and carboplatin. PCR was 37% in the ICI arm and 7% without ICI treatment (this benefit was observed more in patients whose tumor expressed more than one percent PD-L1). The MPR was 57% vs. 14%. At 24 months PFS was 67.2% in the ICI arm and 40.9% in the only chemotherapy arm (HR 0.47). At the same follow-up time, OS was 85.0% in the ICI arm and 63.6% in the non-ICI arm (HR 0.43). In total, 93% of patients in the ICI arm and 69% of patients in the non-ICI group underwent surgery. One surgery was canceled because of ICI-related adverse events. Grade 3 or higher grade side effects were noted in 19% of patients. CtDNA analysis was also regularly performed in 66% of patients before and after neoadjuvant treatment. Pretreatment ctDNA levels were correlated with tumor size. After neoadjuvant treatment, ctDNA was negative in 67% of patients in the ICI arm and in 44% of patients in the non-ICI arm [28].

Checkmate 77T is a phase III trial that is currently in its interim analysis phase. The investigators are evaluating nivolumab with platinum-doublet chemotherapy (4 cycles) followed by surgery and nivolumab (1 year), or placebo with platinum-doublet chemotherapy (4 cycles) followed by surgery and placebo (1 year) in R0 resected stage II-IIIB, NSCLC, ECOG 0-1, EGFR/ALK wild-type, PD-L1 any expression patients. In this study, the follow-up time was no less than 15.7 months. At this time point, the median EFS in the ICI + chemotherapy + adjuvant group was observed. In the ICI arm, the median EFS is 28.9 months, compared to 18.4 months in the chemotherapy + placebo arm (HR: 0.58). PCR rates were improved as well (25.3% vs 4.7%), and MPR rates were higher in the ICI group (35.4% vs 12.1%). In total, 78% and 77% of patients in the two groups underwent definitive surgery and R0 resection was achieved in nearly 90% of cases. Grade 3 or higher adverse events were 32% and 25%, respectively [29].

Impower030 is a phase III trial in which the study population was potentially resectable stage II, IIIA, or select IIIB (T3N2) NSCLC patients with ECOG 0-1 performance status, EGFR wild-type, and without ALK translocation, but PD-L1 expression was not measured. The subjects received neoadjuvant atezolizumab

or placebo plus chemotherapy (platinum-doublet). After surgery patients in the experimental arm received atezolizumab treatment for 16 cycles or until recurrence or unacceptable toxicity, and patients in the control arm received the best supportive care and follow-up. The results of this study are not yet available [30].

Neotorch (phase III) enrolled patients with stage II/III, NSCLC, without EGFR or ALK alterations. Patients received 3 cycles of toripalimab or placebo with chemotherapy, and 13 cycles of toripalimab or placebo treatment after resection Q3W. While the trial is ongoing and EFS has not yet been reached in the ICI arm, it is 15.1 months in the control group (HR 0.40); the outcome is quite promising after 18 months of follow-up. The PCR was higher in the ICI group (24.8% vs. 1%), and MPR was also better in the toripalimab group (48.5%) versus 8.4% in the control group. AEs (grade 3 or higher) were almost equal, with 63.4% in the ICI group and 54.0% in the control group [31].

Keynote-671 is a phase III study in which only interim analysis is available at the moment. The trial enrolled patients eligible for R0 resection of stage II, IIIA or IIIB (N2) NSCLC. Patients received a total of 4 cycles of neoadjuvant pembrolizumab or placebo + cisplatin doublet therapy every 3 weeks and pembrolizumab or placebo (13 cycles) after surgery. With a median follow-up of 25.2 months EFS at 24 months was 62.4% in the ICI arm and 40.6% in the placebo group (HR 0.58). The calculated 24-month OS was 80.9% in the ICI arm and 77.6% in the control arm (not statistically significant). The PCR was 18.1% in the ICI arm and 4.0% in the control arm. MPR was 30.2% in the ICI arm and 11.0% in the control arm. In total, 81.2% of participants in the ICI arm and 79.4% of participants in the control arm underwent surgery. Grade 3 or higher toxicity was 44.9% in the ICI group and 37.3% in the control group. Toxicity that led to cancellation occurred in 12.6% of the patients in the ICI arm and 5.3% of the patients in the control group. Subgroup analysis showed that patients who are smokers, stage III have more benefit in EFS and in contrast to other trials nonsquamous phenotypes have benefitted in terms of EFS compared to squamous phenotypes. Every PD-L1 expression subgroup has benefitted in terms of EFS, but the biggest benefits were observed in the high (TPS>50%) expression group (HR: 0.42) [32].

AEGEAN will be a phase III trial, but only the study design has been published. Eligible patients are: no prior oncotherapy, candidates for complete resection, stage IIA to select (N2) IIIB NSCLC (according to TNM 8), without EGFR mutations or ALK rearrangement, with measured PD-L1 expression. Eligible participants will receive durvalumab or placebo on platinum doublet treatment and durvalumab or placebo after resection for 12 cycles. The primary endpoints are pCR and EFS [33].

The combination of IO + IO in the neoadjuvant setting is an exciting area of inquiry. It has greater immune activation and increased T-cell infiltration into the tumor tissue with ipilimumab + nivolumab therapy but surgical outcomes are not better. A study evaluating the effect of the neoadjuvant ipilimumab and nivolumab combination was stopped early (only 9 participants were selected) because of high rates of toxicity and progression, which canceled resection [17]. Despite a better response, the addition of ipilimumab seems to have a greater risk of serious adverse events leading to the cancellation of a potential complete resection of the tumor [34].

In the neoadjuvant setting there is a two-step approach, the only preoperative treatment as Checkmate816 and after neoadjuvant treatment and surgical resection with "adjuvant" immunotherapy treatment in various cycles. Assessing the efficacy of this treatment is straightforward since the pathological response can be objectively observed in surgical samples. The PCR rate is unexpectedly high after neoadjuvant chemo + ICI therapies (e.g., 18%, 24%, and 37%), and MPR (including pCR as well) is also very promising (37%, 57%, and even 82% were observed) compared to the chemotherapy-only group, where rates are around 2.2% and 4%. It is too early to draw conclusions from the survival data because of the short follow-up time, but the results that have already been presented are very promising. Another question is: were many surgeries canceled because of the high toxicity of this combination of neoadjuvant treatments? The answer is no. The Checkmate816 trial served as the prototype for the neoadjuvant ICI + chemo combination, in this trial, 1%–2% of patients were not operated on because of adverse events, and in NADIM II there are no patients in the same conditions. While in the majority of the trials stage IB-IIIA patients were enrolled, the greatest DFS survival benefit from ICI therapy was seen in stage IIIA patients with more than 1% PD-L1 expression, nonsquamous histology and those who received carboplatin treatment. Surprisingly, this same finding was not found in Keynote-671 in the nonsquamous histology type. The follow of the ctDNA level happened in the trials and they found positive correlation between complete ctDNA clearance and pCR. This approach is very promising and new in both the adjuvant and neoadjuvant settings as well.

Adjuvant therapy

Why is adjuvant immunotherapy useful?

Many trials have compared resection alone with surgery and adjuvant cisplatin-based doublet therapy. In a meta-analysis of adjuvant cisplatin-based doublet therapy, the addition of chemotherapy improved OS (HR 0.89) with a 5.4% risk of disease recurrence [35].

Despite the limited benefit of adjuvant therapy in overall survival (OS), it is administered when indicated. In such cases, the goal of adjuvant therapy is to eradicate potential micrometastases and prevent recurrence of lung cancer [20]. Given the very good results from ICI therapy in metastatic

NSCLC, the question arose as to whether we could achieve similarly good results with ICI therapy in the adjuvant setting. First, the background of the potential effectiveness and the possible targets of adjuvant ICI therapy were examined. It is known that cancer-related immune dysfunction can occur after surgical resection and may be a theoretical target of ICI, since the immune system reacts to surgery with various inflammatory responses and metabolic events [36, 37]. The surgical procedure itself, which includes trauma, blood loss, and hypothermia, may result in immunosuppression. More specifically, Th2 immunity increases in the postoperative period causing the release of growth factors and stress hormones [38]. These changes also lead to the expansion of myeloid-derived suppressor cells, M2 macrophages and T regulatory cells [36], which in turn will lead to suppression of the cellular immune system, resulting in higher expression of PD-L1 and CTLA-4. The altered PD-L1 and CTLA-4 expression appear to make adjuvant ICI treatment highly beneficial and effective in this setting [39]. The synergistic effect of combined ICI and chemotherapy is more effective in the destruction of MRD after surgical resection [40].

Clinical trials

Various immune checkpoint inhibitors have been and are still under examination to prove their effectiveness in the adjuvant setting. Some of these trials are presented below.

BR31/IFCT1401 is a phase III, double-blind trial. The investigators enrolled patients with completely removed stage IB-IIIA NSCLC (according to TNM 7th edition). This trial started in 2014 and is planned to finish in 2024 [41]. It is planned to enroll 1,415 patients and EGFR or ALK alterations are not part of the exclusion criteria. After R0 resection and completion of adjuvant chemotherapy, patients received durvalumab or placebo for 1 year. The primary endpoint was DFS for NSCLC participants with PD-L1 expression (greater than 25%) and without EGFR mutations or ALK rearrangements [20].

In **Impower010** 1,280 patients were enrolled in this phase III trial. Eligible patients for the study included those who had undergone R0 resection, and were in stage IB-IIIA according to the TNM 7 stage and ECOG 0-1. Participants were administered either 16 cycles of adjuvant atezolizumab or received best supportive care following cisplatin doublet chemotherapy (consisting of pemetrexed, docetaxel, vinorelbine, or gemcitabine) for 1-4 cycles. The median follow-up period was 32.8 months. Data processing focused on stage II-IIIA patients, where the primary endpoint was based on PD-L1 expression. Specifically, it looked at the difference in DFS between patients with PD-L1 expression greater than 1%. The analysis revealed a stratified HR for DFS of 0.66 when comparing the ICI group to the control group. In the overall study population (regardless of PD-L1 expression), the difference between the intent-to-treat group and the control group was observed with an HR of 0.79 for DFS. In the stage II–IIIA population with PD-L1 expression ≥1%, the 3-year DFS rates were 60% in the ICI group and 48% in the control group. In the overall population of stage II–IIIA participants, the 3-year DFS rates were 56% in the ICI group and 49% in the control group. The 5-year DFS rates could not be measured because this was an interim analysis. For the secondary endpoint of DFS in patients whose tumors had high PD-L1 expression (>50%), the unstratified HR was 0.43. In further exploratory analyses in stage II–IIIA participants whose tumors expressed 1%–49%, PD-L1 the unstratified HR was 0.87, and in patients with PD-L1<1%, the unstratified HR was 0.97. Unfortunately, OS data were immature in this analysis. Grade 3 or higher grade toxicity occurred in 22% of participants who received ICI and 12% in the control group. Looking at the risk of disease recurrence, new primary tumor appearance or death, it was reduced by 34% with ICI compared to the best supportive care in the PD-L1>1% expressing group and by 21% in the overall patient population. The DFS benefit with atezolizumab was of course highest in patients with tumors expressing PD-L1 >50%, but surprisingly a high DFS benefit could not be seen in the 1%–49% PD-L1 expression subgroup. As EGFR or ALK alterations were not exclusion criteria, it is an interesting question whether there is a difference in the DFS data in these patients. The data indicate that patients with driver mutations did not show a difference in DFS compared to patients without driver mutations. However, these findings should be interpreted with caution due to the low number of participants [42].

PEARLS/Keynote-091 is a triple-blind trial and has now reached its interim analysis. In total, 1,177 participants were enrolled with R0 resected NSCLC, stage IB-IIIA (according to TNM 7), ECOG 0-1 performance status, any verified PD-L1 expression level, and known EGFR and ALK alterations was not a requirement for inclusion. Patients who received adjuvant chemotherapy received ICI treatment within 3–12 weeks after the last dose of chemotherapy. The trial did not exclude patients who did not receive adjuvant chemotherapy previously; therefore, their ICI treatment began within 12 weeks after surgery. Eligible participants received pembrolizumab or placebo every 3 weeks until disease recurrence or intolerable adverse events (up to a maximum of 18 cycles). Crossover was not possible in this trial. Median DFS was 53.6 months in the ICI arm and 42.0 months in the control arm (HR 0.76). When examining the high PD-L1 expressing population, the median DFS was not statistically met in either the ICI arm or the control arm (HR 0.82). It was surprising that the benefit of DFS for ICI was not detected in the PD-L1 > 50% group which is likely due to the relative benefit of ICI treatment increasing with increasing PD-L1 expression in the setting of locally advanced or metastatic NSCLC. Despite this, median DFS in the ICI arm was numerically higher in the PD-L1 >50% population compared with the lower (1%–49% or <1%) PD-L1 expressed populations. What we did not expect was that median DFS in the control arm was also numerically improved in the PD-L1> 50% population compared with the lower (1%–49% and <1%) expressing

group. These statistical imbalances probably occurred due to the short follow-up period. As this was an interim analysis, the median OS data are immature (HR 0.87). Grade 3 or higher side effects occurred in 34% of patients in the ICI arm and 26% of patients in the placebo group [43].

ANVIL is an ongoing trial that started in May 2016 and is planned to be completed in July 2024. Unfortunately, only the study design is accessible. In total, 903 patients were enrolled with operated NSCLC (stage IB-IIIA, according to TNM7). Tumors with an EGFR mutation or ALK rearrangement were excluded. Adjuvant chemotherapy and radiotherapy were not mandatory. Randomized patients received adjuvant nivolumab or were observed for 1 year. The primary endpoints are DFS and OS. The secondary endpoint is the incidence of AEs and their severity [44].

Alchemist is a National Cancer Institute clinical trial platform for biomarker analysis of high-risk resected NSCLC that supports different randomized trials of new adjuvant therapies within the National Clinical Trials Network (NCTN). It includes a screening trial that enrolled participants with stage IB-IIIA (according to TNM7) who underwent R0 surgical resection, and had tissue and blood samples collected for analysis of EGFR and ALK alterations and PD-L1 expression. After the results patients were enrolled to receive adjuvant erlotinib, adjuvant crizotinib or adjuvant nivolumab after adjuvant chemotherapy [45].

ACCIO is a new three-arm trial in the Alchemist portfolio that started in June 2020. The study design is very interesting as it contains 3 arms (Arm A: 4 cycles of platinum doublet and observation, Arm B: 4 cycles of platinum doublet treatment + sequential pembrolizumab therapy for 16 cycles and Arm C: 4 cycles of platinum doublet chemotherapy + pembrolizumab with maintenance pembrolizumab of maximum 12 cycles) with or without postoperative radiotherapy (when needed). Stratification factors were NSCLC histologic type, any stratified PD-L1 expression, smoking habits and stage IB and II vs. IIIA. The primary endpoints are DFS and OS and this study design allowed for the secondary objective of comparing the primary DFS and OS endpoints between arms B and C in the overall population [46].

MERMAID-1 is an interesting phase III parallel-arm, placebo-controlled, double-blind, multicenter trial that was initiated in July 2020. Its estimated completion date is September 2026. Patient enrollment criteria are no EGFR or ALK alterations, stage II-III (according to TNM 8), R0 resection, ECOG 0-1, NSCLC histology, and stratified PD-L1 status. Minimal residual disease (MRD) status was determined by ctDNA analysis of blood samples collected 3–4 weeks after resection. Patients will be randomized 1:1 to get durvalumab or placebo, plus chemotherapy, for 12 weeks. Treatment continues with durvalumab or placebo, until week 48 or disease recurrence. It is very exciting that the primary endpoint (DFS), is determined by the measurement of MRD. Secondary endpoints will be: DFS, DFS in the minimal residual disease positive analysis set; and FAS (blinded independent central review) and OS in the MRD+ analysis set and FAS [47].

MERMAID-2 was launched in November 2020 and is planned to finish in October 2027. Enrolled patients were selected according to the MERMAID-1 requirements (R0 resected, stage II-III (TNM 8), no EGFR or ALK alterations). MRD is monitored by ctDNA levels from plasma samples and patients without visible recurrence but MRD+ with ctDNA levels are selected in this trial, so subjects with definitive therapy (R0 resection + optional neoadjuvant and/or adjuvant therapy) are elected in a 96- week follow-up phase, which means that patients will be examined regularly for MRD with ctDNA level measurement of blood samples. MRD-positive participants are evaluated with negative imaging (no visible tumor) and measured PD-L1 expression to determine eligibility for the trial. Eligible subjects receive durvalumab or placebo for up to 2 years or until disease recurrence. The primary endpoint is DFS in participants whose tumor expresses PD–L1 ≥1%. Secondary endpoints are DFS in the full analysis set, PFS, OS, quality of life questionnaires and rate of side effects [48].

Adjuvant-designed NADIM is an open-label trial that started in January 2021. Enrolled patients receive four cycles of chemotherapy + nivolumab and after 6 cycles of nivolumab or chemotherapy alone 4 cycles. The primary endpoint is DFS. Similar studies have been launched with toripalimab and canakinumab (interleukin-1β blocker) [20].

In the adjuvant setting, many trials are still ongoing; some have only reached the study design and interim analysis phase. The enrollment criteria are similar in many ways, but there are also some differences. In all trials patients were enrolled with completely resected NSCLC with stage II-IIIA (TNM 7 or 8). Impower010 and Keynote-091 did not exclude EGFR and ALK alterations. In Impower010 adjuvant cisplatin base doublet chemotherapy was mandatory while in Keynote-091 it was not, but previous radiotherapy or chemotherapy was prohibited [42, 43]. Despite the relatively immature data, each trial showed a significant EFS benefit compared to adjuvant chemotherapy. In Impower010 the greatest DFS benefit could be seen in the PD-L1 expression ≥50% group, but interestingly in Keynote-091 the PD-L1 ≥50% group did not show this benefit despite the results with pembrolizumab in metastatic disease [42, 43]. There were two interesting study designs that are very promising and may become the basis for future treatments. In ACCIO, the three-arm study design allows a head-to-head comparison of adjuvant chemotherapy versus adjuvant chemotherapy combined with ICI in both sequential and synchronous settings [46]. In Mermaid I and II the use of ctDNA measurement to determine the MRD before adjuvant treatment is quite exciting, but the results of the study are still ongoing [47, 48].

Advanced and metastatic stages

First line

In stage IV lung cancer, the advanced stage itself may be diagnosed primarily at metastatic sites. In practice, we are

TABLE 2 Clinical trials of first line immunotherapy in advanced and metastatic NSCLC.

Trial	ITT treatment regimen vs. cht	N (patients)	mOS (months)	mPFS (months)
Keynote 024	pembrolizumab	305	30 vs. 14.2 HR 0.63	10.3 vs. 6.0 HR: 0.50
EMPOWER-Lung 1	cemiplimab	712	26.1 vs. 13.3 HR 0.57	8.1 vs. 5.3 HR 0.51
IMpower 110	atezolizumab	572	20.2 vs. 13.1 HR 0,59	8.1 vs. 5.0 HR 0.63
Keynote-189 (nonsquamous)	pembrolizumab/pemetrexed/platinum	616	22.0 vs. 10.6 HR 0.49 (at 5 years)	8.8 vs. 4.9 HR 0.52
Keynote-407 (squamous)	pembroliumab/(nab)-paclitaxel/carboplatin	559	15.9 vs. 11.3 HR 0.64	6.4 vs. 4.8 HR0.56
EMPOWER-Lung-3 (squam + nonsqu.)	cemiplimab + cht	466	21.9 vs. 13.0 HR 0.71	8.2/5.5 HR 0.56
IMpower130	atezolizumab + nab-paclitaxel + carboplatin	723	18.6 vs. 13.9 HR 0.79	7.0/5.5 HR 0.64
IMpower150 (nonsquamous)	atezolizumab + carboplatin + paclitaxel + bevacizumab	1,202	19.2 vs. 14.7 HR 0.78	8.4 vs. 6.8 HR 0.57
CheckMate227	nivolumab + ipilimumab	1739	17.1 vs. 13.9 HR 0.79	7.2 vs. 5.5 HR 0.58
CheMate 9LA	nivolumab + ipilimumab + cht (2 cycles)	719	15.6 vs. 10.9 HR 0.66	6.8 vs. 5.0 HR 0.70

generally dealing with small samples. All patients diagnosed with stage IV NSCLC (nonsquamous, squamous) should be tested for driver mutations and for PD-L1 expression. When deciding on the treatment plan for a patient without an oncogene driver, several factors need to be considered. These factors include the histology, the tumor genotype, the level of PD-L1 expression, patient performance status (PS), any existing medical conditions (comorbidities), and the patient's own preferences [49]. ICIs that focus on either PD-1 or PD-L1 have been incorporated into the standard clinical strategy for the treatment of NSCLC. Key phase III studies (Table 2) evaluating various anti-PD-(L)-1 drugs, either alone or in combination with chemotherapy, have established ICI as the primary first-line therapy for metastatic NSCLC without targetable genetic mutations. Despite the progress made, there are unresolved challenges that include determining the best treatment regimen for individual patients. To date, there has been no direct comparison of different ICI-containing therapies in the first-line setting [49, 50].

Checkpoint inhibitor monotherapy

The standard of care for patients with squamous- and nonsquamous NSCLC, who also have a high PD-L1 expression, now involves the use of single-agent ICI. This is now the first-line therapy for patients with PD-L1 ≥50% with no contraindications for ICI. The significance of tumor mutational burden (in both blood and tissue samples) as a predictive indicator of response to cancer immunotherapy in metastatic NSCLC patients is still unclear. While the predictive utility of tumor mutational burden appears to be somewhat limited when it comes to patients receiving a combination of cancer immunotherapy and chemotherapy, recent data indicate that it may have a more meaningful predictive role in the context of immunotherapy alone, without the addition of chemotherapy [51]. If the choice is to use a single checkpoint inhibitor as a standalone treatment, either pembrolizumab, atezolizumab, or cemiplimab can be considered suitable options. The first evidence of enhanced survival outcomes emerged from investigations of NSCLC patients who had already experienced disease progression following platinum-based chemotherapy. This benefit was later extended to the frontline treatment of metastatic disease, whether used as a standalone therapy or in conjunction with chemotherapy. Additionally, this was also observed to be beneficial for patients with locally advanced unresectable disease [52]. A randomized trial has not directly compared the combination of a checkpoint inhibitor and chemotherapy with the use of a checkpoint inhibitor alone in individuals with high PD-L1 levels in NSCLC. Pembrolizumab, cemiplimab and nivolumab are anti-PD1 monoclonal antibodies, while atezolizumab and durvalumab are anti-PD-L1 monoclonal antibodies [18, 53]. To utilize pembrolizumab or cemiplimab as monotherapies in the initial treatment stage, it is necessary to have PD-L1 expression of more than 50%, which means that at least 50% of a minimum of 100 tumor cells (TCs) should show membrane expression of PD-L1. On the other hand, for nivolumab plus ipilimumab in the first line (although not approved by the European Medicines Agency), or pembrolizumab in the second line, a minimum of 1% PD-L1 expression on TCs is required [49].

Pembrolizumab

The KEYNOTE-024 trial demonstrated that pembrolizumab, compared to platinum-based chemotherapy, significantly

extended median PFS in previously untreated NSCLC patients with a PD-L1 tumor proportion score of at least 50% and no EGFR or ALK genetic alterations (10.3 vs. 6.0 HR 0.50). Importantly, the pembrolizumab group displayed an undefined median duration of response, suggesting the potential for long-lasting benefit. Additionally, the incidence of grade 3–5 TRAEs was less frequent in the pembrolizumab-treated patients. This study confirms the efficacy of pembrolizumab in the treatment of advanced NSCLC with high PD-L1 expression, highlighting its potential to provide better outcomes and improved tolerability compared to conventional chemotherapy [4]. Based on the 5-year median OS for pembrolizumab compared to chemotherapy (26.3 months vs. 13.4 months) and its 5-year OS rate (31.9% vs. 16.3%) the US Food and Drug Administration (FDA) approved the utilization of pembrolizumab as an initial treatment option in patients with advanced NSCLC [54, 55]. The KEYNOTE-598 study concludes that incorporating ipilimumab with pembrolizumab does not enhance efficacy and is linked to higher levels of adverse effects compared to using pembrolizumab alone in this group of patients (Grade 3 or higher TRAEs: 62.4% in pembrolizumab-ipilimumab recipients versus 50.2% in pembrolizumab-placebo recipients). Therefore, the findings do not support the use of the pembrolizumab-ipilimumab combination over the use of pembrolizumab alone in this context [56].

Cemiplimab

Cemiplimab demonstrated enhanced OS and PFS in comparison to chemotherapy. The EMPOWER-Lung 1 trial compared the use of cemiplimab alone to the choice of chemotherapy made by the investigators in patients who were newly diagnosed with advanced NSCLC and tumor PD-L1 expression of at least 50%, along with no EGFR mutations or ALK or ROS1 fusions. This study involved 712 participants, 85% of whom were men. The cemiplimab group showed significantly longer median OS (26.1 months vs. 13.3 months) and PFS (8.1 months vs. 5.3 months) compared to the chemotherapy group (HR 0.57 and HR 0.51, respectively). Regarding adverse events, grade 3 or higher TRAEs were less prevalent in the cemiplimab group (18%) in comparison to the chemotherapy group (40%). In conclusion, this study supports the use of cemiplimab as a first-line monotherapy in patients with advanced NSCLC who have a high level of PD-L1 expression. Interestingly, combining chemotherapy with cemiplimab at disease progression showed significant clinical benefit, suggesting a potential novel treatment approach for these patients [11, 57].

Atezolizumab

When considering atezolizumab as a first-line monotherapy treatment, criteria include PD-L1 expression of at least 50% on TCs or at least 10% on tumor-infiltrating immune cells [49]. The FDA approval of atezolizumab was based primarily on the outcomes of the IMpower 110 study. This research aimed to assess the efficacy and safety of atezolizumab compared to platinum-based chemotherapy. It was conducted as an initial treatment for patients with mNSCLC who exhibit PD-L1 expression. During the study 572 treatment-naïve patients were enrolled with metastatic nonsquamous or squamous NSCLC. In the high PD-L1 expression subgroup (205 patients), atezolizumab demonstrated a 7.1-month longer median overall survival (20.2 months vs. 13.1 months; HR for death 0.59) and an 8.1-month longer median PFS compared to chemotherapy (HR 0.63). Notably, grade 3 or higher TRAEs occurred in approximately 30% of atezolizumab patients and 53% of chemotherapy patients. These findings suggest that atezolizumab may be a more effective treatment option compared to traditional chemotherapy for these patients with NSCLC, regardless of histologic type [8]. The outcomes in terms of overall survival based on the degree of PD-L1 expression were consistent with those observed in the KEYNOTE-042 study comparing pembrolizumab to chemotherapy [5]. The main goal of the research was to evaluate the impact of OS on various population subgroups categorized by their PD-L1 expression levels. The study found varying degrees of OS benefit in these subgroups: those with PD-L1 >50%, PD-L1 >20%, and PD-L1 >1%. Notably, no significant PFS improvement was observed in patients with PD-L1 expression between 1% and 49% (HR: 0.92), leading to the approval of pembrolizumab monotherapy for subjects with PD-L1 expression above 50%. In both trials, individuals with high PD-L1 expression experienced the greatest benefit in terms of survival [5].

Nivolumab, durvalumab

Unfortunately, in addition to the remarkable results described above, we also find studies that did not show promising results. The CheckMate026 study conducted a comparison between nivolumab and platinum-based chemotherapy in patients diagnosed with stage IV NSCLC who had PD-L1 expression levels greater than 5% in their tumor cells and did not have EGFR- or ALK-activating mutations. The results in this instance were unfavorable. Nivolumab had a shorter median PFS (4.2 months vs. 5.9 months for CT, HR: 1.15), similar OS (14.4 months vs. 13.2 months for CT, HR: 1.02), but significantly fewer severe adverse effects (17.6% vs. 50.6% for CT) [58]. Another negative result was seen in the MYSTIC trial which sought to evaluate the efficacy of durvalumab (anti- PD-L1), either alone or in combination with tremelimumab, compared to chemotherapy as the initial treatment in treatment-naive metastatic patients. The MYSTIC study did not meet its primary objective of showing

a significant improvement in OS with durvalumab over chemotherapy, although durvalumab patients had a median OS of 16.3 months compared to 12.9 months for chemotherapy. Additionally, there was no statistically significant difference observed in PFS, which was a secondary endpoint of the study [59].

Checkpoint inhibitors + chemotherapy

In accordance with international treatment recommendations for lung cancer, the standard approach for patients with PD-L1 expression levels below 50% involves using a combination of chemotherapy and immunotherapy. This combination has been established as the preferred treatment based on the positive outcomes observed in phase III clinical trials, specifically in terms of improved survival rates, response rates, and the duration of the response [60]. The common approach for the treatment of newly diagnosed stage IV NSCLC is to combine platinum-based chemotherapy with PD-(L)1 inhibition, irrespective of tumor PD-L1 status and in the absence of any contraindication to ICI [49]. Each combination presents its own balance of efficacy and safety, offering valuable options for first-line treatment in this patient population.

Pembrolizumab + chemotherapy

In the KEYNOTE-189 trial involving newly diagnosed metastatic nonsquamous NSCLC patients without EGFR/ALK mutations, chemoimmunotherapy with pembrolizumab, pemetrexed, and platinum-based chemotherapy resulted in a lower risk of death (HR: 0.60), improved 5-year survival (19.4% vs. 11.3%), and better disease control (HR: 0.50, 5-year PFS: 7.5% vs. 0.6%) compared to the placebo group. Interestingly, these benefits remained consistent across various levels of PD-L1 expression in tumor cells. In conclusion, the 5-year results from the KEYNOTE-189 study strongly support the use of pembrolizumab in conjunction with pemetrexed and platinum-based chemotherapy as the standard therapy in previously untreated nonsquamous mNSCLC patients, providing substantial and long-lasting enhancements in overall and progression-free survival [6, 61]. In the KEYNOTE-407 study on untreated metastatic squamous NSCLC patients, 559 participants were randomly divided into two groups. One group received pembrolizumab with chemotherapy (paclitaxel/nab-paclitaxel + carboplatin), while the other received a placebo with chemotherapy. After 56.9 months, the pembrolizumab group showed significant improvements in both OS and PFS. Notably, the 5-year OS rate was 18.4% with pembrolizumab, nearly double that of the placebo group at 9.7%. The study reported manageable toxicity levels with a 3-year OS rate of 69.5%. Pembrolizumab with chemotherapy is now the standard first-line treatment for untreated metastatic squamous NSCLC, regardless of PD-L1 expression, showcasing significant survival benefits and becoming the preferred first-line option [7, 62].

Cemiplimab + chemotherapy

Cemiplimab, an anti PD-1 inhibitor, was studied in the EMPOWER-Lung 3 trial in patients with advanced mNSCLC without EGFR, ALK, or ROS1 abnormalities. A total of 466 patients were randomly assigned (2:1 ratio) to receive cemiplimab with specific platinum-doublet chemotherapy or a placebo with chemotherapy. After 28.4 months, the cemiplimab group had a median OS of 21.1 months, compared to 12.9 months in the chemotherapy-only group (HR 0.65), with a median PFS of 8.2 months for cemiplimab vs. 5.5 months for chemotherapy alone (HR 0.55). Overall, the combination of cemiplimab with chemotherapy improved OS, PFS, and overall response rate (ORR) in patients with advanced NSCLC, irrespective of histological subtype and PD-L1 expression levels, but with a higher incidence of TRAEs [63]. In the KEYNOTE-407 study, the median OS in patients with mNSCLC treated with pembrolizumab plus chemotherapy was 17.2 months. These data were derived from a median follow-up period of 40.1 and 56.9 months [64]. In the 2-year analysis of the EMPOWER-Lung 3 studies [63], the HR for overall survival OS in patients with squamous NSCLC was 0.61, while in the final analysis of KEYNOTE-407, the HR for OS in patients with metastatic squamous NSCLC was 0.71 [65]. Cemiplimab stands out as the second PD-(L)1 inhibitor that has demonstrated efficacy in advanced NSCLC, either on its own or in combination with chemotherapy, regardless of whether the cancer is of squamous or nonsquamous histology [12].

Atezolizumab plus chemotherapy ± bevacizumab

In addition to pembrolizumab and cemiplimab, there are other approved treatment options available. One such alternative is the combination of atezolizumab with carboplatin and nab-paclitaxel chemotherapy for mNSCLC lung cancer. This approach was studied in the IMpower130 trial, which aimed to assess the efficacy and safety of this combination compared to chemotherapy alone as a first-line treatment. The study involved 724 patients. The co-primary goals focused on assessing PFS and OS in the intention-to-treat population that lacked EGFR or ALK mutations. The trial ran from 2015 to February 13, 2017. The results showed significant improvements in both OS (18.6 vs. 13.9 months; HR 0.79) and PFS (7.0 vs. 5.5 months; HR 0.64) with atezolizumab plus chemotherapy compared to chemotherapy alone. The most common grade 3 or higher

TRAEs were myelosuppression-related events. These findings suggest that the combination of atezolizumab with carboplatin and nab-paclitaxel chemotherapy may be a valuable first-line treatment option for patients with mNSCLC lung cancer, and it was generally well tolerated [9]. The IMpower150 trial assessed the combination of atezolizumab with bevacizumab and chemotherapy as a first-line treatment for mNSCLC patients, including those with varying levels of PD-L1 expression and previous EGFR or ALK alterations. After 1,202 patients were enrolled, they were divided into three groups: ACP (atezolizumab-carboplatin-paclitaxel), ABCP (atezolizumab-bevacizumab-carboplatin-paclitaxel), and BCP (bevacizumab-carboplatin-paclitaxel). Results revealed a median OS of 19.0 months for ACP vs. 14.7 months for BCP (HR 0.84), with ABCP also showing a longer OS (19.5 months) compared to BCP (HR 0.80). Exploratory analyses suggested a longer OS with ACP and ABCP in PD-L1–high and PD-L1–positive subgroups, while PD-L1–negative subgroups had similar OS. The safety profile remained consistent. While ACP showed a numerical OS improvement over BCP, it was not statistically significant. However, with additional follow-up data, further OS improvement was observed with ABCP. This study supports the combination of immunotherapy, chemotherapy, and angiogenesis inhibitors like bevacizumab as an effective treatment for certain lung cancers, with FDA approval as an alternative option for advanced nonsquamous NSCLC patients without driver mutations [66]. In addition to FDA approval this first-line treatment option is also approved by the EMA [48]. The advantage of ABCP over BCP in terms of PFS was evident even in patients who had liver metastases at the beginning of the study. In the KEYNOTE-189 trial, patients with liver metastases had positive outcomes with pembrolizumab and chemotherapy, suggesting that this combination may also be a viable treatment option for this subset [67, 68].

Dual ICI

Anti-PD1 + anti-CTLA-4

Among the use of a combination chemotherapy and immunotherapy, researchers have investigated the efficacy of using anti-PD-1 and anti-CTLA-4 in different situations related to NSCLC [69]. First, researchers examined anti-PD-1/PD-L1 and anti-CTLA-4 combinations in metastatic melanoma, and found that these combinations generated enduring positive responses, irrespective of PD-L1 expression levels [70]. Researchers in the CheckMate227 trial observed a significant improvement in OS across all levels of PD-L1 expression, including patients with less than 1% expression. This study was a groundbreaking phase III trial evaluating the efficacy of combining nivolumab (anti-PD1) and ipilimumab (anti-CTLA-4) in the treatment of advanced NSCLC. The trial included a total of 1739 patients regardless of their PD-L1 expression. In patients with PD-L1 expression ≥1%, nivolumab plus ipilimumab resulted in a median OS of 17.1 months, a higher objective response rate of 35.9%, and a significantly longer duration of response (median 23.2 months) compared to chemotherapy (OS: 14.9 months, response rate: 30.0%, duration: 6.2 months). In terms of TRAEs, 32.8% of patients experienced grade 3 or 4 events with nivolumab plus ipilimumab, while 36.0% of patients had these events with chemotherapy. Discontinuation due to TRAEs was more common with dual immunotherapy (18%) compared to chemotherapy (9%). The most common immun-related adverse effects observed in individuals receiving nivolumab plus ipilimumab were skin-related issues (occurring in 34% of cases) and endocrine events (experienced by 24% of patients). This study supports the use of dual immunotherapy as a highly effective first-line treatment for advanced NSCLC, offering a substantial improvement in OS and response duration compared to traditional chemotherapy, irrespective of the patient's PD-L1 expression level [12]. In the CheckMate-227 study, the 5-year overall survival rate for patients with advanced NSCLC with PD-L1 ≥1% was 24% when treated with the nivolumab-ipilimumab combination, as opposed to 14% for those receiving chemotherapy alone. The use of dual ICI demonstrated enhanced OS in both histologic subcategories, with a greater advantage in squamous compared to nonsquamous. Moreover, within the squamous subtype, the benefit was more pronounced in patients with PD-L1 expression less than 1% than for those with PD-L1 expression greater than or equal to 1% in lung cancer [71].

Dual ICI + Cht

Anti-PD-(L)1 + anti-CTLA-4 + chemotherapy

Building on the findings of CheckMate227, researchers in the CheckMate-9LA trial found that adding a short course of two cycles of platinum-doublet chemotherapy to the combination of nivolumab and ipilimumab had a significant impact on overall survival compared to using chemotherapy alone. This combination also showed a favorable risk-benefit profile. Results showed a median OS of 15.6 months with nivolumab/ipilimumab/chemotherapy, compared to 10.9 months in the control group, representing a significant OS improvement (HR 0.66 in favor of the experimental group). Moreover, there were improvements in PFS (6.8 months vs. 5.0 months in the control group, HR 0.70) and a higher overall response rate (38% vs. 25% in the control group) in the experimental group. It is worth noting that the most common grade 3–4 TRAEs included neutropenia (7% vs. 9%) and anemia (6% vs. 14%) [14]. At three-year follow-up, the combination of nivolumab, ipilimumab, and two cycles of chemotherapy maintained significant OS benefit (mOS 15.8 months vs. 11.0 months, HR 0.7) compared to chemotherapy alone in the intent-to-treat population.

Additionally, the three-year overall survival rate (3-year OS) was notably higher in the nivolumab-ipilimumab group (27% vs. 19%). In patients with baseline brain metastases, nivolumab-ipilimumab showed impressive efficacy, including a median OS of 19.3 months (HR 0.45), significantly improved systemic PFS (9.7 vs. 4.1 months, HR 0.44), and substantial intracranial PFS benefit (11.4 vs. 4.6 months, HR 0.42). These results indicate the efficacy of combination therapy in patients with pretreated baseline brain metastases [72]. The POSEIDON trial investigated the combination of tremelimumab (anti-CTLA-4) with durvalumab (anti-PD-L1) and chemotherapy (T + D + CT) and durvalumab with chemotherapy (D + CT) versus chemotherapy alone (CT) as the initial treatment for mNSCLC in patients with EGFR/ALK wild-type tumors. Results showed that D + CT significantly improved PFS over CT alone (HR 0.74; median PFS 5.5 vs. 4.8 months), while T + D + CT significantly enhanced both PFS (HR 0.72; median PFS 6.2 vs. 4.8 months) and OS (HR 0.77; median OS 14.0 vs. 11.7 months) compared to CT alone. However, the improvement in OS for D + CT versus CT did not reach statistical significance (HR 0.86; median OS 13.3 vs. 11.7 months). The 24-month OS rates were also significantly higher with T + D + CT (32.9% vs. 22.1%). TRAEs of maximum grade 3-4 were observed in 51.8% for T + D + CT, 44.6% for D + CT, and 44.4% for CT.

In summary, the combination of durvalumab with chemotherapy enhanced PFS compared to chemotherapy alone, and the addition of a short course of tremelimumab to durvalumab and chemotherapy resulted in significant improvements in both OS and PFS compared to chemotherapy, without a significant increase in tolerability issues [15]. This suggests that it may be a promising new option for the first-line treatment of mNSCLC. It is important to mention that regulatory approval has been granted for the combined use of tremelimumab and durvalumab alongside platinum-based chemotherapy in patients with metastatic NSCLC with no EGFR or ALK genetic alterations. In the Checkmate-227 study, when patients had to stop treatment due to TRAEs, the median OS was 41.5 months, and the 5-year OS rate was 39% [72]. Similarly, in the CheckMate-9LA trial, 48% of patients experienced grade 3–4 TRAEs, and 18% of them had to discontinue treatment. The median OS in this group was 27.5 months, with a 4-year OS rate of 41% [73]. Comparable findings were observed in the POSEIDON trial, where 58% of patients experienced grade 3 TRAEs, and 9.4% had to discontinue treatment [15]. Thus, the safety profile should not be an obstacle to the widespread application of dual ICI, whether with or without chemotherapy, in routine clinical practice.

In the IMpower 131 and POSEIDON trials, the combination of atezolizumab and durvalumab with chemotherapy led to median OS times of 14.2 and 14.0 months, respectively, but no survival benefit over chemotherapy was observed in either trial [15, 74]. Cemiplimab in combination with platinum-based doublet chemotherapy (EMPOWER-Lung 3), the combination of durvalumab, tremelimumab, and platinum-based doublet chemotherapy (POSEIDON), and the combination of nivolumab with ipilimumab (CheckMate 227, specifically for PD-L1≥1% tumors) have obtained FDA approval but are awaiting approval by the EMA [49].

Among the previously mentioned studies with predominantly favorable results, there are a few exceptions where the outcomes were not favorable. In MYSTIC, durvalumab plus tremelimumab did not improve OS or PFS in patients with ≥25% PD-L1 expression. Similarly, in the NEPTUNE trial (which included metastatic NSCLC patients with a blood tumor mutational burden of ≥20 mutations per megabase), durvalumab and tremelimumab did not enhance overall survival compared to chemotherapy [59, 75].

Role of immunotherapy in NSCLC with driver mutation

Mutations such as EGFR, ALK, KRAS, and other genetic changes (including MET, RET, BRAF, and ROS1) have brought about a significant shift in the way this type of NSCLC is treated. In the era of immuno-oncology, there is growing evidence suggesting that prominent oncogenes have different impacts on the immune microenvironment within tumors, which in turn affects the clinical advantages of using ICIs as a treatment approach.

EGFR

EGFR-activating mutations, which are common in NSCLC, are treatable with targeted therapies and more prevalent in non-smokers, light smokers, young, Asian, and female patients. ICIs have limited efficacy in EGFR-mutant NSCLC based on data from studies like CA209-012 and Keynote 001, where response rates were lower than in wild-type patients. The ATLANTIC and PACIFIC studies also found that EGFR- or ALK-positive patients had worse outcomes with durvalumab treatment compared to wild-type patients. The IMpower 150 trial showed improved OS in EGFR-mutant patients treated with ABCP compared to BCP, but overall, ICIs alone or with chemotherapy have limited efficacy in EGFR-mutant NSCLC [10, 76, 77].

ALK

Studies like ATLANTIC and IMMUNOTARGET did not find immunotherapy to be effective. These findings were further supported by retrospective analyses conducted in Massachusetts and a multicenter study in France. In the IMpower150 trial, the combination of chemotherapy with atezolizumab and

bevacizumab did not show a significant difference in PFS in ALK-positive patients compared to bevacizumab/chemotherapy (8.3 vs. 5.9 months; HR 0.65; not significant), consistent with the results of IMpower130. In ALK-rearranged NSCLC, ICIs alone do not appear to be promising; chemotherapy remains the standard after ALK tyrosine kinase inhibitors (TKIs) lose efficacy [10, 78].

KRAS

In advanced NSCLC, KRAS mutations stand out as the most common molecular abnormalities. These KRAS mutations exhibit considerable diversity and involve substitutions at codons 12, 13, or 61. The most frequent of these substitutions which is present in 41% of KRAS-mutant NSCLC cases is KRAS p.G12C. Studies have shown that immunotherapy consistently demonstrates clinical activity that is at least equivalent to that seen in patients who have wild-type KRAS. In a meta-analysis, it was observed that KRAS-mutant patients responded more favorably to ICI treatment when compared to receiving docetaxel monotherapy [79–81].

BRAF

BRAF mutations, present in approximately 2% of NSCLC cases, with p.V600E being the most common type, have shown clinical efficacy with ICIs in two retrospective cohorts of 39 and 38 patients, including those with p.V600E variants. PFS ranged from 3.0 to 4.1 months, and OS was 13.1 months in the second cohort. Smoking-related factors, such as higher mutational burden and PD-L1 expression, may contribute to increased ICI sensitivity in BRAF mutant NSCLC, similar to KRAS mutations [82, 83].

ROS1

ROS1 rearrangements occur in approximately 2.5% of lung adenocarcinoma patients, with various fusion partners. Tyrosine kinase inhibitors like crizotinib, entrectinib and lorlatinib are approved for treatment, but the efficacy of ICIs remains uncertain. The IMMUNOTARGET registry, which included six patients, reported a low response rate (17%) and a median OS of 18.4 months in ROS1 fusion-positive NSCLC. Negrao et al suggested in their study that PD-L1 may not be an independent predictor of immunotherapy response [81, 84, 85].

MET

MET, a receptor tyrosine kinase, is crucial in cell processes and is implicated in NSCLC, with MET exon 14 skipping mutations in 3%–5% of cases and amplifications in 1%–5% of NSCLC patients, effectively treated with drugs like crizotinib, capmatinib, savolitinib, and tepotinib. In the IMMUNOTARGET registry it was observed that the efficacy of ICIs was not influenced by either high PD-L1 expression or a high TMB. If targeted therapy for MET is available, it is advisable to consider its use as a first-line treatment [81, 84, 86–88].

Her2

HER2 mutations and amplifications are found in 2%–4% of lung adenocarcinomas, often in non-smokers, and are effectively treated with drugs like ado-trastuzumab emtansine (T-DM1) and TKIs. Lung tumors with Her2 amplification have high TMB and low PD-L1, which may explain the reduced efficacy of immunotherapy. In the IMMUNOTARGET study, Her2-mutant patients showed lower ORR, median PFS, and OS, suggesting that chemo-immunotherapy should not be the initial treatment option [81, 89].

TP53

TP53, a well-studied gene, is vital for cell cycle regulation, DNA repair, and apoptosis. TP53 mutations, when co-occurring with KMT2C, may lead to a better ICI response in patients with advanced NSCLC, while mutations in STK11 and KEAP1 do not notably affect ICI response in this genetic context [90, 91].

RET

RET rearrangements are found in approximately 1%–2% of patients with NSCLC. Patients with RET fusions are generally never smokers with lung adenocarcinoma and early development of intracranial metastases. The FDA and EMA have granted approval for the use of highly selective RET inhibitors, such as selpercatinib and pralcetinib, specifically for NSCLC cases with RET fusion. While there is a lack of prospective data, existing evidence indicates that NSCLC with RET rearrangements characterized as biologically “cold” tumors do not respond well to ICIs. Therefore, it is advisable to prioritize targeted therapies when they are available for these RET-rearranged NSCLC patients [84, 92].

NTRK

NTRK gene fusions occur in approximately 0.2% of cases without a clear correlation to sex, age, or smoking history. Larotrectinib and entrectinib are approved for the treatment of these tumors. In NSCLC, NTRK gene fusions exhibit higher

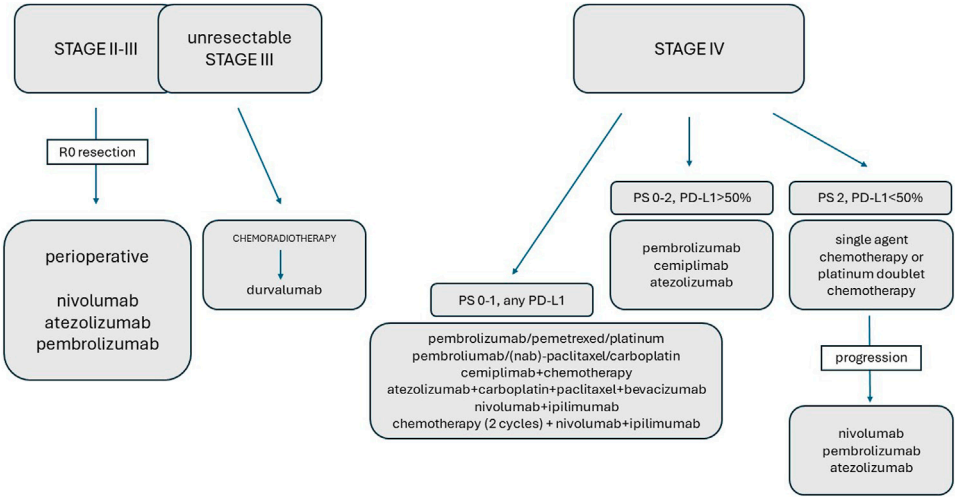

FIGURE 1
The role of immunotherapy in the treatment of NSCLC.

TMB and PD-L1 expression than EGFR, ALK, and ROS1 alterations, suggesting a combination of chemotherapy and immunotherapy for comprehensive treatment [93, 94].

Beyond the first line, rechallenge

When patients cannot receive first-line immunotherapy for any reason or show progression after platinum doublet treatment, and they become eligible for ICI therapy, anti-PD-(L)1 monotherapy is the preferred choice. The FDA and EMA subsequently approved the anti-PD-1 antibody nivolumab and the anti PD-L1 antibody atezolizumab irrespective of PD-L1 status and the anti PD-1 antibody pembrolizumab only in patients with PD-L1 expression. Nivolumab was the first approved agent to show efficacy in patients with advanced and metastatic NSCLC in the second-line and beyond setting. These results are based on two trials, CheckMate-017 and -057. CheckMate-017 evaluated nivolumab at a dose of 3 mg/kg Q2W compared versus docetaxel 75 mg/m^2 Q3W in 272 patients with advanced or metastatic squamous cell lung carcinoma. Both OS (9.2 vs. 6 months; HR: 0.59) and PFS (3.5 vs. 2.8 months; HR: 0.62) showed a significant improvement for the nivolumab arm independent of PD-L1 status [95]. The CheckMate-057 trial also demonstrated an OS benefit (12.2 vs. 9.4 months; HR: 0.73) of nivolumab (3 mg/kg Q2W) over docetaxel (75 mg/m^2 Q3W) showed inferiority in terms of PFS (2.3 vs. 4.2 months), but it was superior at 1 year (19% vs. 8%; HR: 0.92). The higher the PD-L1 expression the greater the survival benefit [96]. Based on these data the drug received approval from both medical agencies in both histologic subtypes. Another optional anti-PD-1 agent for second-line treatment is pembrolizumab which was found to be superior to docetaxel. In the KeyNote-010 trial the effects of pembrolizumab at 2 or 10 mg/kg Q3W were compared to docetaxel (75 mg/m^2 Q3W) in patients with advanced or metastatic NSCLC with PD-L1 expression (≥1%). Both doses of pembrolizumab demonstrated an OS benefit (10.4 months for pembrolizumab 2 mg/kg (HR: 0.71), 12.7 months for pembrolizumab 10 mg/kg (HR: 0.61), and 8.5 months for docetaxel) but no PFS benefit. In a subgroup of patients with higher PD-L1 expression (TPS ≥50%), the PFS was also significantly better (HR: 0.59) [97]. Based on the above data pembrolizumab was also approved by the FDA and EMA for second-line use in PD-L1 positive (≥1%) patients. The third agent which is an anti PD-L1 antibody and therefore slightly different from the other two agents was atezolizumab. The OS (13.8 vs. 9.6 months; HR: 0.73) superiority and PFS non-inferiority over docetaxel was demonstrated in the OAK trial [98]. Based on these results, atezolizumab was also approved by the european (EMA) and american (FDA) medical agencies. In selected patients rechallenge with ICI (especially with pembrolizumab) may be an option if the reason for previous discontinuation was not disease progression or toxicity and clinical benefit was achieved during ICI administration [54, 99].

Discussion

The future landscape of NSCLC immunotherapy is promising and continually evolving, revolutionizing the treatment paradigm for this aggressive disease. Immunotherapy has emerged as a game-changer, offering new hope and improved outcomes for these patients. ICIs targeting PD-1/PD-L1 have become a cornerstone of this treatment. They have shown significant improvements in OS,

PFS, and durable responses compared to conventional chemotherapy. Initially limited to patients with high PD-L1 expression, ICIs are now being considered for a broader patient population, including those with low or no PD-L1 expression. The future lies in combining immunotherapeutic agents with other immunotherapies, chemotherapy, targeted therapies, or even radiation. These combinations aim to enhance the immune response and address tumor heterogeneity and resistance mechanisms. Research into novel biomarkers beyond PD-L1 and TMB is ongoing. Identifying more precise predictors of response to immunotherapy will enable better patient selection and personalized treatment strategies. Research is underway to develop and validate new checkpoint inhibitors targeting different immune checkpoints other than PD-1/PD-L1 and CTLA-4. These could potentially offer improved responses and reduced resistance. The role of immunotherapy is expanding beyond advanced stages. The neoadjuvant approach with an adjuvant ICI combination may be the best option for patients who have no contraindication to immunotherapy, but another ICI + chemotherapy combination may be better than standard-of-care chemotherapy alone even in the adjuvant setting. It is anticipated that ICI will become a mandatory part of the perioperative treatment of resectable NSCLC and ctDNA level measurement may become critical in deciding whether or not patients require adjuvant therapy or not (with or without prior neoadjuvant treatment). This is being explored in the adjuvant setting after surgery or in combination with chemoradiotherapy for locally advanced disease, potentially preventing recurrence. These therapeutic possibilities are summarized in Figure 1. Resistance mechanisms limit the efficacy of immunotherapy. Strategies to overcome this hurdle include combination therapies, the development of novel drugs, and a better understanding of the tumor microenvironment. The landscape is shifting to a more patient-centered approach that emphasizes quality of life, management of treatment-related toxicities, and addressing the unique needs of each patient. Patients with BRAF or KRAS/TP53 mutations benefit most from ICIs, while those with EGFR or ALK/ROS1 rearrangements show lower PD-L1 and mutational burden, leading to ICI resistance. Understanding the genomics of NSCLC will help select ICI candidates. Targeting immunosuppressive mechanisms alongside oncogene signaling may sensitize NSCLC to ICIs. Tumors with driver mutations are diverse, therefore they require precision medicine. Frontline TKIs are preferred to ICIs, with chemo-immunotherapy as an alternative. Combinations of targeted therapy and ICIs are being studied. Broader oncogenic factors should be considered in future NSCLC ICI studies. Ongoing clinical trials are exploring new treatment modalities, innovative drug combinations, and novel therapeutic targets. These trials are crucial for advancing the field and bringing new therapies to the clinic. Despite advancements, the high cost of immunotherapies poses a challenge to accessibility. Efforts to balance innovation and affordability through biosimilars and health policy interventions remain critical. The future of NSCLC immunotherapy is bright, driven by a deep understanding of tumor biology, rapid advancements in technology, and collaborative efforts across the scientific community. As research continues, the goal remains to further improve outcomes, extend survival, and ultimately transform NSCLC into a manageable, chronic condition for more patients.

Author contributions

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Conflict of interest

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Prognostic value of lung immune prognostic index in non-small cell lung cancer patients receiving immune checkpoint inhibitors: a meta-analysis

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Background and Purpose: Until now, it has been difficult to accurately predict the efficacy of immunotherapy in patients with non-small cell lung cancer (NSCLC). A novel indicator, the lung immune prognostic index (LIPI), has shown relatively high prognostic value in patients with solid cancer. Therefore, this study aimed to further identify the association between LIPI and the survival of patients with NSCLC who receive immune checkpoint inhibitors (ICIs).

Methods: Several electronic databases were searched for available publications up to April 23, 2023. Immunotherapy outcomes included overall survival (OS), progression-free survival (PFS), and hazard ratios (HRs) with 95% confidence intervals (CIs). Subgroup analysis based on the study design and comparison of the LIPI was conducted.

Results: In this meta-analysis, 21 studies with 9,010 patients were included in this meta-analysis. The pooled results demonstrated that elevated LIPI was significantly associated with poor OS (HR = 2.50, 95% CI:2.09–2.99, $p < 0.001$) and PFS (HR = 1.77, 95% CI:1.64–1.91, $p < 0.001$). Subgroup analyses stratified by study design (retrospective vs. prospective) and comparison of LIPI (1 vs. 0, 2 vs. 0, 1–2 vs. 0, 2 vs. 1 vs. 0, 2 vs. 0–1 and 2 vs. 1) showed similar results.

Conclusion: LIPI could serve as a novel and reliable prognostic factor in NSCLC treated with ICIs, and elevated LIPI predicts worse prognosis.

KEYWORDS

lung immune prognostic index, non-small cell lung cancer, immune checkpoint inhibitor, prognosis, meta-analysis

Introduction

Lung cancer remains the most common malignancy and leading cause of tumor-related deaths worldwide [1, 2]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases [3]. Despite great advances in early screening, surgical techniques, and adjuvant therapies for NSCLC, the overall prognosis remains poor, representing a relatively high risk of recurrence and therapeutic resistance [4, 5]. In the last few years, immunotherapy has become an important treatment option for NSCLC, especially for patients with advanced-stage and metastatic NSCLC. Unfortunately, less than 20% of patients could benefit from immunotherapy [6].

In clinics, immune checkpoint inhibitors (ICIs), particularly anti-programmed death ligand 1 (PD-L1)/programmed death 1 (PD-1) antibodies, are widely used as first- or second-line treatments for metastatic/advanced NSCLC alone or in combination with chemotherapy. However, as mentioned above, the number of patients who benefit from ICIs is fairly limited [6]. Thus, accurate and effective indicators to predict the efficacy of ICIs are urgently needed to help select potential beneficiaries of ICIs. Overall, PD-L1 expression and tumor mutation burden (TMB) are the most commonly used biomarkers to select ICI-advantaged populations and predict prognosis. Nevertheless, the predictive effect of these two biomarkers on ICI efficacy is not satisfactory in clinical practice [7, 8]. Patients with high PD-L1 expression are more likely to experience better survival, but a subset of patients do not benefit from immunotherapy [9, 10]. Therefore, further exploration of effective predictive indicators for the prognosis of ICIs treated NSCLC is required.

Since the immune checkpoint pathway includes an important circulatory phase, changes in some parameters based on peripheral blood may be associated with the response to immunotherapy. Increasing evidence suggests that inflammatory responses play an essential role in the development and progression of cancers [11, 12]. The inflammatory process in the body is considered to be the immune resistance mechanism in cancer patients, which promotes the growth and spread of tumor cells and activates the carcinogenic signaling pathway [11, 12]. Some biomarkers, such as the neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-to-lymphocyte ratio (dNLR), and platelet-to-lymphocyte ratio (PLR) have been used to detect inflammatory status and predict prognosis in various cancers, including NSCLC [11–14]. The lung immune prognostic index (LIPI) is a novel indicator based on a dNLR >3 and lactate dehydrogenase (LDH) > upper limit of normal range (ULN), which was first reported by Mezquita et al. [15]. Patients were divided into three groups based on the number of risk factors from the LIPI: low-risk, intermediate-risk, and high-risk groups with 0, 1, and 2 risk factors, respectively. Previous studies have revealed that pretreatment LIPI play a role in predicting the therapeutic outcomes of ICIs in patients with solid cancers. However, whether it can predict the prognosis of ICIs ICI-treated NSCLC remains unclear.

Therefore, this meta-analysis aimed to further identify the association between LIPI and survival of NSCLC patients receiving ICIs, which might contribute to the selection of an advantaged population and improvement of the therapeutic efficacy of immunotherapy among NSCLC patients.

Materials and methods

The current meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 [16].

Literature search

The PubMed, EMBASE, Web of Science, and CNKI databases were searched from inception to April 23, 2023. The following terms were used for the search: PD-1, PD-L1, CTLA-4, ICIs, immune checkpoint inhibitor, lung, pulmonary, cancer, tumor, carcinoma, neoplasm, LIPI, lung immune prognostic index, survival, prognosis, and prognostic. The detailed search strategies were as follows: (PD-1 OR PD-L1 OR CTLA-4 OR ICIs OR immune checkpoint inhibitor) AND (lung OR pulmonary) AND (cancer OR tumor OR carcinoma OR neoplasm) AND (LIPI OR lung immune prognostic index) AND (survival OR prognosis OR prognostic). Free text and Medical Subject Headings terms were also applied. All the references cited in the included studies were reviewed.

Inclusion criteria

Studies that met the following criteria were included:1) patients were pathologically diagnosed with primary NSCLC; 2) patients who received ICIs with or without other combined therapies such as chemotherapy; 3) LIPI score was assessed according to the dNLR values and LDH level before immunotherapy, and the association between LIPI and efficacy of immunotherapy was evaluated; 4) the overall survival (OS) and (or) progression-free survival (PFS) were defined as outcomes of immunotherapy; 5) hazard ratios (HRs) with 95% confidence intervals (CIs) for OS and PFS were directly reported in articles.

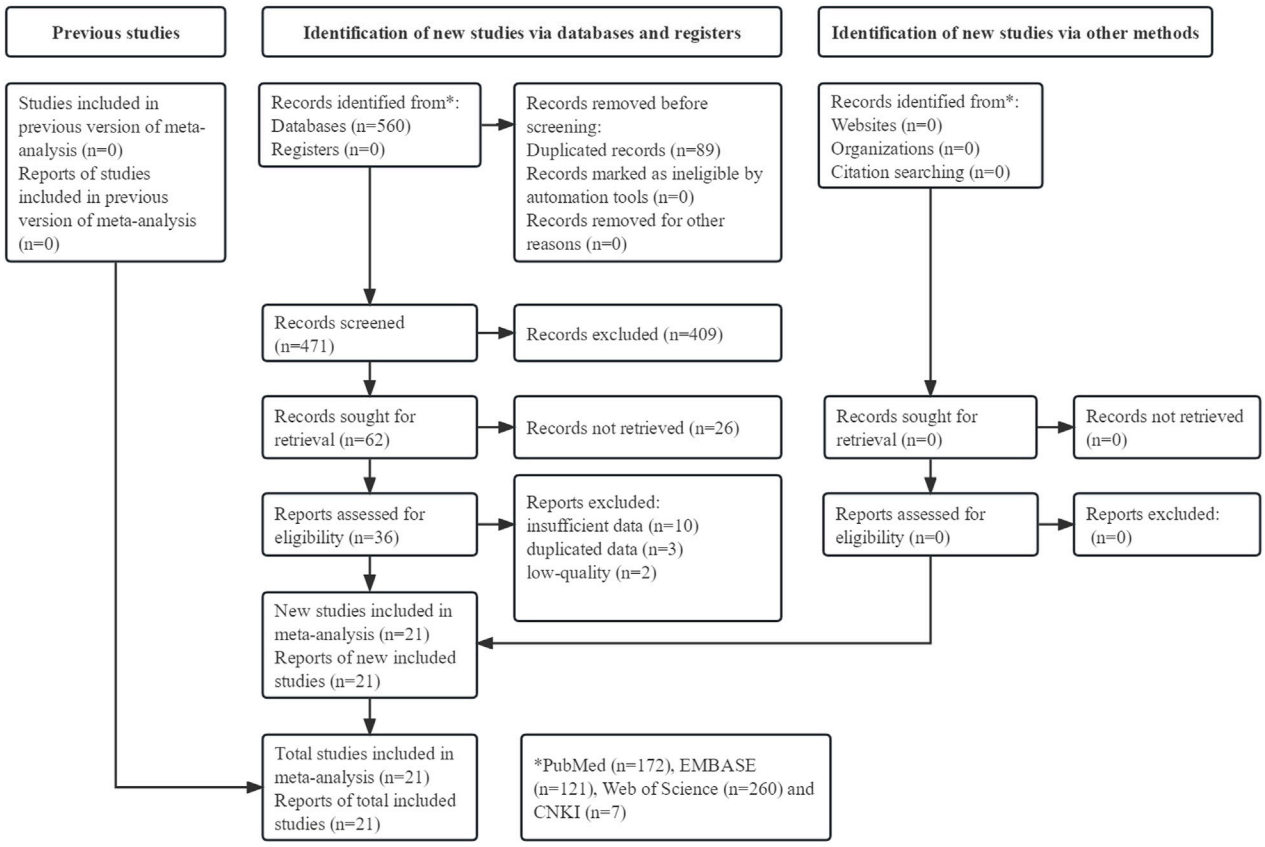

FIGURE 1
Prisma flow diagram of this meta-analysis.

Exclusion criteria

Studies that met any of the following criteria were excluded:1) low-quality studies; 2) letters, editorials, reviews, case reports, or animal trials; and 3) studies with insufficient or duplicated data.

Data extraction

The following information was collected from the included studies: name of first author, publication year, country, study design (retrospective or prospective), sample size, TNM stage, pathological type, detailed drugs of ICIs, threshold and comparison of LIPI, endpoint, HR, and 95% CI.

Quality assessment

Owing to the nature of the included studies, the Newcastle-Ottawa Scale (NOS) score system was used to evaluate the quality of the included studies. As mentioned above, only high-quality studies with an NOS score ≥6 were included.

The literature search, selection, information collection, and quality assessment were conducted by two authors independently and any disagreement was resolved by team discussion.

Statistical analysis

All statistical analyses were performed using STATA 12.0. Heterogeneity between studies was evaluated using I^2 statistics and Q test. If significant heterogeneity was observed ($I^2 > 50\%$ and/or $p < 0.1$), the random-effects model was applied; otherwise, the fixed-effects model was used. HRs and 95% CIs were combined to evaluate the association between the LIPI, OS, and PFS. Subgroup analysis based on study design (retrospective vs. prospective) and comparison of LIPI (1 vs. 0, 2 vs. 0, 1–2 vs. 0, 2 vs. 1 vs. 0, 2 vs. 0–1 and 2 vs. 1) were conducted. Sensitivity analysis was conducted to detect the sources of heterogeneity and assess the stability of the overall results. Furthermore, Begg's funnel plot and Egger's test were conducted to detect publication bias, and significant publication bias was defined as $p < 0.05$ [17–19].

TABLE 1 Basic characteristics of included studies.

Author	Year	Country	Study design	Sample size	TNM stage	Pathological type	ICIs	Threshold and comparison of LIPI	Endpoint	NOS
Mezquita [15]	2018	France	R	466	IIIB-IV	Mixed	Nivolumab, pembrolizumab, atezolizumab, durvalumab, and durvalumab-ipilimumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1 and 0 vs. 2	OS, PFS	7
Kazandjian [20]	2019	United States	P	1,368	IV	Mixed	NR	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 2 and 1 vs. 2	OS, PFS	8
Ruiz-Bañobre [21]	2019	Spain	R	188	IIIB-IV	Mixed	Nivolumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1 vs. 2	OS, PFS	8
Sorich [22]	2019	Australia	P	1,489	Advanced	Mixed	Atezolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1 and 0 vs. 2	OS, PFS	7
Mazzaschi [23]	2020	Italy	P	109	IV	Mixed	NR	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1 vs. 2	OS, PFS	8
Wang [24]	2020	China	R	330	IIIB-IV	Mixed	Nivolumab, pembrolizumab, atezolizumab, and other PD-1/PD-L1 inhibitors	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0vs 1 and 0 vs. 2	OS, PFS	8
Ali [25]	2021	China	R	73	IV	Mixed	Pembrolizumab, nivolumab, camrelizumab and atezolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS, PFS	6
Galland [27]	2021	France	R	231	NR	Adenocarcinoma	PD-1/PD-L1 inhibitors	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS, PFS	6

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TABLE 1 (*Continued*) Basic characteristics of included studies.

Author	Year	Country	Study design	Sample size	TNM stage	Pathological type	ICIs	Threshold and comparison of LIPI	Endpoint	NOS
Grosjean [28]	2021	Canada	R	327	I-IV	Mixed	Pembrolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS	6
Hopkins [29]	2021	Australia	P	1,148	Advanced	Mixed	Atezolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1 and 0 vs. 2	OS, PFS	6
Mountzios [30]	2021	Greece	R	672	IV	Mixed	PD-L1 inhibitors	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS, PFS	6
Chen [26]	2021	China	R	84	IIIB-IV	Mixed	PD-1/PD-L1 inhibitors	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS, PFS	6
Chen [31]	2022	China	R	85	IV	Mixed	PD-1 inhibitors	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS, PFS	6
De Giglio [32]	2022	Italy	R	182	IV	Mixed	NR	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS	6
Holtzman [33]	2022	Israel	R	423	III-IV	Mixed	Pembrolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	OS	6
Ortega-Franco [34]	2022	United Kingdom	R	113	III-IV	Mixed	Pembrolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1	OS, PFS	6

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TABLE 1 (*Continued*) Basic characteristics of included studies.

Author	Year	Country	Study design	Sample size	TNM stage	Pathological type	ICIs	Threshold and comparison of LIPI	Endpoint	NOS
Tanaka [35]	2022	Japan	R	237	I-IV	Mixed	NR	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0–1 vs. 2	OS, PFS	6
Zhou J [36]	2022	China	R	51	IIIB-IV	Mixed	PD-1 inhibitors	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1–2	PFS	6
Zhou S [37]	2022	China	R	53	IV	Mixed	Pembrolizumab, nivolumab, sintilimab, camrelizumab, tislelizumab, and atezolizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0–1 vs. 2	PFS	6
Zhou Y [38]	2022	China	R	86	I-IV	Mixed	Pembrolizumab, nivolumab and sindillimab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 1 and 0 vs. 2	PFS	7
Huang [39]	2023	China	R	147	IIIB-IV	Mixed	Nivolumab, pembrolizumab, atezolizumab, durvalumab, treprizumab, carrelizumab, sintilimab and tislelizumab	0: dNLR≤3 and LDH ≤ ULN; 1: dNLR >3 or LDH > ULN; 2: dNLR >3 and LDH > ULN; 0 vs. 2 and 1 vs. 2	PFS	7

ICIs: immune checkpoint inhibitors; LIPI: lung immune prognostic index; NOS: Newcastle-Ottawa Scale; R: retrospective; P: prospective; NR: not reported; PD-1: programmed death-1; PD-L1: programmed cell death 1 ligand 1; dNLR: derived neutrophil-to-lymphocyte ratio; ULN: upper limit of normal level; LDH: lactate dehydrogenase; OS: overall survival; PFS: progression-free survival.

Results

Literature search and selection

The detailed process is illustrated in Figure 1. Initially, 560 records were searched from four databases and a total of 21 studies were included [15, 20–39].

Basic characteristics of included studies

A total of 9,010 participants were enrolled in 21 studies published between 2018 and 2023. Most of the included studies were retrospective and focused on patients with advanced NSCLC. The sample size ranged from 51 to 1,489, and all studies applied the same definition of LIPI risk grading: LIPI 0, dNLR≤3 and LDH ≤ ULN; LIPI 1, dNLR >3 or LDH > ULN; and LIPI 2, dNLR >3 and LDH > ULN. Specific data are presented in Table 1.

Association between LIPI and OS and PFS

Seventeen studies explored the relationship between LIPI and OS in NSCLC patients receiving ICIs. The pooled results showed that elevated LIPI predicted poorer OS (HR = 2.50, 95% CI: 2.09–2.99, $p < 0.001$; $I^2 = 68.6\%$, $p < 0.001$), and subgroup analysis based on the study design showed the same results (Figure 2A).

Eighteen studies identified a relationship between LIPI and PFS in immunotherapy-treated NSLC. The pooled results demonstrated

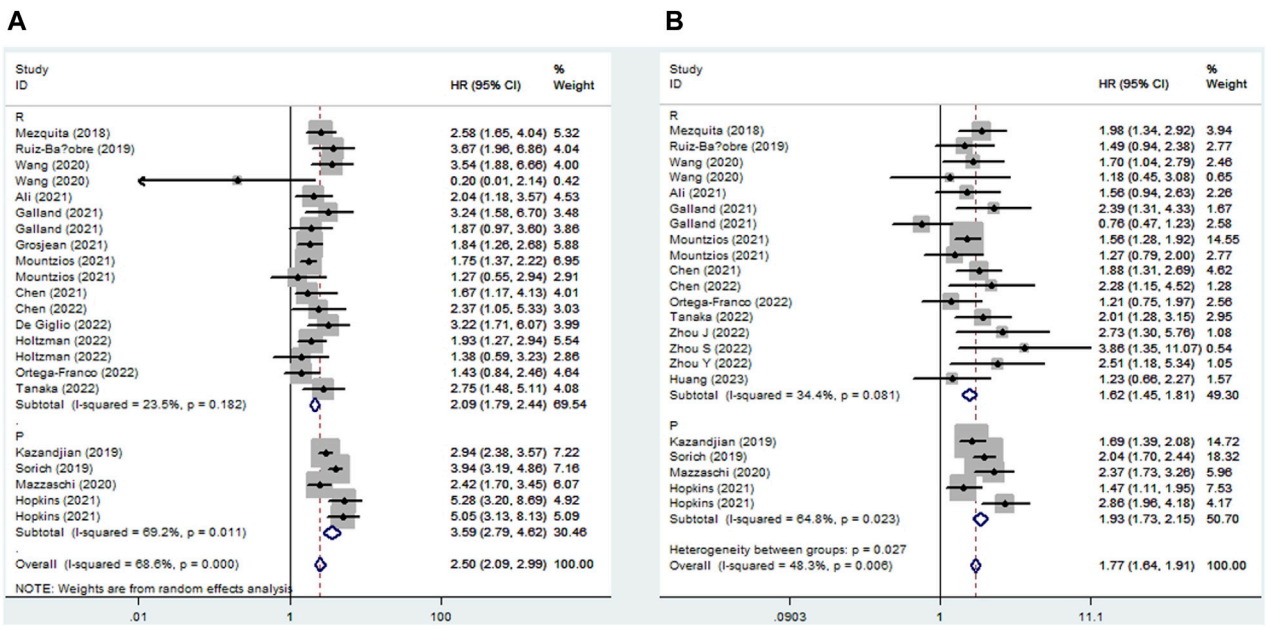

FIGURE 2
Subgroup analysis based on study design for the association between LIPI and overall survival **(A)** and progression-free survival **(B)** of non-small cell lung cancer patients receiving immune checkpoint inhibitors.

that elevated LIPI was obviously associated with poor PFS (HR = 1.77, 95% CI:1.64–1.91, $p < 0.001$; $I^2 = 48.3\%$, $p = 0.006$), and subgroup analysis stratified by study design further verified the significant relationship between LIPI and PFS (Figure 2B).

Subgroup analysis for OS

In this meta-analysis, we conducted a subgroup analysis based on a comparison of the LIPI and study design. The pooled results further demonstrated that elevated LIPI was significantly related to worse OS (LIPI 1 vs. 0: HR = 1.72, 95% CI: 1.52–1.94, $p < 0.001$; LIPI 2 vs. 0: HR = 3.81, 95% CI: 2.84–5.10, $p < 0.001$; LIPI 1–2 vs. 0: HR = 1.90, 95% CI: 1.64–2.20, $p < 0.001$; LIPI 2 vs. 1 vs. 0: HR = 2.68, 95% CI: 1.97–3.64, $p < 0.001$; LIPI 2 vs. 0–1: HR = 2.75, 95% CI: 1.48–5.11, $p < 0.001$; LIPI 2 vs. 1: HR = 1.69, 95% CI: 1.37–2.08, $p < 0.001$). In addition, a more specific subgroup analysis based on study design for the comparison of LIPI 1 vs. 0 (Figure 3A), LIPI 2 vs. 0 (Figure 3B), LIPI 1–2 vs. 0 (Figure 3C), and LIPI 2 vs. 1 vs. 0 (Figure 3D) further identified the above findings. Detailed results are presented in Table 2.

Subgroup analysis for PFS

Similarly, subgroup analysis for PFS based on the comparison of LIPI and the study design was performed. Pooled results revealed that elevated LIPI was obviously associated with poorer PFS (LIPI 1 vs. 0: HR = 1.44, 95% CI: 1.31–1.57, $p < 0.001$; LIPI 2 vs. 0: HR = 1.91, 95% CI: 1.69–2.16, $p < 0.001$; LIPI 1–2 vs. 0: HR = 1.60, 95% CI: 1.26–2.04, $p < 0.001$; LIPI 2 vs. 1 vs. 0: HR = 1.94, 95% CI: 1.24–3.05, $p = 0.004$; LIPI 2 vs. 0–1: HR = 2.22, 95% CI: 1.47–3.36, $p < 0.001$; LIPI 2 vs. 1: HR = 1.25, 95% CI: 1.02–1.53, $p = 0.030$). Furthermore, specific subgroup analysis based on study design for the comparison of LIPI 1 vs. 0 (Figure 4A), LIPI 2 vs. 0 (Figure 4B), LIPI 1–2 vs. 0 (Figure 4C), and LIPI 2 vs. 1 vs. 0 (Figure 4D) further confirmed the above findings. The detailed results are presented in Table 2.

Sensitivity analysis

Sensitivity analysis for the association between LIPI and OS and PFS was performed, which demonstrated that the pooled results of this meta-analysis were stable, and none of the included studies had an obvious impact on the relationship between LIPI and OS (Figure 5A) and PFS (Figure 5B) among immunotherapy-treated NSCLC patients.

Publication bias

The Begg's funnel plots for OS (Figure 6A) and PFS (Figure 6B) were both symmetrical, and the *p*-values of

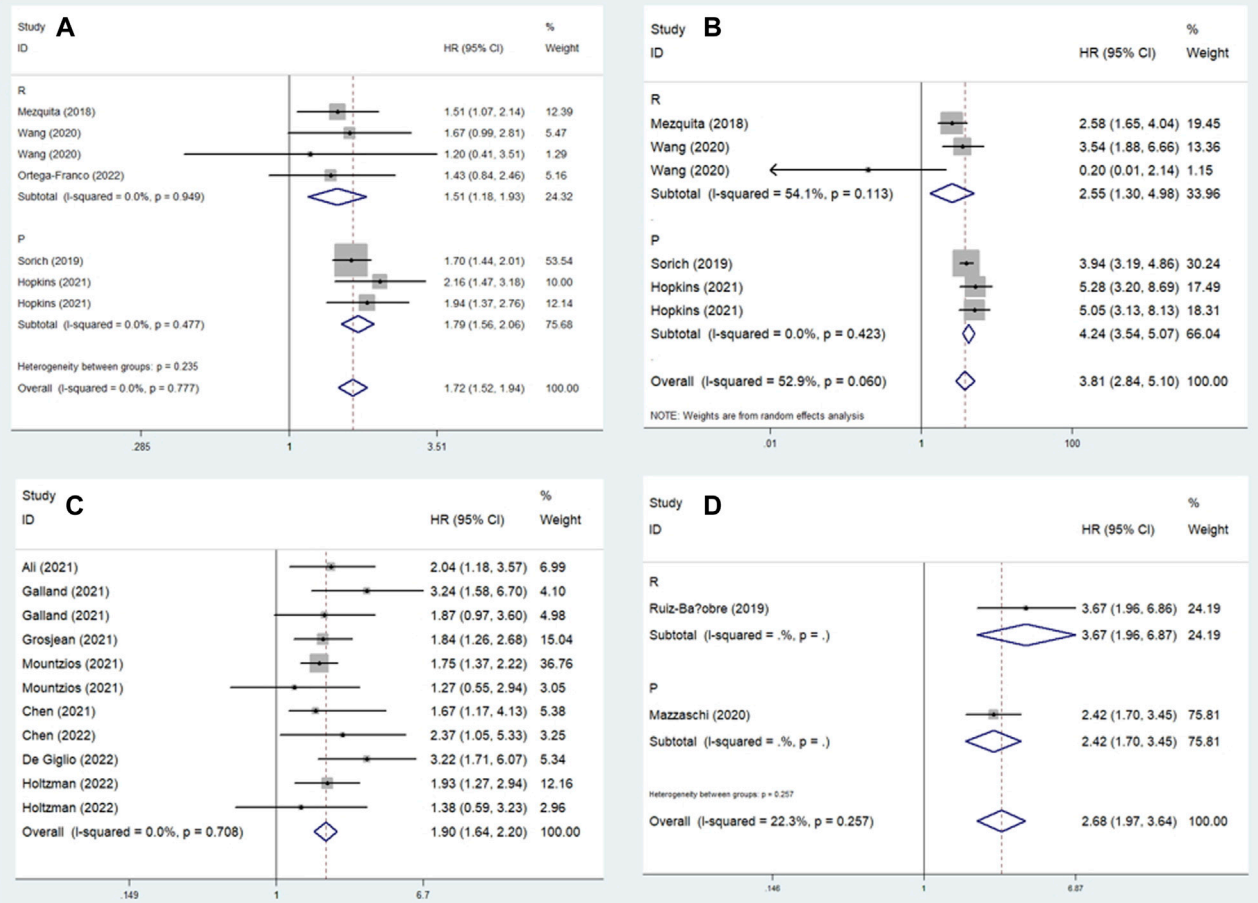

FIGURE 3
Subgroup analysis based on study design for the association between LIPI and overall survival of non-small cell lung cancer patients receiving immune checkpoint inhibitors. **(A)** LIPI 1 vs. 0; **(B)** LIPI 2 vs. 0; **(C)** LIPI 1–2 vs. 0; **(D)** LIPI 2 vs. 1 vs. 0.

Egger's test for OS and PFS were 0.208 and 0.992, respectively. Thus, no significant publication bias was observed in this meta-analysis.

Discussion

This meta-analysis explored the predictive role of LIPI for the long-term survival of NSCLC patients who received ICIs based on current evidence, and the pooled results showed that LIPI was significantly associated with OS and PFS in this group of patients. Patients with elevated LIPIs were more likely to have a worse prognosis than patients with good LIPIs. Therefore, the LIPI could serve as a novel and reliable prognostic indicator in patients with NSCLC receiving ICIs.

The invasiveness of malignant tumors depends on the nature of tumor cells and their microenvironment. Previous studies have indicated that inflammation is a recognized feature of cancer, and inflammatory reactions play a crucial role in the process of carcinogenesis [40]. On the one hand, in malignant solid tumors, inflammatory stimulation leads to immune cell infiltration, angiogenesis, and fibroblast proliferation [41, 42]. In contrast, it is one of the mechanisms of immune tolerance, promoting tumor growth and dissemination, and activating oncogenic signaling pathways in cancer patients [43]. The dNLR was calculated using the neutrophil and lymphocyte counts. Neutrophils are key participants in tumor inflammation and immunity, and participate in tumor progression. Studies have found that neutrophils can produce vascular endothelial growth factor (VEGF), which plays an important role in mediating tumor angiogenesis and is a powerful immunosuppressive factor of natural and adaptive anti-tumor immunity [44]. In addition, neutrophil-derived proteases can degrade cytokines and chemokines and reshape the extracellular matrix, and neutrophil elastase in tumor cells can overactivate the PI3K pathway, further accelerating uncontrolled tumor proliferation [45]. It has been reported

TABLE 2 Results of meta-analysis.

	No. of studies	Hazard ratio	95% confidence interval	*p*-Value	I^2 (%)	*p*-Value
Overall survival						
Overall	17	2.50	2.09–2.99	<0.001	68.6	<0.001
Retrospective	13	2.09	1.79–2.44	<0.001	23.5	0.182
Prospective	4	3.59	2.79–4.62	<0.001	69.2	0.011
LIPI 1 vs. 0	5	1.72	1.52–1.94	<0.001	0.0	0.777
Retrospective	3	1.51	1.18–1.93	0.001	0.0	0.949
Prospective	2	1.79	1.56–2.06	<0.001	0.0	0.477
LIPI 2 vs. 0	4	3.81	2.84–5.10	<0.001	52.9	0.423
Retrospective	2	2.55	1.30–4.98	0.006	54.1	0.113
Prospective	2	4.24	3.54–5.07	<0.001	0.0	0.423
LIPI 1–2 vs. 0	8	1.90	1.64–2.20	<0.001	0.0	0.708
Retrospective	8	1.90	1.64–2.20	<0.001	0.0	0.708
LIPI 2 vs. 1 vs. 0	2	2.68	1.97–3.64	<0.001	22.3	0.257
Retrospective	1	3.67	1.96–6.87	<0.001	—	—
Prospective	1	2.42	1.70–3.45	<0.001	—	—
LIPI 2 vs. 0–1	1	2.75	1.48–5.11	<0.001	—	—
Retrospective	1	2.75	1.48–5.11	<0.001	—	—
LIPI 2 vs. 1	1	1.69	1.37–2.08	<0.001	—	—
Prospective	1	1.69	1.37–2.08	<0.001	—	—
Progression-free survival						
Overall	18	1.77	1.64–1.91	<0.001	48.3	0.006
Retrospective	14	1.62	1.45–1.81	<0.001	34.4	0.081
Prospective	4	1.93	1.73–2.15	<0.001	64.8	0.023
LIPI 1 vs. 0	6	1.44	1.31–1.57	<0.001	0.0	0.979
Retrospective	4	1.35	1.13–1.61	0.001	0.0	0.927
Prospective	2	1.47	1.32–1.63	<0.001	0.0	0.982
LIPI 2 vs. 0	6	1.91	1.69–2.16	<0.001	41.0	0.105
Retrospective	4	1.75	1.36–2.24	<0.001	0.0	0.529
Prospective	2	1.97	1.71–2.27	<0.001	75.0	0.018
LIPI 1–2 vs. 0	6	1.60	1.26–2.04	<0.001	55.3	0.028
Retrospective	6	1.60	1.26–2.04	<0.001	55.3	0.028
LIPI 2 vs. 1 vs. 0	2	1.94	1.24–3.05	0.004	61.8	0.106
Retrospective	1	1.49	0.94–2.37	0.092	—	—
Prospective	1	2.37	1.73–3.25	<0.001	—	—
LIPI 2 vs. 0–1	2	2.22	1.47–3.36	<0.001	19.9	0.264
Retrospective	2	2.22	1.47–3.36	<0.001	19.9	0.264
LIPI 2 vs. 1	2	1.25	1.02–1.53		0.0	0.652
Retrospective	1	1.09	0.58–2.04	0.788	—	—
Prospective	1	1.27	1.03–1.57	0.027	—	—

LIPI: lung immune prognostic index.

that T cells producing interleukin (IL)-17 can release CXC chemokines to supplement neutrophils, and IL17a is involved in resistance to ICIs [46]. Therefore, higher dNLR levels may reflect negative inflammation and contribute to resistance to ICIs. Peripheral blood lymphocyte count is considered a predictive factor for the prognosis of various cancers [47]. Lymphocytes play an important role in tumor-related immunity, have potential anti-tumor immune functions to inhibit tumor development, participate in cytotoxic cell death and cytokine production, and inhibit tumor cell proliferation and metastasis through the immune response to cancer [48].

LDH is widely distributed in major human organs and catalyzes the conversion of lactate and pyruvate. It is an indicator of tumor burden, cell damage, and necrosis. Studies have shown that elevated LDH levels are an adverse prognostic factor for tumors [49, 50]. Elevated LDH levels are a product of enhanced tumor glycolysis and hypoxia-induced tumor necrosis [51]. On one hand, in tumors with increased glycolytic activity, both aerobic and anaerobic glycolysis under hypoxia can affect immune cell function due to glucose deficiency or tumor acidity [52]. Furthermore, hypoxia itself or the excessive expression of hypoxia-

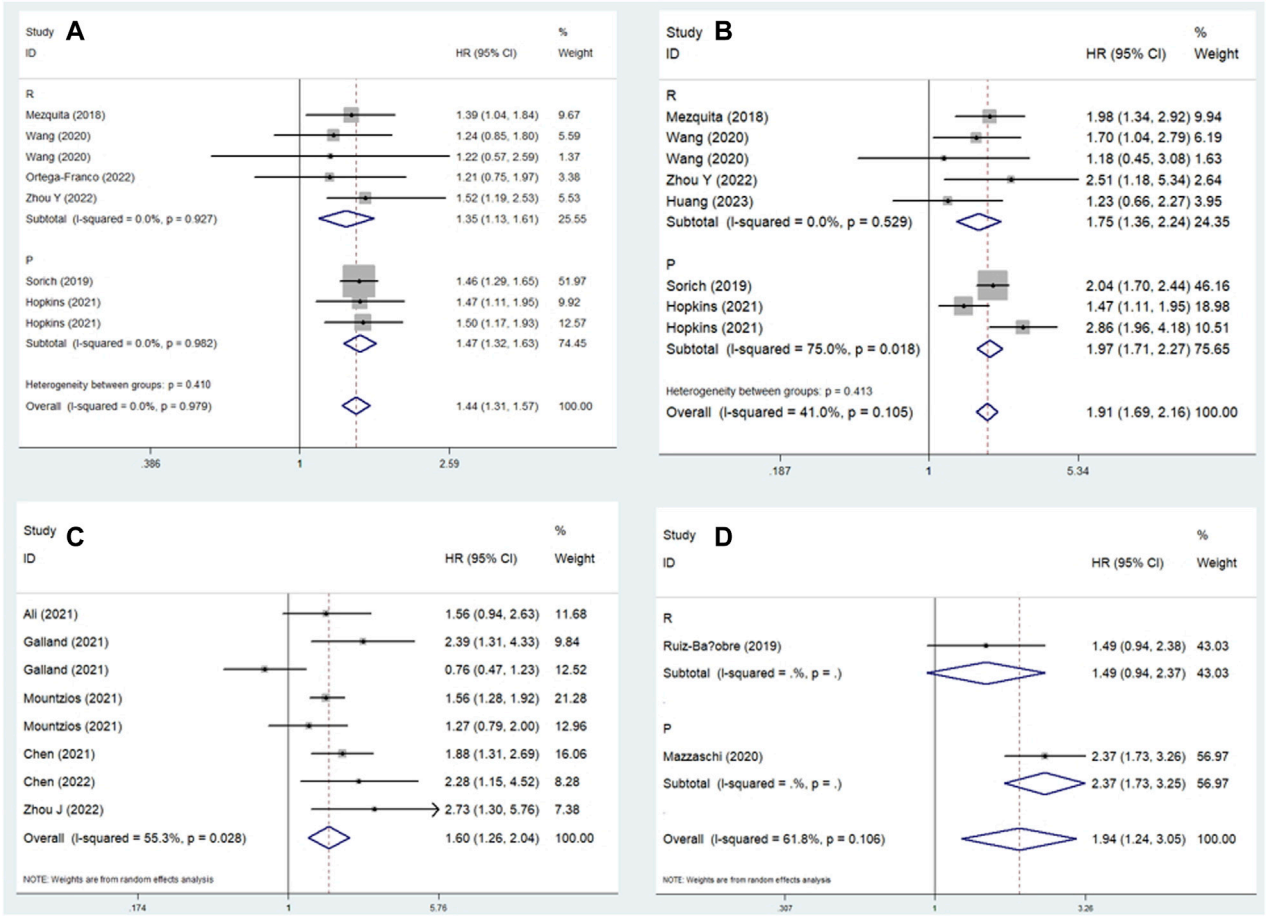

FIGURE 4
Subgroup analysis based on study design for the association between LIPI and progression-free survival of non-small cell lung cancer patients receiving immune checkpoint inhibitors. **(A)** LIPI 1 vs. 0; **(B)** LIPI 2 vs. 0; **(C)** LIPI 1–2 vs. 0; **(D)** LIPI 2 vs. 1 vs. 0.

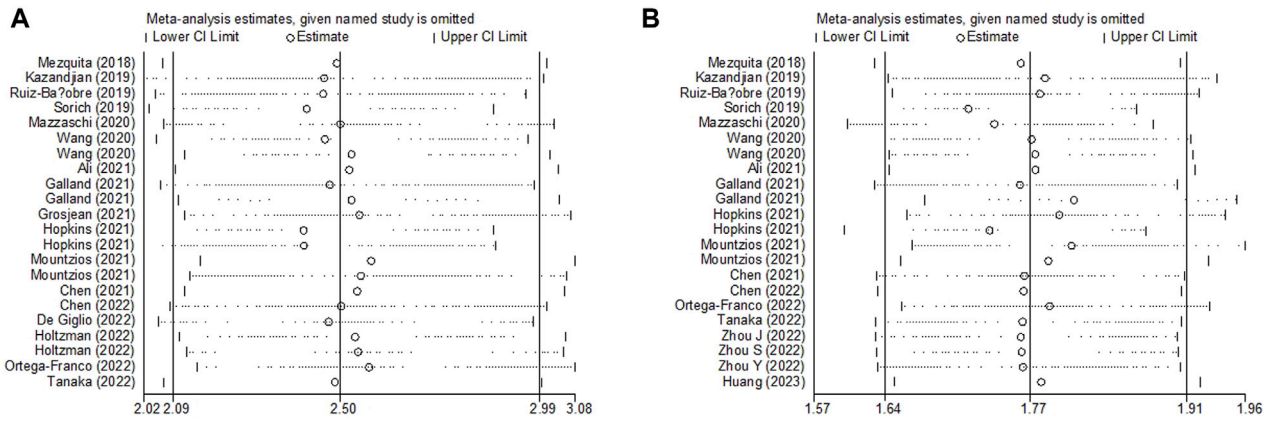

FIGURE 5
Sensitivity analysis about the association between LIPI and overall survival **(A)** and progression-free survival **(B)** of non-small cell lung cancer patients receiving immune checkpoint inhibitors.

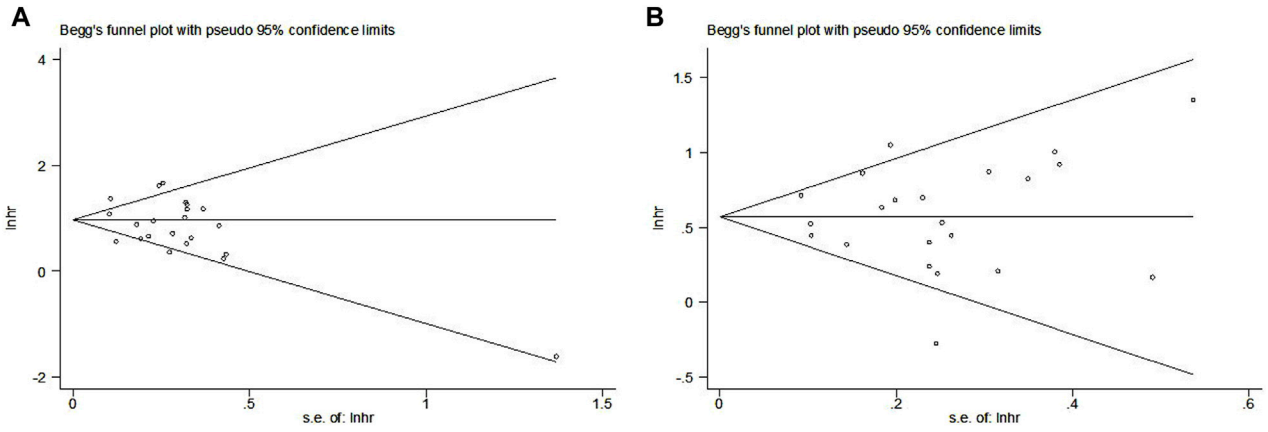

FIGURE 6
Begg's funnel plots for the association between LIPI and overall survival **(A)** and progression-free survival **(B)** of non-small cell lung cancer patients receiving immune checkpoint inhibitors.

regulated factors in highly glycolytic tumors may affect antitumor immunity [53]. In addition, the main switch for hypoxia-induced angiogenesis, hypoxia-inducible factor-1 (HIF-1), is activated by hypoxia and upregulates VEGF in tumors [54]. VEGF promotes tumor angiogenesis by inducing the proliferation and survival of endothelial cells, forming a large number of malformed and dysfunctional neovasculatures in the tumor [55]. These tumor blood vessels interfere with the active anticancer immune system and inhibit the therapeutic effect of ICI treatment. Therefore, LDH levels can affect the efficacy of ICIs.

Liu et al. included 12 studies with 4,883 solid cancer patients who received ICIs treatment and demonstrated that elevated pretreatment LIPI was significantly associated with worse OS (HR = 3.33, 95% CI:2.64–4.21, $p < 0.001$; HR = 1.71, 95%CI 1.43–2.04, $p < 0.001$) and PFS (HR = 2.73, 95% CI: 2.00–3.73, $p < 0.001$; HR = 1.43, 95%CI 1.28–1.61, $p < 0.001$) [56]. However, only six studies explored the relationship between pretreatment LIPI and survival, and five studies were included in the pooled analysis [56]. In another meta-analysis by Xie et al., four studies involving 7,373 advanced NSCLC patients receiving ICIs, targeted therapy, or chemotherapy and their results revealed that intermediate and poor LIPI predicted worse OS (HR = 1.61, 95% CI: 1.48–1.75, $p < 0.01$; HR = 2.74; 95% CI:2.26–3.33, $p < 0.01$) [57]. However, only three of the included studies identified the predictive role of pretreatment LIPI for OS in immunotherapy-treated NSCLC patients. Therefore, we performed this meta-analysis to determine the predictive value of LIPI for prognosis among patients with NSCLC receiving ICIs, and the pooled results indicated that LIPI could serve as a reliable prognostic factor in this group of patients.

This meta-analysis had several limitations that should be noted. First, all included studies were observational, and most of them were retrospectively conducted. Second, some of the included studies had relatively small sample sizes, which might have caused bias. Third, we were unable to conduct more subgroup analyses based on other parameters such as the pathological subtype, drugs of ICIs, and combinations of other therapies due to the lack of original data and sufficient information reported in the included studies.

Conclusion

Overall, LIPI could serve as a novel and reliable prognostic factor in NSCLC treated with ICIs, and intermediate LIPIs predict a worse prognosis. However, further high-quality studies are required to verify our findings.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

XX designed the study. YW, YL, and DZ performed the literature search and selection, collected the data, and wrote the paper. YY, LL, and JL performed statistical analysis and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Advancing neoadjuvant therapies in resectable non-small cell lung cancer: implications for novel treatment strategies and biomarker discovery

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The delivery of neoadjuvant and perioperative therapies for non-small cell lung cancer has been radically altered by significant advances and by the incorporation of targeted therapies as well as immune checkpoint inhibitors alone or alongside conventional chemotherapy. This evolution has been particularly notable in the incorporation of immunotherapy and targeted therapy into the treatment of resectable NSCLC, where recent FDA approvals of drugs such as nivolumab and pembrolizumab, in combination with platinum doublet chemotherapy, have led to considerable improvements in pathological complete response rates and the potential for enhanced long-term survival outcomes. This review emphasizes the growing importance of biomarkers in optimizing treatment selection and explores the impact of emerging studies that challenge existing treatment paradigms and investigate novel therapeutic combinations poised to redefine standard of care practices. Furthermore, the discussion extends to the unmet needs within perioperative treatment assessment and prognostication, highlighting the prospective value of biomarkers in evaluating treatment responses and prognosis.

KEYWORDS

NSCLC, perioperative, neoadjuvant, adjuvant, lung cancer

Abbreviations: ALK: Anaplastic Lymphoma Kinase; CHIP: Clonal hematopoiesis of indeterminate potential; dMMR: Deficient mismatch repair; EFS: Event free survival; EGFR: Epidermal Growth Factor Receptor; mAb: Monoclonal Antibody; MAF: Mutant allele fraction; mPR: major pathological response; MSI: Microsatellite instability; NSCLC: Non-small cell lung cancer; OS: Overall survival; pCR: Pathologic complete response; PDC: Platinum doublet chemotherapy; TMB: Tumor mutation burden; TME: Tumor microenvironment.

Introduction

Lung cancer, with non-small cell lung cancer (NSCLC) representing about 80% of cases, continues to pose a formidable health issue, ranking as the second highest in new cancer cases and the leading cause of cancer mortality worldwide [1]. The landscape of NSCLC management has undergone dramatic changes in recent years, driven by the advent of biomarker-targeted therapies and immunotherapies. These advances have not only transformed the treatment of advanced and locally advanced disease but are now rapidly reshaping the approach to resectable NSCLC as well. In the perioperative setting for resectable NSCLC, nivolumab and more recently pembrolizumab with platinum doublet chemotherapy have been approved in neoadjuvant/perioperative settings (Table 1) and pembrolizumab, atezolizumab, osimertinib, and alectinib all approved in the adjuvant setting, respectively. These approvals in the perioperative settings have markedly improved the management of resectable NSCLC, heralding a new era where molecularly targeted therapies and immune checkpoint inhibitors are poised to optimize treatment efficacy. This transformative phase is set against a contrasting historical context of two decades marked by numerous attempts to augment the standard of adjuvant chemotherapy, most of which failed to improve outcomes significantly. High-profile endeavors like the integration of radiation therapy, angiogenesis inhibition through VEGF targeting [2], and cancer vaccines targeting specific antigens such as MAGE [3] have been rigorously investigated but ultimately did not achieve a new standard of care, reflecting the complexity and resilience of NSCLC to therapeutic advances. This review seeks to provide a comprehensive overview of the current state and future directions of perioperative treatment in NSCLC, highlighting biomarker identification that could refine treatment selection and improve clinical outcomes, as well as exploring novel therapeutics to redefine the standards of care for NSCLC.

Current FDA approved preoperative standard of care for resectable NSCLC

The current standard of care for the neoadjuvant treatment of resectable NSCLC has evolved substantially, now increasingly utilizing multimodal strategies to improve surgical outcomes and

TABLE 1 Key phase III studies for preoperative regimens.

Trial	Regimen and FDA approval	Stage	ALK/EGFR included	Patients	PFS/EFS/RFS/OS	pCR/mPR
CheckMate 816 (8)	Neoadjuvant **nivolumab** + CT vs. CT for 3 cycles **Approved**	IB-IIIA (AJCC 7th ed)	No	358	**EFS** at 2 years 63.8% vs. 45.3% (**HR** 0.65 CI 0.43–0.91) **mEFS** 31.6 vs. 20.8 months (HR 0.63 CI 0.43–0.91 $p = 0.005$) **OS** at 2 years not reached in either arms (HR 0.57 CI 0.30–1.07 $p = 0.008$)	**pCR** 24% vs. 2.2% (OR 13.94 CI 18.0–31.0 $p < 0.001$) **mPR** 36.9% vs. 8.9% (OR 5.7 CI 3.16–10.26)
NADIMII (12)	Neoadjuvant **nivolumab** + CT vs. CT for 3 cycles R0 resections → adjuvant **nivolumab** for 6 months	IIIA-IIIB	No	86	**PFS** at 2 years 67.2% vs. 40.9% (HR 0.47 CI 0.25–0.88) **OS** at 2 years 85% vs. 63.6% (HR 0.43 CI 0.19–0.98)	**pCR** 37% vs. 7% (RR 5.34 CI 1.34–21.23 $p = 0.02$) **mPR** 53% vs. 14% (RR 3.82 CI 1.49–9.79)
KEYNOTE-671 (9)	Neoadjuvant **pembrolizumab** + CT for 4 cycles vs. placebo + CT Adjuvant **pembrolizumab** monotherapy up to 13 cycles vs. placebo **Approved**	II-IIIB N2 (AJCC 8th ed)	Yes	797	**EFS** at 2 years 62.4% vs. 40.6% (HR 0.58 CI 0.46–0.72 $p < 0.001$) **OS** at 2 years 80.9% vs. 77.6% **Median OS** NR vs. 45.5 months ($p = 0.02$)	**pCR** 18.1% vs. 4.0% (difference 14.2% CI 10.1–18.7 $p < 0.0001$) **mPR** 30.2% vs. 11.0% (difference 19.2% CI 13.9–24.7 $p < 0.0001$)
AEGEAN (17)	Neoadjuvant **durvalumab** + CT for 4 cycles vs. placebo + CT Adjuvant **durvalumab** monotherapy up to 12 cycles vs. placebo	II-IIIB N2 (AJCC 8th ed)	No	740	**EFS** at 2 years 63.3% vs. 52.4% (HR 0.68 CI 0.53–0.88 $p = 0.04$)	**pCR** 17.2% vs. 4.3% **mPR** 33.3% vs. 12.3%
CheckMate 77T (20)	Neoadjuvant **nivolumab** + CT for 4 cycles vs. placebo + CT Adjuvant **nivolumab** vs. placebo for 1 year	IIA-IIIB (N2)	No	461	**mEFS** not reached vs. 18.4 months (HR 0.58 CI 0.42–0.81 $p = 0.00025$)	**pCR** 25.3% vs. 4.3% (OR 6.64 CI 3.4–12.97) **mPR**35.4% vs. 12.1% (OR 4.01 CI 2.48–6.49)

CT, chemotherapy; DFS, disease-free survival; EFS, event-free survival; HR, hazard ratio; mEFS, median event-free survival; OR, odds ratio; OS, overall survival; PFS, progression free survival; R0, complete resection; RFS, recurrence-free survival; RR, relative risk.

hopefully to extend overall survival (OS). While studies in the past have indicated similar benefits between neoadjuvant and adjuvant chemotherapy [4, 5], logistical considerations sustained the latter as the prevailing practice pattern. Nonetheless, for patients with stage IIA to IIIA resectable NSCLC, the standard neoadjuvant protocol has conventionally incorporated platinum-based doublet chemotherapy, which has proven to enhance survival rates when compared with surgery alone [6].

Recent developments have also introduced immune checkpoint inhibitors into the neoadjuvant setting for NSCLC, either as monotherapy, concomitantly with chemotherapy, or in the context of dual immunotherapeutic strategies (Table 1). The unique aspect of the neoadjuvant approach is that it provides clinicians with the opportunity to directly observe the patient's tumor response to treatment through the assessment of the postsurgical specimen. This pathological assessment can offer invaluable insights into the efficacy of the neoadjuvant regimen and the tumor's biological behavior under therapeutic pressure. In addition, the native tumor serving as an *in situ* "tumor vaccine" might provide optimal T cell responses as opposed to administration post-operatively in a micrometastatic setting. Monotherapy with immune checkpoint inhibitors has allowed for novel translational studies, but have yielded moderate efficacy [7] while combination chemotherapy and immunotherapy has shown more promising results from clinical trials, specifically significant improvements in pathological complete response rates and potentially long-term outcomes [8, 9]. Recent investigations predominantly gravitate towards the synergistic potential of perioperative chemotherapy combined with immune checkpoint inhibitors, underscored by clinical trial evidence suggesting substantial improvement in rates of pathological complete response, with the prospective to confer sustained survival benefits.

Current neoadjuvant immunotherapy studies

Nivolumab in combination with platinum doublet chemotherapy (PDC) was approved in the neoadjuvant setting for resectable NSCLC (Stage IIA to IIIA per AJCC eighth edition) without known driver mutations. The approval was based on the pioneering CheckMate 816 study which demonstrated a significantly improved EFS at 2 years of 63.8% versus 45.3% and HR of 0.65 (95% CI 0.47–0.90) of neoadjuvant chemo/immunotherapy versus PDC alone. Median EFS was 31.6 (95% CI 30.2-not reached) vs. 20.8 months (95% CI 14–26.7). The pathological complete response (pCR) after neoadjuvant chemoimmunotherapy (3 cycles) was also notably higher at 24% compared to 2.2% with chemotherapy alone demonstrating a dramatic effect on improving tumor response [8].

Several perioperative studies involving both use of neoadjuvant and adjuvant immunotherapy offer further new insights into management and treatment in the perioperative setting. The NADIM trial utilized the anti-PD1 agent, nivolumab in the perioperative setting along with PDC (carboplatin/paclitaxel) in the neoadjuvant setting followed by adjuvant nivolumab monotherapy for 1 year in 46 patients with stage IIIA NSCLC and showed 83% major pathological response (mPR), 63% pCR, with OS of 81.9% at 36 months [10, 11]. In the subsequent NADIM II trial, cohorts were expanded to stage IIIA and IIIB disease comparing chemoimmunotherapy versus PDC alone in the neoadjuvant setting followed by adjuvant nivolumab post-surgery for 6 months in those who underwent R0 resections and received nivolumab preoperatively. This trial has similarly demonstrated impressive findings [12], further supporting the argument for the use of perioperative use of immunotherapy in resectable NSCLC (see Table 1).

The recent FDA approval for perioperative use of pembrolizumab was based on results of the pivotal KEYNOTE-671 study where pembrolizumab along with PDC significantly improved pathological response as well as event free (EFS) and overall survival (OS). KEYNOTE-671 using neoadjuvant (4 cycles) and adjuvant (up to 13 cycles) pembrolizumab and platinum-based chemotherapy in stage II-IIIB (N2 stage) also showed a significant EFS HR of 0.58 with 62.4% versus 40.6% EFS at 24 months in the experimental versus the control arm of neoadjuvant PDC alone. Additionally, mPR was 30.2% versus 11.0%, pCR 18.1% versus 4%, and OS at 2 years of 80.9% versus 77.6% in the pembrolizumab compared to placebo group respectively. Interestingly, exploratory analysis has shown potential benefits in the perioperative use of pembrolizumab in those without mPR or pCR as well (HR 0.73 and 0.69 respectively) [9]. Also notably, the KEYNOTE-671 study included some patients with Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase (ALK) mutations—subgroups historically with limited benefit from immunotherapy [13–16].

Other perioperative studies such as AEGEAN and NEOTORCH have demonstrated similar benefits. The randomized AEGEAN trial which studied durvalumab versus placebo along with PDC in the neoadjuvant setting (4 cycles) followed by adjuvant durvalumab or placebo monotherapy up to 12 cycles in stage II-IIIB (N2 stage) NSCLC showed EFS at 24 months of 63.3% compared to 52.4% with HR of 0.68 with median EFS not met in durvalumab group versus 25.9 months in the placebo group. mPR was 33.3% versus 12.3% while pCR was 17.2% versus 4.3% [17]. Toripalimab (3 cycles in neoadjuvant and 1 cycle in adjuvant setting with PDC followed by toripalimab alone up to 13 cycles) was also administered to stage II-IIIB (N2 stage) NSCLC without EGFR or ALK mutations in NEOTORCH trial in China which also showed significant benefits with EFS at 2 years was 64.7% versus 38.7% with HR of 0.4 with median EFS not reached in toripalimab versus

15.1 months in the placebo arm with mPR of 48.5% versus 8.4% and pCR of 24.8% versus 1.0% [18].

Dual neoadjuvant immunotherapy with nivolumab and ipilimumab was also studied in the phase II NEOSTAR trial. Combination of ipilimumab and nivolumab compared to nivolumab alone improved mPR rate to 50% vs. 24% respectively [19]. The addition of chemotherapy to neoadjuvant ipilimumab plus nivolumab resulted in a mPR rate of 50%, compared to 32% with nivolumab alone [20].

Emerging perioperative studies

Unresolved challenges in the management of perioperative NSCLC include a need for deeper understanding of patient selection and better methods to determine treatment duration, in particular an improved prognostication of whether adjuvant therapy is needed in patients who received neoadjuvant chemoimmunotherapy. The design of the CheckMate 816 trial stands out for its focused examination of neoadjuvant treatment, demonstrating the clear benefits of incorporating neoadjuvant immunotherapy. This approach contrasts with other perioperative studies that combine both neoadjuvant and adjuvant treatments, a methodological choice that cannot separate the contribution of each component of therapy. The targeted approach used in CheckMate 816 trial was endorsed as the preferred design schematic by the FDA [21], setting a new standard for the design of perioperative clinical trials in this domain. Preliminary interim analysis from CheckMate 77T which compares neoadjuvant nivolumab with PDC and adjuvant nivolumab for 1 year to neoadjuvant placebo with PDC and adjuvant placebo for 1 year has met its primary endpoint of EFS (not reached vs. 18.4 months, HR 0.58), pCR of 25.3% vs. 4.7%, mPR of 35.4% vs. 12.1% with comparable tolerability as of now and awaits data maturation [22]. As such, many additional neoadjuvant immunotherapy (NCT06269211 (toripalimab)) and chemoimmunotherapy trials (NCT05962021 (toripalimab+PDC), NCT05157776 (sintilimab+PDC), NCT05882513 (serplulimab+PDC), NCT06241807 (camrelizumab+PDC)) are underway as well as perioperative studies (NCT05925530 (durvalumab+PDC followed by surgery and adjuvant durvalumab vs. chemoradiotherapy), NCT05116462 (sintilimab + PDC pre and post operatively followed by maintenance sintilimab vs. placebo). The results of these studies are awaited to further define the best use of perioperative therapy. However, none of them have a design that will allow clear understanding of the added value of the adjuvant treatment component.

There is consequently a pressing need for expanded research focused on patients who do not achieve a pCR following neoadjuvant therapy and for new studies incorporating novel biomarkers and experimental strategies. Additionally, despite the quite excellent outcomes of this group of patients, the role of continuing immunotherapy post-operatively in patients who have achieved pCR remains an open question. Refined biomarkers and prognostic tools are essential for precisely selecting patients likely to derive maximal benefit from adjuvant immunotherapy post-curative resection, thereby minimizing cumulative toxicities, alleviating treatment-related burdens, and reducing financial toxicity.

Perioperative treatment with EGFR/ALK mutations

Concerted efforts are similarly underway to advance therapies targeting driver mutations in earlier settings. Postoperative use of osimertinib for 3 years with or without adjuvant chemotherapy, as evidenced by the pivotal ADAURA trial in patients with resected stage IB-IIIA NSCLC who have EGFR exon 19 deletions or exon 21 L858R mutationshas significantly extended both DFS (90% vs. 44% at 2 years, 73% vs. 38% at 4 years) with HR of 0.17 at 2 years and 0.23 at 4 years as well as OS and furthermore CNS disease free rate was also significantly improved- 92% vs. 81% compared to placebo [19, 20]. Alectinib use of up to 2 years in adjuvant setting, evaluated in the ALINA study for stage IB-IIIA NSCLC patients with ALK rearrangements, was compared against adjuvant platinum based chemotherapy for 4 cycles and has also shown a considerable prolongation in DFS of 93.6% vs. 63.7% at 2 years with HR 0.24 [23]. Its assessment has led to recent FDA approval of alectinib in ALK-positive NSCLC in the adjuvant setting.

Some immunotherapy-focused studies, such as KEYNOTE-671 allowed patients harboring mutations in EGFR and ALK to participate and some subset analyses based on small numbers of patients are suggestive of potential benefits. However, given the outstanding activity of targeted agents for EGFR and ALK mutation harboring patients, the focus at present should revolve around optimal perioperative utilization of targeted therapies. Indeed, several other early studies also demonstrated potential use of targeted therapies in preoperative studies. NCT03433469 using 2 cycles of neoadjuvant osimertinib in stage IA-IIIA NSCLC with EGFR mutations demonstrated 15% mPR and 44% achieved lymph node downstaging [24] as well as NCT04201756 which utilized 2 to 4 cycles of neoadjuvant afatinib, achieved 9.1% mPR and 57.6% had pathological downstaging for stage III NSCLC [25]. Combinatory studies using targeted therapies with or without chemotherapy are also being explored: NCT04351555 (NeoADAURA; phase III osimertinib vs. osimertinib plus PDC vs. placebo plus PDC neoadjuvantly followed by physician's choice adjuvant treatment of targeted therapy with or without chemotherapy) [26], NCT04302025 (NAUTIKA1; single arm phase II neoadjuvant use of alectinib for 8 weeks) [27],

and NCT05015010 (ALNEO; single arm phase II neoadjuvant use of alectinib for 2 cycles followed by adjuvant use up to 24 cycles) [28].

Unmet needs in preoperative settings

One of the unmet needs in the preoperative setting for NSCLC is for accurate assessment of pathological response, which is integral to formulating decisions regarding postoperative therapy. Currently, surrogate endpoints such as pCR and mPR have been utilized to predict EFS and even more importantly OS. Although achieving mPR was observed to significantly correlate with improved survival in neoadjuvant chemotherapy trials [29, 30], further studies were needed to validate this in the era of immunotherapies and other therapeutics in resectable NSCLC. Recent meta-analysis of seven neoadjuvant randomized controlled trials showed that while pCR results were strongly correlative (R^2 = 0.82, β = 0.96) with EFS at 2 years, but that OS was only moderately correlative (R^2 = 0.55, β = 0.26). In addition, the association between hazard ratio of OS and EFS was poorly correlative (R^2 = 0.27, β = 0.11). This suggests that pCR, despite its strong linkage with EFS, may not be a completely accurate surrogate for the full clinical picture in assessing the long-term outcomes of neoadjuvant treatments [31]. Furthermore, given the potential for interobserver discrepancies due to the nature of estimating 0 or 10% residual tumor and non-standardized guidelines across trials and centers, the International Association for the Study of Lung Cancer (IASLC) published a guideline for pathologic assessment in neoadjuvant studies for NSCLC in 2020 to increase tumor sampling and assessment for tumors greater than 3 cm as well as inspection of the entire specimen for samples less than 3 cm in size [32, 33]. However, the impact of this guideline on clinical practice and patient outcomes remains uncertain, as its adoption and effectiveness in enhancing the precision of pathological assessments have yet to be thoroughly evaluated in diverse clinical settings. Developing and implementing universal pathological response assessment to facilitate more precise and informed clinical decisions that is timely, accurate, and reproducible in early-stage NSCLC is a critical and urgent need.

Lastly, there is a pressing need for novel treatments and innovative trial designs in the perioperative NSCLC landscape. While recent advances have introduced more effective treatment options, there remains a vast potential for discovering and integrating new therapies that could further enhance patient outcomes. The synergistic application of radiotherapy and immunotherapy has been observed to enhance immune priming, potentially contributing to new treatment avenues. Preclinical and clinical studies (in metastatic or recurrent settings) have shown that co-administration of immune check point inhibitor and radiation therapy may amplify release of major histocompatibility complex-1, tumor specific T cell response, as well as generating immune memory cells in tumor draining lymph nodes and potentially offer added clinical benefit [34–37]. Findings from a phase II clinical trial revealed that combination of neoadjuvant durvalumab with immunomodulatory doses of stereotactic radiation resulted in a higher mPR of 53.3% vs. 6.7% and although not statistically significant, three-year DFS rate of 83% compared to 69%, underscoring the potential of these combinatory approaches in improving patient outcomes [38]. Notably, however, the PACIFIC-2 trial with concurrent durvalumab and chemoradiotherapy compared to chemoradiotherapy for the treatment of unresectable stage III NSCLC did not meet its primary end point of PFS [39]. Similarly, JAVELIN trial in locally advanced head and neck cancer, the addition of avelumab to chemoradiotherapy also did not meet its primary end point of PFS [40]. A potential hypothesis behind several of these failures could be due to changes in tumor specific T cells after radiotherapy that negatively impacts the effect of immunotherapy. Select studies currently investigating the combination of radiation therapy and various immunotherapy include NCT05500092 (neoadjuvant nivolumab and chemotherapy with or without radiation), NCT04245514 (SAKK 16/18 chemotherapy followed by durvalumab followed by various radiation regimens then adjuvant durvalumab), NCT05798845 (neoadjuvant toripalimab plus radiotherapy), and NCT04933903 (NEO Rad neoadjuvant nivolumab, ipilimumab, and radiation).

Mechanistically novel therapeutics are being explored in the metastatic setting that potentially offer opportunities for patients with resectable NSCLC. Combination of different immune checkpoint inhibitors are under evaluation including a series of studies focused on T cell immunoreceptor with Ig and ITIM (TIGIT) antibody and lymphocyte-activation gene 3 (LAG-3) antibody and other novel checkpoints in a multidrug platform such as NEOCOAST which combines durvalumab with oleclumab (anti-CD73 monoclonal antibody (mAb)), monalizumab (anti-NKG2A mAb), or danvatirsen (anti-STAT3 antisense oligonucleotide) [41] (see Table 2). A phase II trial using combination of neoadjuvant nivolumab with or without relatlimab, a LAG-3 inhibitor, in stage IB-IIIA NSCLC was able to demonstrate mPR of 27% vs. 30%, DFS at 12 months of 89% vs. 93%, and OS at 12 months of 93% vs. 100% demonstrating potential for novel combinatory regimens [42]. Notably, antibody drug conjugates (ADC) targeting trophoblast cell surface antigen 2 (Trop-2), a transmembrane glycoprotein prevalent in NSCLC, are also gaining traction [43]. Sacituzumab govitecan, a Trop-2 targeted ADC, currently approved for metastatic breast and urothelial cancer based on improved PFS and OS [44–46], is now being studied in a range of lung cancer-focused studies. Furthermore, TROPION-Lung-02 phase 1b study

TABLE 2 Ongoing representative perioperative studies with novel immune checkpoint inhibitors and biomarkers.

Upcoming perioperative chemoimmunotherapy trials					
Trial	**Phase**	**Stage**	**Neoadjuvant treatment arm(s)**	**Adjuvant treatment arm(s)**	**Primary endpoint(s)**
NCT04316364	III	II-IIIB	Adebrelimab + PDC	Adebrelimab	mPR, EFS
NCT05116462	III	II, IIIA or IIIB (resectable N2 only)	Sintilimab + PDC	Sintilimab + PDC, followed by Sintilimab	EFS
NCT04158440	III	II-IIIB (N2 only)	Toripalimab + PDC	Toripalimab vs. placebo	mPR, EFS
NCT04606303	II	IIB-IIIB without driver mutations	Toripalimab + PDC	NA	mPR
NCT05882513	II	IIA-IIIB (no N3 patient)	Serplulimab + PDC	NA	pCR
NCT05157776	III	IIIA	Sintilimab + PDC	NA	pCR
NCT04865250 (iREP)	II	II, IIIA or select IIIB (T3N2 only)	Atezolizumab + PDC	NA	mPR
Emerging novel combination trials					
Trial	**Phase**	**Stage**	**Neoadjuvant treatment arm(s)**	**Adjuvant treatment arm(s)**	**Primary endpoint(s)**
NCT04832854 (SKYSCRAPER-05)	II	II, IIIA, or select IIIB (T3N2 only)	Arm 1 (high PD-L1 expression): Atezolizumab + Tiragolumab. Arm 2 (Any PD-L1 expression): Atezolizumab + Tiragolumab + PDC	Arm 1 (high PD-L1 expression): Atezolizumab + Tiragolumab or PDC Arm 2 (Any PD-L1 expression): Atezolizumab + Tiragolumab	mPR, surgical delays, operative and post-operative complications, surgical cancellations related to study treatment, AE
NCT05061550 (NeoCOAST-2)	II	IIA-IIIB	Arm 1: Oleclumab + durvalumab + PDC	Arm 1: Oleclumab + durvalumab	pCR, AE
			Arm 2: Monalizumab + durvalumab + PDC	Arm 2: Monalizumab + durvalumab	
			Arm 3: Volrustomig (Dose Exploration) + PDC	Arm 3: Volrustomig	
			Arm 4: Dato-DXd + durvalumab + single agent platinum	Arm 4: durvalumab	
			Arm 5: AZD0171 + durvalumab + PDC	Arm 5: AZD0171 + durvalumab	
NCT05360979	II	II, IIIA, IIIB (T3N2)	Envafolimab + Recombinant human endostatin + PDC	Envafolimab	mPR
NCT05891080	II	IIIB-IIIC	Arm 1: Toripalimab + JS004 + PDC Arm 2: Toripalimab + PDC	NA	pCR
NCT05387109	IV	II-IIIB (IIIB only T3N2)	Penpulimab + Anlotinib	individualized per patient	pCR

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TABLE 2 (*Continued*) Ongoing representative perioperative studies with novel immune checkpoint inhibitors and biomarkers.

Upcoming perioperative chemoimmunotherapy trials					
Trial	**Phase**	**Stage**	**Neoadjuvant treatment arm(s)**	**Adjuvant treatment arm(s)**	**Primary endpoint(s)**
NCT05742607 (MATISSE)	II	IIA-IIIA	Durvalumab + IPH5201 + PDC	Durvalumab + IPH5201	pCR, AE
NCT04040361 (EAST ENERGY)	II	IB-IIIA	Pembrolizumab + Ramucirumab	NA	mPR
NCT06088771	I, II	T1b or more advanced (>1 cm) and resectable	Dupilumab + Cemiplimab	standard of care	DLTs, mPR
NCT05577702	II	II-IIIA	Arm 1a: Tislelizumab Arm 1b: Tislelizumab and Ociperlimab Arm 1c: Tislelizumab and LBL-007 Arm 2a: Tislelizumab + PDC Arm 2c: LBL-007 + Tislelizumab + PDC	NA	mPR
NCT06077760	III	Resected Stage II, IIIA, IIIB (N2)	-	V940 + Pembrolizumab	DFS
Emerging novel biomarker/imaging focused trials					
Trial	**Phase**	**Stage**	**Neoadjuvant treatment arm(s)**	**Adjuvant treatment arm(s)**	**Primary endpoint(s)**
NCT04158440	III	II-III	Toripalimab + PDC	Toripalimab	PD-L1 in tissue specimen, TMB, WES and change of ctDNA in peripheral blood sample
NCT06221462 (PRIORITY)	II	IB-IIIB	Sintilimab + Anlotinib	Sintilimab	MRD ctDNA
NCT05429463 (neoSCORE II)	III	cIB-IIIA	Sintilimab + PDC	+/− RT, ± Sintilimab	PD-L1, ctDNA, TIIC
NCT04061590	II	I-IIIA	Treatment Arm-1: Pembrolizumab Treatment Arm-2: Pembrolizumab + PDC	NA	Proportion of patients with a ≥2-fold increase in the number of TIICs in post- versus pre-pembrolizumab treatment tumor specimens
NCT04638582	II	IA3 - IIA	Arm 1: Pembrolizumab Arm 2: Pembrolizumab + PDC	Arm 1 and 2: Pembrolizumab ± PDC	ctDNA resolution, imaging measures of response in correlation with pCR
NCT05925530 (MDT-BRIDGE)	II	IIB - IIIB (N2 only)	Durvalumab or Durvalumab followed by CRT	Durvalumab up to 12 months	ctDNA clearance
NCT02818920 (TOP1501)	II	IB (>/= 3 cm per CT), cIIA/IIB, IIIA (N0-2)	Pembrolizumab	Adjuvant CT ± RT f/b Pembrolizumab x 4	change in biomarkers, TIL, T cells specific against TAA, change in immunomodulatory effects, circulating T cells, gene expression of the PD-1/PD-L1 axis, correlation of pathologic response to the presence of TILs
NCT05882513	II	IIA-IIIB (no N3 patient)	Serplulimab + PDC	NA	ORR before surgery - the proportion of subjects with imaging PR or CR

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TABLE 2 (*Continued*) Ongoing representative perioperative studies with novel immune checkpoint inhibitors and biomarkers.

Upcoming perioperative chemoimmunotherapy trials					
Trial	**Phase**	**Stage**	**Neoadjuvant treatment arm(s)**	**Adjuvant treatment arm(s)**	**Primary endpoint(s)**
NCT04875585 (IMNWOP1)	II	IA3-IIIA (max. single station N2)	Pembrolizumab + Lenvatinib	Pembrolizumab	radiologic response, surgical resection rate
NCT04586465 (DYNAPET)	II	IIA-IIIB	Pembrolizumab + PDC	NA	mPR, dynamic PET-CT SUV change, ORR, uptake rate constant changes
NCT06241807	II	IIIA-IIIB (T3-4N2)	Camrelizumab + PDC	NA	ctDNA, serum metabolomics testing, 18F-FDG uptake value, circulating immunological biomarkers, and observation of tumor immune microenvironment

AE, adverse events; CR, complete response; CT, chemotherapy; ctDNA, circulating-tumor DNA; DFS, disease free survival; DLTs, dose-limiting toxicities; EFS, event-free survival; mPR, major pathological response; MRD, minimal residual disease; ORR, objective response rate; ORR, objective response rate; pCR, pathological complete response; PD-L1, programmed cell death ligand 1; PDC, platinum doublet chemotherapy; PET-CT SUV, positron-emission tomography and a computed tomography standardized uptake values; PR, partial response; RT, radiation therapy; SAE, serious adverse events; TAA, tumor-associated antigen; TTICs, tumor-infiltrating immune cells; TIL, tumor-infiltrating lymphocytes; TMB, tumor mutation burden; WES, whole exome sequence.

demonstrated promising results with datopotamab deruxtecan (Dato-DXd), another Trop-2 ADC, in combination with pembrolizumab with or without chemotherapy with objective response rate of 60% (with chemotherapy) and 55% (without chemotherapy) suggesting a potential synergistic effect that enhances antitumor immunity [47]. Building on these findings, the TROPION-Lung-01 study compared Dato-DXd with docetaxel in advanced and metastatic NSCLC, revealing a median PFS of 5.6 versus 3.7 months. Significantly, the HR was 0.63 in non-squamous histology types, suggesting that newer therapeutics like Dato-DXd could be promising agents for further study in earlier stages of NSCLC [48]. Results from further studies like TROPION-Lung07 and TROPION-Lung08 are awaited to confirm its efficacy in advanced NSCLC [49, 50]. Concurrently, innovative trials are incorporating these therapeutics into early-stage treatment. For instance, the NeoCOAST-2 trial (NCT05061550) evaluates a multidrug platform including neoadjuvant Dato-DXd, durvalumab, and platinum, while NCT06055465 explores the combination of neoadjuvant sacituzumab govitecan and pembrolizumab.

Furthermore, personalized mRNA vaccines encoding tumor-specific neoantigens used alongside immunotherapy are under investigation. The KEYNOTE-942 trial has demonstrated an improved recurrence-free survival (RFS) rate of 79% compared to 62% at 18 months (HR 0.561) by combining the V940 vaccine with pembrolizumab in a population of patients with resected high-risk melanoma [51]. Additionally, ongoing studies like INTerpath-002 (NCT06077760) are examining the role of the V940 messenger RNA vaccine in conjunction with pembrolizumab in the adjuvant setting for patients with completely resected stage II-IIIB NSCLC. Another trial, YE-NEO-001 (NCT03552718), is investigating a personalized neoepitope vaccine using a yeast vector in a similar adjuvant context. Collectively, these innovative modalities offer a more tailored and potentially more effective approach to cancer therapy, targeting the specific characteristics of individual tumors and potentially triggering a more robust immune response.

Biomarkers-current and future

In the effort to optimize perioperative immunotherapy, biomarker studies aim to identify potential prognostic and predictive correlates of treatment outcomes (Figure 1). For example, integration of circulating tumor DNA (ctDNA) assays has shown promise in perioperative trials. One such application of ctDNA includes monitoring ctDNA dynamics following neoadjuvant chemoimmunotherapy. In operable NSCLC, the NADIM trial showed that pretreatment mutant allele fraction (MAF) < 1% correlated with PFS and OS

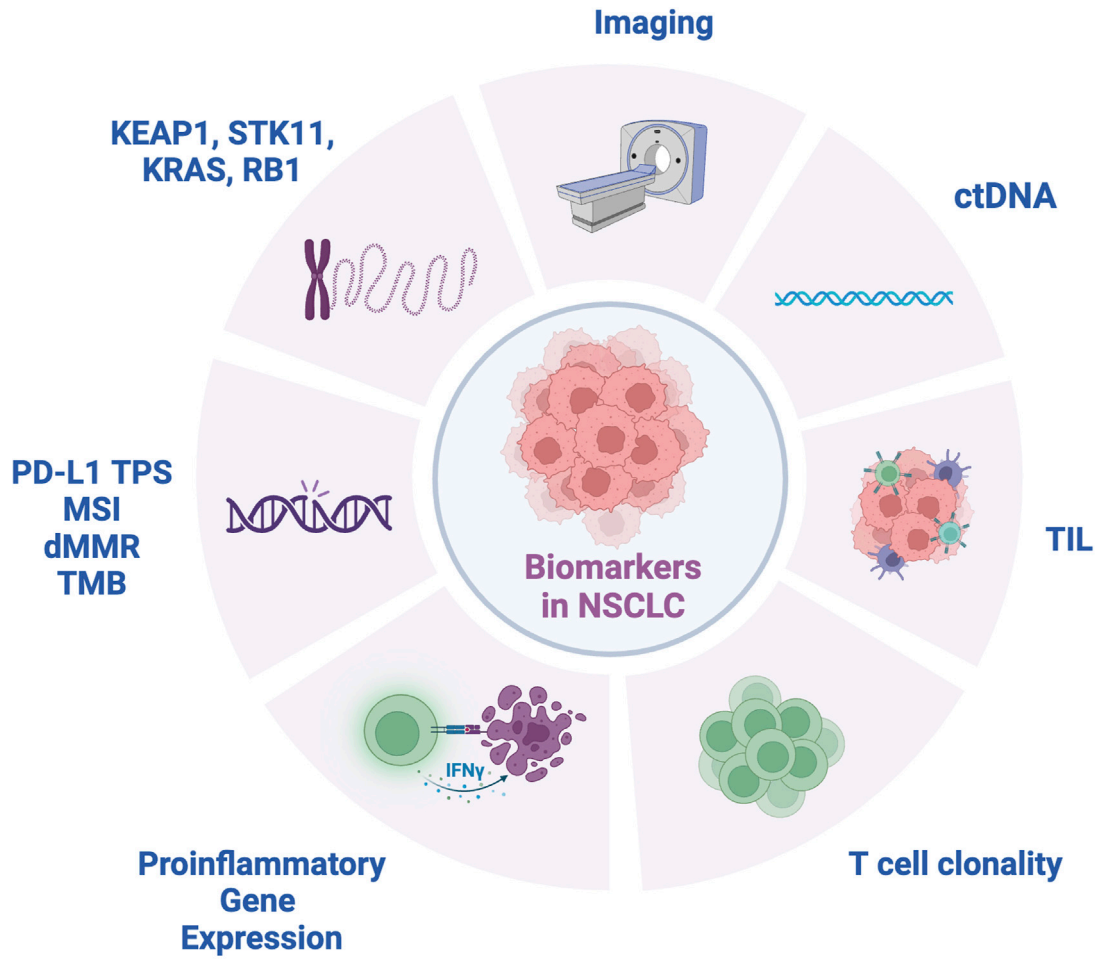

FIGURE 1
Biomarkers for NSCLC. ctDNA, circulating tumor DNA; dMMR, mismatch repair deficient; IFNγ, interferon-gamma; KEAP1, Kelch-like ECH-associated protein 1; KRAS, Kirsten ras sarcoma oncogene; MSI, microsatellite instability; RB1, retinoblastoma 1 gene; STK11, serine/threonine kinase 11; TIL, tumor infiltrating lymphocytes; TMB, tumor mutation burden; TPS, tumor proportion score.

benefits following neoadjuvant nivolumab and chemotherapy. In addition, clearance of ctDNA after neoadjuvant treatment was associated with improved PFS and OS [10]. CheckMate-816 similarly applied ctDNA dynamics to neoadjuvant chemotherapy, showing higher ctDNA clearance in preoperative nivolumab plus chemotherapy compared to chemotherapy alone. In addition, undetectable ctDNA following neoadjuvant treatment was positively associated with EFS and pCR [8].

Furthermore, ctDNA has also been applied to the assessment of minimal residual disease (MRD) (Figure 2). Indeed, in early-stage NSCLC treated with surgery and adjuvant chemotherapy and/or radiotherapy, ctDNA detection following resection was associated with clinical recurrence [34]. Similarly, the LUNGCA-1 study has shown a temporal association post-operatively between ctDNA presence and RFS [52]. In the perioperative immunotherapy space, the Impower010 trial showed that ctDNA detection postoperatively was similarly associated with a trend towards worse DFS in both patients treated with adjuvant atezolizumab and best supportive care following adjuvant chemotherapy [53].

Several studies are underway to further elucidate the role of MRD detected via sensitive MRD platforms in perioperative settings. One such trial is NCT04367311, a phase II study using chemoimmunotherapy with atezolizumab looking at ctDNA clearance in MRD-positive patients with resected stage I/IIA NSCLC. ADAPT-E is another phase II study assessing the utility of adjuvant durvalumab for stage I-III NSCLC with ctDNA positivity after definitive surgery or radiation and have completed standard of care chemotherapy as to achieving ctDNA clearance (NCT04585477). Future work using designs similar to the ADAPT-E trial are necessary to investigate whether MRD-positivity using ctDNA can better identify patients at risk of

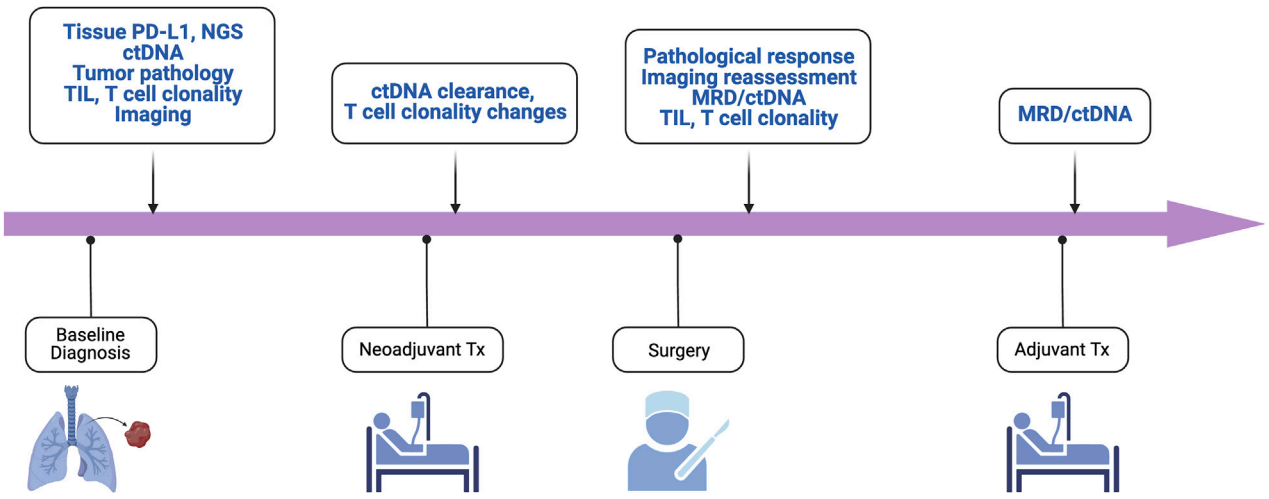

FIGURE 2
Key biomarkers for enhanced management and trial enrichment. ctDNA, circulating tumor DNA; mPR, major pathological response; MRD, minimal residual disease; NGS, next-generation sequencing; pCR, pathologic complete response; TIL, tumor infiltrating lymphocytes; TMB, tumor mutation burden.

recurrence postoperatively and guide the use of adjuvant immunotherapy to minimize that risk.

Despite the promise that ctDNA may hold as a biomarker of interest, there are important limitations to its use influenced by the inherent sensitivity of ctDNA detection methods [54] and clonal hematopoiesis of indeterminate potential (CHIP) to a lesser extent [55]. For example, ctDNA's utility is constrained by its limited sensitivity in cases of low tumor burden such as early-stage NSCLC [56] where the sparse release of tumor DNA into the bloodstream may fall below the detection threshold of current technologies. In addition, most trials do not specify CHIP, a condition characterized by the accumulation of somatic mutations in hematopoietic stem cells, which can interfere with the accurate interpretation of ctDNA mutations. This interference is especially problematic when the variant allele frequency (VAF) of ctDNA mutations is low, as DNA shed from white blood cells harboring CHIP mutations may be mistakenly attributed to tumor-derived DNA. Together, these challenges underscore the need for enhanced detection methods and interpretative strategies to accurately discern ctDNA's true clinical value in the management of cancer patients in neoadjuvant and perioperative contexts.

In metastatic NSCLC, recognized predictive biomarkers for immunotherapy response include PD-L1 tumor proportion score, microsatellite instability (MSI)/deficient mismatch repair (dMMR), and tumor mutational burden (TMB). Based on evidence for these biomarkers in multiple solid tumors, the FDA granted approval for pembrolizumab for MSI-high, dMMR, and TMB-high tumors regardless of tissue type [57]. Nonetheless, in the perioperative immunotherapy space TPS and TMB score have shown inconsistent results. While high PD-L1 TPS was associated with higher mPR rates in the LCMC3 and NEOSTAR studies, later phase trials have not reliably reproduced these findings although certainly general trends are observed of better results in patients with TPS score high positive tumors [58, 59]. In the phase III Checkmate 816 study, both PD-L1 positive and PD-L1 negative patients showed improved pCR rates with neoadjuvant chemoimmunotherapy. Of note, the PD-L1 high patients had a highly impressive close to 50% pCR rate and showed the greatest improvement in EFS(8). In addition, inconsistent results were noted in the adjuvant setting as well where TPS score appeared to correlate with DFS in the Impower 010 study, while the Phase III PEARLS trial found that adjuvant pembrolizumab was associated with longer DFS across all PD-L1 subgroups [60]. As for TMB, its role remains unclear in the perioperative space as several studies thus far including the Checkmate 816, LCMC3, and NADIM trials evaluating TMB and its association with pCR have failed to show a significant relationship [8, 10, 59].

The perioperative setting is ideal for studying novel biomarkers by examination of both pre-treatment and post-treatment tissue obtained following surgical resection. For example, several trials have investigated how the immunophenotype of the tumor microenvironment and in circulating peripheral blood may relate to perioperative immunotherapy outcomes. T-cell repertoire was evaluated in an early-phase trial of neoadjuvant nivolumab showing that tumors demonstrating a mPR showed a higher clonality of the T-cell population in both the tumor and peripheral blood [7].

LCMC3 employed similar methods to evaluate T-cell responses in resected NSCLC following neoadjuvant atezolizumab finding that tumors with mPR were significantly associated with an expansion of peripheral blood-activated $CD8^+$ T cells [59]. The NEOSTAR trial studying neoadjuvant nivolumab and ipilimumab versus nivolumab evaluated the immune cell infiltration of pre- and post-therapy tumor specimens using multiplex immunofluorescence and demonstrated that dual-immunotherapy combination induced greater overall tumor infiltration of $CD3^+$ and $CD3^+CD8^+$ T lymphocytes, tissue-resident memory cells, and effector memory T cells than single-agent nivolumab [58]. In the NADIM trial investigating neoadjuvant nivolumab plus chemotherapy, tumors achieving pCR were associated with a proinflammatory gene expression profile and higher upregulation of IFN-γ-responsive genes involved in antitumor response [61]. Those tumors without pCR, however, showed upregulation of genes related to proliferation. Additionally, peripheral blood collected from patients enrolled in the NADIM trial showed a differential profile of immune parameters based on pCR or non-pCR, such as higher $CD4^+$ PD-1+ cells and lower monocyte CTLA-4 expression in patients with pCR [62]. Future studies are necessary to elucidate how pathologic correlates such as T cell clonality, immune cell infiltration, and immune gene expression in the tumor microenvironment and peripheral blood relate to perioperative immunotherapy response.

Another group of biomarkers of uncertain significance are several key somatic mutations associated with poor response to immunotherapy. For example, a *post hoc* analysis of the POSEIDON trial evaluating combined PD-L1 and CTLA-4 inhibition with durvalumab and tremelimumab plus chemotherapy in metastatic NSCLC showed that patients with KEAP1, STK11, and KRAS mutations benefited more from combination immunotherapy [63]. In addition, results from the perioperative NADIM trial showed that tumors with KEAP1, STK11, and RB1 mutations were less likely to show a benefit from preoperative immunotherapy [10]. Further efforts will be required to determine how the presence such molecular alterations can guide perioperative immunotherapy treatment strategies.

Lastly, radiomic biomarkers have shed light to predicting response to immunotherapy in the perioperative settings. Literature shows imaging biomarkers such as maximum standardized uptake value (SUVmax), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) from positron emission tomography/computed tomography (PET/CT) scans have demonstrated prognostic significance in resectable NSCLC. A meta-analysis found that high SUVmax, MTV, and TLG correlated with lower disease free survival (HR of SUVmax = 2.43, MTV = 2.49, TLG = 2.97) and OS (HR SUVmax = 1.52, MTV = 1.91, TLG = 1.94) in resectable NSCLC [64]. Sun et al also demonstrated high radiomic based biomarker of tumor infiltration with CD8 cells was associated with better overall survival of 24.3 months compared to 11.5 months in those with low radiomic score (HR 0.58) [65]. Zerunian et al assessed CT-derived texture parameters less than 56.2 mean value of positive pixels to be associated with lower OS and PFS with HR 0.89 with pembrolizumab use [66]. Further work is needed to explore how imaging biomarkers can be systematically integrated into perioperative trials to enhance prognostication and therapeutic approaches.

The development of precise biomarkers is critical in the perioperative setting to optimally treat patients with NSCLC. Current limitations may hinder personalized treatment planning and often lead to a trial-and-error approach, potentially delaying the identification of the most effective treatment for individual patients. The development and validation of reliable predictive biomarkers is essential to optimize treatment selection, enhance response rates, and avoid unnecessary toxicity from ineffective therapies.

Conclusion

In this review, we summarize the current rapidly evolving landscape of perioperative therapy for NSCLC. The evidence gathered from recent clinical trials underscores the potential of neoadjuvant approaches to improve and augment surgical outcomes, enhance pathological response rates, and ultimately, increase overall survival rates for patients with resectable NSCLC. Further challenges in optimizing patient selection to identify ideal candidates for neoadjuvant treatments, duration of treatment, and optimal treatment regimen are still ongoing and need to be supported. Integration of molecular profiling and the development of predictive biomarkers hold promise for personalizing neoadjuvant treatment approaches, potentially enabling the tailoring of therapy to individual patient characteristics and tumor biology. Moreover, the exploration of novel therapeutic agents and combinations, as well as the innovative endpoints in trial designs, will be crucial in overcoming resistance mechanisms and improving patient outcomes.

Author contributions

HJ and BH conceptualized the manuscript. HJ and AD'A drafted the manuscript. HJ, RG, AD'A, BS, PI, and BH revised the manuscript. HJ drafted the figures. HJ and RG drafted the tables. BH provided overall supervision of this manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The rapidly changing field of predictive biomarkers of non-small cell lung cancer

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Lung cancer is a leading cause of cancer-related death worldwide in both men and women, however mortality in the US and EU are recently declining in parallel with the gradual cut of smoking prevalence. Consequently, the relative frequency of adenocarcinoma increased while that of squamous and small cell carcinomas declined. During the last two decades a plethora of targeted drug therapies have appeared for the treatment of metastasizing non-small cell lung carcinomas (NSCLC). Personalized oncology aims to precisely match patients to treatments with the highest potential of success. Extensive research is done to introduce biomarkers which can predict the effectiveness of a specific targeted therapeutic approach. The EGFR signaling pathway includes several sufficient targets for the treatment of human cancers including NSCLC. Lung adenocarcinoma may harbor both activating and resistance mutations of the EGFR gene, and further, mutations of KRAS and BRAF oncogenes. Less frequent but targetable genetic alterations include ALK, ROS1, RET gene rearrangements, and various alterations of MET proto-oncogene. In addition, the importance of anti-tumor immunity and of tumor microenvironment has become evident recently. Accumulation of mutations generally trigger tumor specific immune defense, but immune protection may be upregulated as an aggressive feature. The blockade of immune checkpoints results in potential reactivation of tumor cell killing and induces significant tumor regression in various tumor types, such as lung carcinoma. Therapeutic responses to anti PD1-PD-L1 treatment may correlate with the expression of PD-L1 by tumor cells. Due to the wide range of diagnostic and predictive features in lung cancer a plenty of tests are required from a single small biopsy or cytology specimen, which is challenged by major issues of sample quantity and quality. Thus, the efficacy of biomarker testing should be warranted by standardized policy and optimal material usage. In this review we aim to discuss major targeted therapy-related biomarkers in NSCLC and testing possibilities comprehensively.

KEYWORDS

NSCLC, driver oncogenes, immune checkpoint inhibitor, gene fusion, biomarker

Introduction

Primary lung cancer used to be a rare tumor in the past, however today it is the most common cause of cancer mortality worldwide. In 2020, lung carcinoma was the second most common malignancy, with around 2.2 million newly diagnosed cases. Moreover, with 1.796 million cases, it was the number one cause of cancer deaths [1]. The incidence in both of US and EU is now declining, that follows the trend of shrinking smoking prevalence [2]. The incidence rate in male patients increased from 1973 to 1984 (83.5 and 97.9/100,000 person-years, respectively) followed by a gradual decrease till 2015 (55.3/100,000 person-years), while in female patients the incidence increased in a more extended period from 1973 to 2007 (20.2–51.3/100,000 person-years) and then subsequently decreased to 2015 (44.2/100,000 person-years). The trend in the incidence of lung carcinoma in men in Central Europe is similar to that seen in Western countries, but with a slight delay. Unfortunately, according to the latest data, the incidence of lung cancer is still rising in women [3].

About 85% of lung cancer cases are non-small cell carcinoma, the remaining 15% belong to the clinically separate category of small cell carcinoma. The group of non-small cell carcinomas is further subdivided as adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and several other relatively rare histological types [4]. The relative frequency of histological types of lung carcinoma has also changed significantly in recent decades. In the past the squamous cell carcinoma was the most common type of lung cancer but their relative frequency declined and the general occurrence of adenocarcinoma increased. Today, adenocarcinoma is the most common type, accounting for about 50%–56% of all lung cancer cases and is the most frequent histological subtype in never-smokers [5]. Increased risk of adenocarcinoma in smokers is a result of changes in design and composition of tobacco products. The introduction of ventillated filters in cigarettes and the increased levels of tobacco-specific nitrosamines both have played a role [6–10]. The decline of squamous cell carcinoma follows the trend of declining smoking prevalence in industrialized countries [2].

As incidence and mortality data indicate, lung cancer is one of the most aggressive cancers and has an unfavorable outcome. Classical treatment options for lung cancer include surgical resection or chemo- and radiotherapy, depending on clinic-pathological variables. For patients with early-stage lung cancer, surgical resection is the optimal treatment option, while patients with locally advanced or metastatic NSCLC and most SCLC patients are treated with chemo-radiotherapy. However, in the last decades, significant progress has been made in understanding the molecular pathogenesis of lung tumours, both NSCLC and SCLC groups. Even SCLC, which was previously thought to be uniform, has been shown by recent data to be divided into at least 4 distinct molecular subgroups [11].

Thanks to the massive increase in genetic and immunological knowledge the variety of treatment methods has also shifted over the decades. Therapeutic targeting of the EGF-receptor introduced the era of biological therapies, and a growing list of specifically acting agents is now effectively used in selected cancer patients. So-called oncogene-addicted NSCLC is a molecular genetically distinct group of lung cancers in which well-defined driver mutations direct the pathogenesis, and pharmacological blockade of this target is expected to result in a significant therapeutic response. These patients also form a clinically well-defined group, mostly non-smokers, female predominance, and a relatively younger age are the main characteristics.

The frequency of currently known clinically significant driver gene defects is shown in Figure 1.

In addition, another group of lung tumours is also emerging, lacking targetable driver mutations to our present knowledge. However, due to marked immunogenicity, these patients may well benefit from immune checkpoint inhibitor therapies [12].

The introduction of molecularly targeted therapies promise a major advance, however lung cancer still has a poor prognosis and the 5-year survival rate remains at a very low level, the 5-year OS was 10.7 in 1973 for all lung cancer patients, which increased to 19.8% in 2010 [13, 14]. As seen in Western countries, survival rates for lung cancer patients in Central Europe have improved over the past decade, particularly after the introduction of immunotherapy. For non-squamous NSCLC, the 3-year survival in 2019 was 28.7% compared to 14.5% in 2011, and for squamous cell carcinoma 22.3% versus 13.37%. Unfortunately, for SCLC, there was no significant improvement over the study period [15].

The emergence of new molecular targets has also challenged diagnostic pathology by requiring the identification of appropriate predictive biomarkers and by the development of reliable, cost-effective testing options.

In this study, we aim to review the main events in the molecular pathogenesis of NSCLC also serving as therapeutic targets. Furthermore, we aim to present the status of biomarker testing options.

Gene mutations and copy number changes

EGFR mutations

Adenocarcinomas harbor mutations of genes of EGFR signaling pathway. EGFR belongs to the epidermal growth factor RTK (receptor tyrosine kinase) family. The EGF receptor has an extracellular ligand-binding, a transmembrane and an intracellular domain, the latter having tyrosine kinase

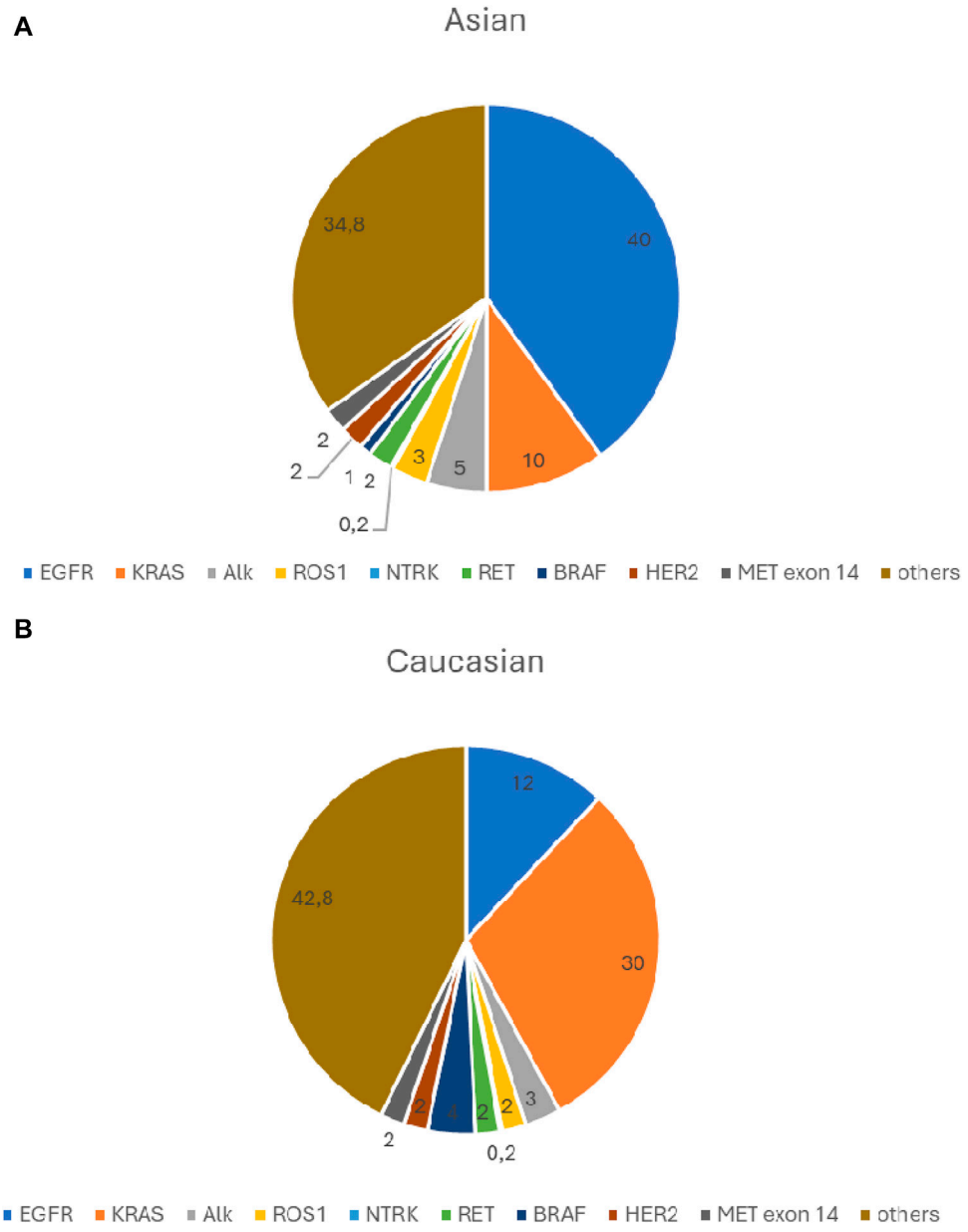

FIGURE 1
(A) Significant driver gene defects frequency- Asian population. **(B)** Significant driver gene defects frequency- Caucasian population.

activity. The intracellular protein kinase domain contains a small N-terminal lobe and a larger C-terminal lobe. The two parts form the active site cleft that serves as a binding site for ATP. In physiological conditions the extracellular ligand binding (EGF) activates the receptor and the downstream signaling results in cellular transactivation, cell proliferation and survival.

The use of the EGFR receptor as a potential therapeutic target was already suggested in the 1980s by Mendelshon and colleagues [16, 17]. As in many other tumors, EGFR expression has been shown to be increased in lung adenocarcinomas, an early event in carcinogenesis [18]. In 2002, the first data on an EGFR inhibitor treatment in NSCLC were published [19, 20]. The first clinical results showed considerable variability in the response rate [21, 22]. Benefits were mostly observed in non-smokers, women and in the Far Eastern population. The level of EGFR expression detected by immunohistochemistry has not been shown to be of predictive value [23, 24]. Mutations in EGFR were first identified in 2004 and have also been associated with response to therapy [25–27]. All the detected genetic abnormalities were

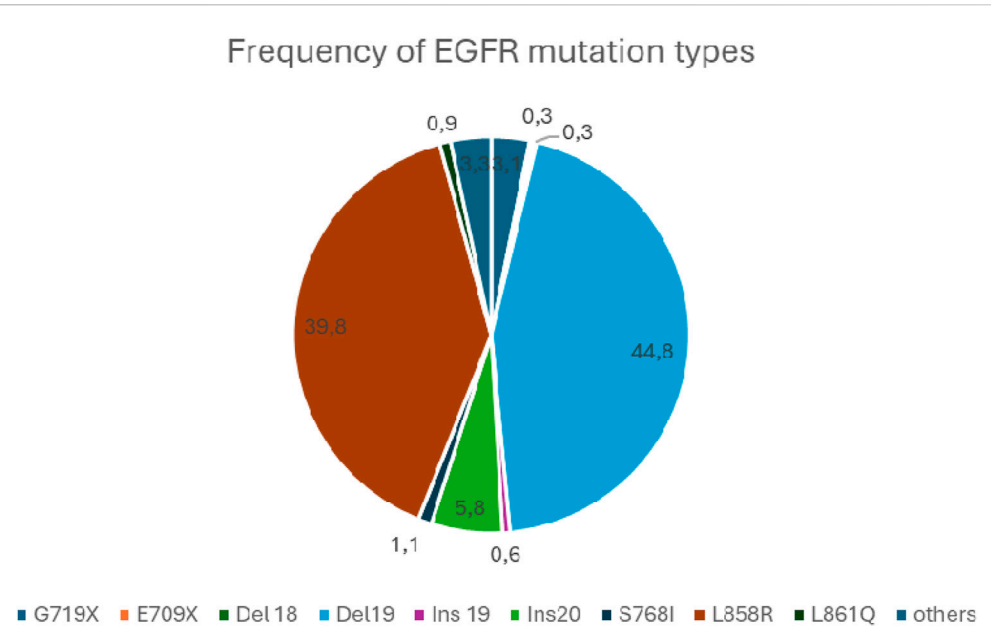

FIGURE 2
The relative frequency of EGFR mutations.

heterozygous, and wild-type EGFR was found in normal lung tissue adjacent to the tumor, suggesting that these mutations are somatic [25]. The EGFR mutation could also be detected in the benign epithelium surrounding the tumor, demonstrating that it is an early event in carcinogenesis [28]. Mutations were demonstrated to affect the kinase domain of the receptor protein causing constitutive activation and downstream signaling in the absence of the receptor ligand. Women and never-smokers were preferentially involved. EGFR mutations show ethnic differences, prevalence ranges 10%–15% in Caucasians and 30%–40% in Asians [29]. Genetic alterations of other major oncogenes, such as KRAS, ALK, ROS1 are mutually exclusive with EGFR mutations. Among adenocarcinomas EGFR mutations are frequently detected in cases with lepidic and papillary growth patterns. The two most common mutations, the so called classical EGFR mutations are i) mutation at codon 858, replacing leucine 858 with arginine (L858R) and ii) the in-frame deletion in exon 19 causing removal of amino-acid residues 746–750 of the expressed protein [30]. These two mutations account for 85%–90% of all EGFR mutations.

Both classical EGFR mutations affect the kinase domain. In the inactive form of the wild-type EGFR molecule an outward rotation of the alphaC helix in the N lobe is provided, which is stabilized by the helical turn of the A-loop. This conformation inhibits the association of amino acids K745 with E762 and the consecutive binding and orientation of ATP. The L858R mutation occurs in the C lobe N-terminal portion of the activation loop resulting the destabilization of the inactive state thereby promoting the conversion in a more active state [31, 32]. The deletion in exon 19 (746ELREA750) occurs immediately before the αC-helix in the N lobe and desrupts the inactive conformation through the shortening of the alpha C loop. The L858R mutant is approximately 50-fold more active than the wild-type enzyme, and the G719S mutant shows about ten times more activity over the wild-type.

In addition to the classic mutations mentioned above (L858R and del19), about 600 other rare EGFR mutations have been described, accounting for about 10%–15% of all EGFR mutations [33]. These include exon 18 E709x, del18, G719x, exon19 insertion, exon20 insertion, and S768I, as well as L861Q affecting exon21. Of these, exon 20 insertions are the most common (4%–10% of all EGFR mutations).

The relative frequency of EGFR mutations based on the COSMIC database is shown in Figure 2 [34].

EGFR mutations show variable sensitivity to EGFR tyrosine kinase inhibition. The specific genotype obtained by DNA sequencing gains special importance as the type of mutation carries the basic potential of resistance to given inhibitor agents. It is of note that resistance or low response to TKI today indicates the consideration of an alternative inhibitor. No response to EGFR TKI treatment has been observed in case of exon 20 insertions [35] except A763-Y764insFQEA [36–38]. For others, the therapeutic effect is less than observed for the classical mutations, e.g., G719x, exon 19 insertions, S768I, L861Q. For these mutations 2nd generation TKI treatment may show improved results.

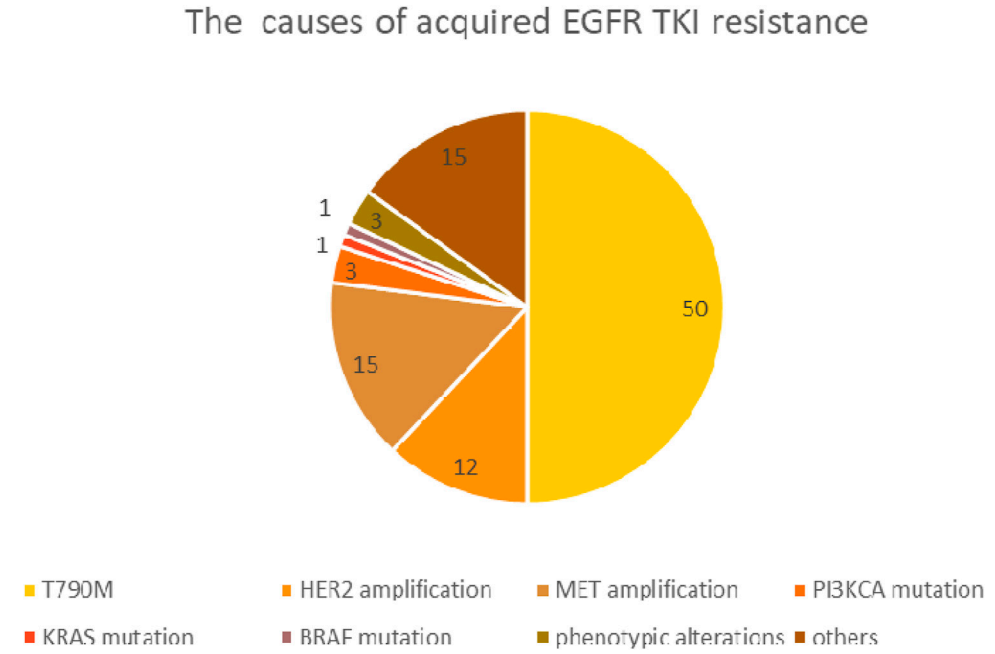

FIGURE 3
The most common causes of acquired EGFR TKI resistance.

Germline mutations of the EGFR gene were also described [39], which, if present, increase the incidence of adenocarcinoma, mostly in the presence of additional somatic mutations. The 4 most common germline mutations are T790M (which is one of the most important causes of acquired TKI resistance), V843I, R776H, P848L, all point mutations.

An EGFR activating mutation is a prognostic factor indicating unfavorable outcome. On the other hand, it is a factor predicting the response to tyrosin kinase inhibitor treatment. T790M mutations and mutations in exon 20 are associated with resistance to tyrosin kinase inhibitors.

Although EGFR-TKI treatment has been shown to be effective in most patients with EGFR mutant lung adenocarcinomas, no response is observed in 5%–25% of patients due to intrinsic resistance to these drugs. In addition to the pharmacokinetic complications of the drug, intrinsic resistance is frequently due to some genetic variation. These include most of the exon20 insertions, with the exception of A763-Y764insFQEA- [36–38], T790M, exon 2-7 variant III(vIII) in frame deletion [40] and some other secondary genetic events.

Patients with an initially good therapeutic response may show unfortunate disease progression after 9–19 months of treatment initialization due to acquired resistance (first line TKI: 9–12 months, 19 months first line third generation TKI) [41, 42]. Acquired resistance is often the result of a secondary EGFR mutation, while in many cases it occurs due to activation of an alternative signaling pathway. The most common (49%–63%) acquired mutation is T790M, which results in a threonine-methionine substitution at position 790, the ATP binding site of the receptor protein. This amino acid exchange prevents EGFR-TKI from binding to the kinase domain and results in increased ATP affinity. Further to *de novo* mutagenesis it is assumed, that a co-existing, drug-resistant EGFR T790M positive subclone has been selected by TKI treatment. In addition to the T790M mutation, EGFR gene amplification and several rare second/third EGFR mutations are considered as resistance mechanisms, including the C797S, L792, L718Q, SV768IL genotypes. The activation of alternative signaling pathways may also occur through a gene amplification, such as MET, HER2 or other rare gene mutations. Resistance may also result from tumor phenotypic alterations, such as transformation towards squamous or small cell carcinoma and epithelial-mesenchymal transition [43, 44]. The most common causes of acquired EGFR TKI resistance and their relative frequency are shown in Figure 3.

It is surprising that, despite the wealth of data that has been accumulated on the incidence and therapeutic significance of EGFR mutations, very little is known about the origin and causes of EGFR mutations. Few publications correlate different etiological factors and EGFR mutations. It appears that air pollution, including exposure to microparticles, can induce EGFR mutations [45]. In addition, exposure to radon in the residential environment may also have a potential EGFR mutation-inducing effect [46].

EGFR genotyping is generally based on sequence analysis of tumor DNA isolated from biopsy samples. As a fast alternative approach to predict the efficacy of EGFR inhibitory treatment, the expression of total EGFR protein was first attempted for immunohistochemistry, but as already mentioned, this remained inconclusive [23, 47] Mutation-specific monoclonal antibodies against exon 19 E746-A750del and exon 21 L858R mutations are available, which represent approx. 90% of all EGFR mutations (clone 6B6 for exon19 deletions; and clone 43B2 against point mutation of exon21 L858R, Cell Signaling Technology) [48]. Tests with these antibodies have shown relatively high sensitivity and specificity for the two mutations indicated, especially for L858R. The lower sensitivity for exon19 deletions is mostly explained by the fact that the exon19 antibody only detects the most common deletion of exon19 (deletion E746-A750). This deletion is of 15 base pairs and represents 50%–65% of all exon19 deletions. However, a variety of deletions, including 9, 12, 16, 18, and 24base-pairs variants have been identified, each producing slightly different protein and antigen epitope structure not detectable by the commercial exon19 antibody [49]. According to the literature the EGFR mutation-specific antibodies have a fair sensitivity and high specificity in identifying lung adenocarcinomas with classic EGFR mutations, while they do not recognise uncommon EGFR mutations. They did not provide sufficient sensitivity (about 40%–60%) or specificity (70%) for the detection of all EGFR mutations, compared to gold standard sequencing methods [50–53]. Sanger DNA sequencing has been widely used, but its disadvantages, primarily its low sensitivity (requirement of 40%–50% mutant DNA in samples) [54], has before led to the development of more sensitive detecting methods including real-time quantitative PCR (RT-PCR). However, this method is relatively expensive, time consuming, and not incorporated in routine diagnostic procedures in many departments of pathology. In contrast, immunohistochemistry has lower costs, shorter turnaround time, and is available in the majority of laboratories. IHC is a rapid, cheap and well-known assay that does not require huge tumor cell content and performs quite well even in degraded tissue (e.g., decalcified bone tissue) or cytology samples. EGFR-mutant-specific antibodies cannot replace conventional molecular methodologies, but they could be very helpful in small tumor samples with poor material [53].

KRAS mutations

KRAS is one of the longest known oncogenes and activating mutations play a crucial role in the early oncogenesis of several types of tumors, such as pancreatic ductal adenocarcinoma, colon cancer and lung cancer [55]. HRAS and NRAS are the other two members of the RAS gene family with clinical impact [56]. The human homologue of the RAS gene, HRAS, located on the short arm of chromosome 11 at position 11p15.1-11p15.3, was first described in the early 1980s in a human bladder cancer cell line. A short time later KRAS, a gene showing homologue features was detected in lung adenocarcinoma, located on the short arm of chromosome 12 at position 12p11.1-12p12.1. The NRAS gene is located on chromosome 1 [57]. All three RAS genes have 4 exons and broadly similar structures [58]. The KRAS gene encodes two protein isoforms composed of 188 and 189 amino acids (KRAS-4B and 4A), the single amino acid difference is a result of alternative splicing [59]. The KRAS protein is a cell membrane-associated G protein with GTP-ase activity [60,61], coupling cell surface growth factor receptors such as EGFR to various intracellular (mitogenic) signaling pathways. The most common signaling pathways involved are the mitogen activated protein kinase (MAPK) [62, 63] and phosphatidylinositol 3-kinase (PI3K) pathways [64]. The active RAS protein binds GTP, in which guanine nucleotide exchange factors (GEFs) participate through GTP-GDP exchange. GTPase activating proteins (GAPs) inactivate RAS by enhancing the GTPase activity of the RAS [60, 61, 65]. RAS gene mutations at specific sites result in spontaneous activation of the RAS protein without mitogen signaling, leading to uncontrolled cell proliferation and cell survival. As these play an important role from the earliest phase of carcinogenesis they are called oncogenic, or hot-spot mutations [55, 66] In the case of KRAS G12C or G12V mutations, intracellular levels of RAS-related proteins (RALs) are elevated and AKT phosphorylation is reduced [67].

The earliest and most frequent mutations in lung adenocarcinoma occurs in the KRAS gene and affects the EGFR/RAS/RAF signaling pathway [68–70]. The prevalence of the KRAS mutation is around 30% in the Western population, compared to around 10% in the Far East. In lung cancer, mutations in NRAS or HRAS are rare, with a prevalence of less than 1% each. The vast majority of KRAS mutations (about 80%) affect codon 12 within exon 2 of the gene and is close to the section encoding the nucleotide binding site of the KRAS protein. The most common change of codon 12 mutations is a G>T transversion, which results in a glycine-cysteine (G12C) or glycine-valine (G12V) substitution at the protein level. Another type of codon 12 mutation is G>A transition, replacing amino acid glycine by aspartic acid (G12D). Less frequent codon 12 mutations are also known: G12A, G12S, G12R, G12F. Occasionally, mutations can be detected in codon 13 (G13C) or codon 61 (Q61H) [71].

Mutations generally inhibit the interaction of KRAS with GAPs and the hydrolysis of GTP bound by KRAS, thereby keeping the KRAS protein in an active conformation [72]. Different mutations activate different intracellular signaling pathways to different degrees. In KRAS G12C mutant tumors, the classical mitogenic signaling pathway (RAS-RAF-MEK-ERK) is activated, whereas the PI3K-AKT-mTOR pathway is dominant for the other mutant genotypes. This may be due to a different RAF affinity in each mutation type [73] The G>T transversions

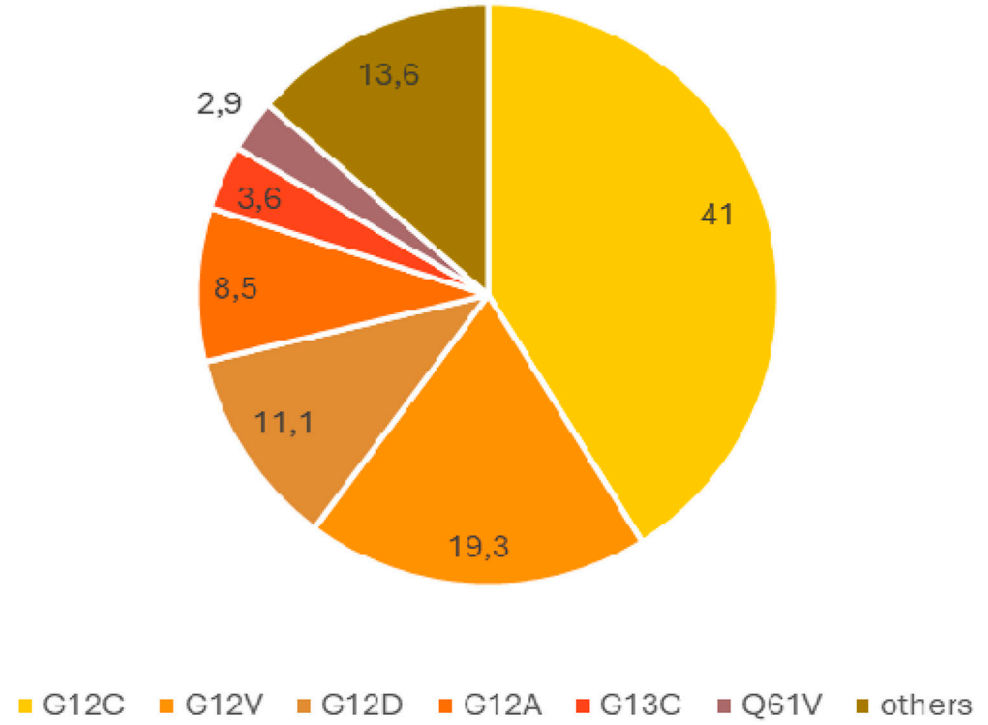

FIGURE 4
Average relative frequency of the most common KRAS mutation types.

resulting in G12C exchange are supposedly associated with polycyclic aromatic hydrocarbons (PAH) exposure in tobacco smoke. On the other hand, G>A mutations resulting in KRAS G12D are more common in non-smokers. The prevalence of KRAS mutations is 40% in heavy smokers, 30% in former current non-smokers and 20% in never-smokers [74]. The most common type is G12C, which accounts for 40% of all KRAS mutations, while G12V and G12D account for 19% and 11%, respectively. The average relative frequency of KRAS mutation types, based on large databases, is shown in Figure 4.

There are also significant geographical and social differences in the prevalence of KRAS G12C, with a higher prevalence in Western countries and a lower prevalence in the Far East (8.9%–19.5% and 1.4%–3%, respectively), which may be related to smoking habits. Our own observation also suggests this, as KRAS G12C is more common in Eastern Hungary, the care area of our centre, than in the better developed western part of the country, which may be explained by the smoking habits of the population here (unpublished data). Although KRAS mutations, especially G12C, are strongly associated with smoking, KRAS mutations are surprisingly unfrequent in small cell lung cancer, which occurs almost exclusively in heavy smokers [74, 75]. In squamous cell carcinomas, which are also strongly associated with smoking, the occurrence of KRAS mutations is rare or only occurs in mixed tumors, such as adenosquamous carcinoma [71]. The frequency of KRAS mutations also varies in the histological subtypes of adenocarcinoma. KRAS mutations are most often detected with mucinous morphology, but different genotypes show variable frequencies (G12C rarely, whereas G12V and G12D more often) [76]. Among mucinous tumors, KRAS mutations are almost exclusively found in invasive mucinous adenocarcinomas (69%), with no KRAS mutations detected in adenocarcinoma *in situ* and colloid carcinoma. However, mucinous carcinomas do not harbour EGFR mutations [77]. Interestingly, the KRAS mutation is more common in women [78]. The metastatic pattern of the tumor also differs with the mutant genotype, with G12C tumors having a higher incidence of intrapulmonary metastasis (38% vs. 21%) and a lower incidence of pleural metastasis (4% vs. 39%). It was also observed that brain metastasis is less frequent in KRAS mutations (33% vs. 40%), but the frequency of brain metastasis is similar for each KRAS mutation type [79, 80].

The presence of EGFR and KRAS mutations are mutually exclusive. In theory they affect the same signaling pathway and the presence of one mutation is sufficient for tumourigenesis. Despite this generally accepted view, KRAS and EGFR mutations can rarely occur within the same tumor [76, 81]. A recent large retrospective study of data from 3,774 patients found the concomitant presence of two or three driver mutations in 1.7% of cases, most commonly EGFR/KRAS mutations (in 0.53% of cases). It should be noted that this study examined a patient population from the Far East, where the prevalence of EGFR mutations is inherently high, 43% in this study [82].

The particular role of KRAS in tumorigenesis is also suggested by the observation that in one of the most common congenital pulmonary malformations, congenital pulmonary airway malformation type I (CPAM), KRAS mutations (G12V or G12D) are often detected, predominantly in intracystic mucinous cell clusters. In fact, these mutations can be identified in nonmucinous cystic regions as well, but not in healthy lung tissue. In these patients, KRAS mutant adenocarcinoma of mucinous character frequently appears later, indicating to the oncogenic nature of the mutation [83–85].

The prognostic role of KRAS mutations in lung adenocarcinoma has long been the subject of intensive studies. Although many data have been published on this issue, its prognostic significance is still not clearly understood. Investigations of prognostic significance are complicated by the fact that the term "KRAS mutation" itself is not uniform, mutation types, ethnic, gender and histological differences as well as treatment mode should also be considered. In addition, other genetic abnormalities, such as EGFR mutations, modify the behavior of KRAS wild-type cases and affect prognosis [86]. However, most of the large case-control studies published in recent years support the hypothesis that the presence of KRAS mutations, particularly KRAS G12C mutations, has a negative prognostic significance, and reduce both OS and PFS [87–90]. There is also evidence that increased mutated KRAS levels in circulating tumour DNA also have a negative prognostic significance [91].

The predictive significance of the KRAS mutation has also been extensively studied. Many reports suggest that mutant KRAS is not a negative predictor of conventional chemotherapy [92] However, as with prognostic significance, the question is more nuanced, with many factors to consider, such as the type of KRAS mutation, the patient population studied and treatment characteristics. In early-stage resected NSCLC patients, no significant predictive value of KRAS mutation status was found for adjuvant treatment [93], and similar results were obtained in the neoadjuvant setting [94]. A more recent study showed that KRAS mutation is a negative predictor of cytotoxic chemotherapy in advanced NSCLC [95].

Another important question is the impact of KRAS mutations on targeted therapies, especially EGFR targeting. Many conflicting observations have been reported on this topic, too. Most studies have shown that the presence of a KRAS mutation has a negative predictive effect in this respect, EGFR TKI treatment in these patients having a worse objective response rate. However, no difference in survival has been found between KRAS mutant/EGFR wild-type and KRAS wild-type/EGFR wild-type patients, therefore, the significance of KRAS mutation to select patients for EGFR TKI treatment appeared to be limited [96]. The results of individual studies are significantly affected by the type of KRAS mutation present: while poor treatment efficacy is seen with G12C and G12V, better response rates are seen with G12D and G12S [97].

Testing for KRAS mutations is possible from tumor DNA isolated from tumor tissue, bronchial brush smears, plasma or pleural fluid (cfDNA analysis), using either a single-gene PCR-based approaches, classical or a next-generation sequencing [98, 99].

BRAF mutations

BRAF is a member of the rapidly accelerated fibrosarcoma (RAF) kinase family. Its role is signal transduction from the RAS protein to the mitogen-activated protein kinase cascade (MAPK) [100]. The RAF protein is composed of three main domains: CR1, CR2, CR3. CR1 functions as an auto-inhibitor of the kinase CR3 domain and is also responsible for RAS-GTP binding. The CR2 region forms a flexible link between the CR1 and CR3 domains. Upon activation the RAS-GTP binds to the RAS-GTP binding site (RBD) of the CR1 domain. BRAF is then phosphorylated on amino acids T599 and S602, which results in a protein conformational change. This active form homo- or heterodimerizes with other RAF family proteins, also contributing to the stabilization of the active conformation. The results is the activated BRAF kinase domain, which phosphorylates MEK1, the downstream member of the MAPK signaling pathway [101].

BRAF is one of the most frequently mutated genes in human tumors. Mutations are most frequently detected in melanoma (40%–50% of cases), but are also common in papillary thyroid cancer, colorectal cancer and NSCLC [102–105].

(It is interesting to note that Davies [102] was the first to report the BRAF mutation, but in her publication she uses a different nomenclature, V599E, to refer to the mutation he detected - now called V600E-because the sequence of the protein had previously been misinterpreted, A31 G32 A33 was mistaken for R31 P32. Because A33 was missing from earlier sequences, some studies incorrectly assigned wrong numbers to coding mutations and amino acids.)

BRAF mutations most commonly occur at codon 600 in exon 15 of the gene, resulting in the exchange of amino acid valine to glutamate (V600E) of the protein. Other substitutions, such as V600D/K/R, can be also rarely seen at this site. These mutations are also known as class I mutations [106]. The V600E mutation

results in a marked increase in BRAF kinase activity (up to 500-700-fold compared to wild-type BRAF), with a consequent stimulation of the MAPK signaling pathway. The V600E mutation results in the conformational change of the monomer BRAF protein already possessing with an active kinase function that would otherwise gain through dimerization of the wild-type conformation [107]. In melanoma, V600E is the most common BRAF mutation, whereas in NSCLC only half of the mutations affecting BRAF belong to this group. NonV600E mutations form a heterogeneous group and can be further classified into class II and class III [101].

BRAF mutations play an important role in lung carcinogenesis, as also demonstrated experimentally *in vivo* [108]. Data from large case-control studies suggest that BRAF mutations occur in 2.2%–4.9% of NSCLC. Owsley found 772 BRAF mutations (4.1%) out of 18,944 NSCLC cases, of which 30.7% (237 cases) were V600E mutations [103]. In Villaruz's study, 21 cases (2.2%) of 951 adenocarcinomas proved to be BRAF mutant, 81% of which were V600E [109]. V600E mutations occur mainly in women, non-smokers, are associated with micropapillary morphology and have a worse prognosis than wild type [110]. Non-V600E mutations are exclusively detected in smokers, in equal proportions in men and women, and are not associated with a prognosis worse than the wild type (Marchetti, 36 BRAF mutations detected in 739 adenocarcinomas- 4.9%- of which 56.8% were V600E) [111]. The results of individual studies are contradictory regarding the prognostic role. Villaruz et al. observed a better outcome for V600E, while others did not observe any difference in prognosis [109, 112] The contradictory results are likely explained by the fact that BRAF mutations are relatively rare and only small cohorts of patients could be examined despite extended studies. Co-mutations are relatively common, mainly mutations in KRAS and PI3K, while in nonV600E, mutations in p53 and STK11 are common [106].

The conformational change through the mutation enables the differentiation of the BRAF protein from the wild-type form. Thus, the BRAF status can also be assessed by immunohistochemistry for V600E mutations. The commercially available monoclonal diagnostic antibody was raised against a synthetic version of the V600E-encoded protein fragment located around the amino acid affected by the mutation [113]. This antibody detects the BRAF V600E mutant epitope with sufficient sensitivity and specificity, as has been demonstrated in several tumor types such as colorectal carcinoma, papillary thyroid cancer and melanoma [114–118] However, it is not applicable to V600D/K/R or class II and III non-V600E mutations. Since almost half of the BRAF mutations in NSCLC are nonV600E, the IHC test is of limited use to identify tumors harbouring BRAF mutations. However, according to current NCCN recommendations, specific TKI inhibitor treatment should be used for V600E mutation positive tumors [119]. Thus, the IHC methodology may be considered as a screening test for the identification of these patients in histological conditions. In addition, the same guidelines (and updated version also) recommend an NGS-based methodology to determine a comprehensive BRAF status [120].

Gene fusions with clinical relevance

In addition to the now "classic" MAP-kinase pathway mutations, several clinically relevant chromosomal rearrangements have also been identified in NSCLC. It has long been known that specific gene fusions determine the development of several haematological malignancies and sarcomas. The earliest such gene fusion identified was bcr-abl characteristic for chronic myeloid leukaemia, discovery finally leading to the pioneering concept of tyrosine kinase inhibitor therapies [121]. Oncogenic gene translocations play a special role in NSCLC carcinogenesis, especially if their functionality can be therapeutically blocked. The most important ones are ALK, ROS1 and RET rearrangements and the significantly less frequent NTRK gene fusions, with prevalence rates of 4%–6%, 2%, 1%–2% and 0.1%–0.23%, respectively. These fusions occur in a patient population clinically distinct from classical NSCLC (predominantly younger, non-demented patients with adenocarcinoma histology).

Due to the availability of effective targeted TKI drugs with FDA or EMA approval it is particularly important to identify these patients within the confines of predictive molecular testing. The selection of sufficiently effective and validated, yet rapid and not least relatively inexpensive methodology is a major challenge for pathology laboratories and molecular geneticists. In addition to the "big four," additional gene fusions have recently become known, such as those involving NRG1, SMARCA4, BRAF, FGFR1 and EGFR, further complicating the everyday molecular diagnostic practice of NSCLC.

ALK rearrangements

The ALK gene (anaplastic lymphoma kinase) was discovered as a result of genetic studies in anaplastic large cell lymphoma. The gene is located on the short arm of chromosome 2, in the 2p23 region. As a member of the insulin receptor superfamily, ALK encodes a tyrosine kinase-activated transmembrane receptor protein whose function is only partially understood. In humans, ALK expression is detected intermittently during neural development, with a decline in expression during postnatal life. In adults, it is expressed only scattered in some neurons, endothelium and in pericytes of the brain. The ALK protein contains an extracellular ligand-binding, a transmembrane, and a cytoplasmic kinase domain [122]. The

ligand(s) for the ALK receptor have not been unequivocally identified. The role of pleiotrophin, midkine was hypothesized [123], followed by other candidates, including heparin, FAM150A and FAM150B [124].

The main types of ALK gene alterations include rearrangements (fusions), gene amplification, and point mutations [125].

Fusions of the ALK gene, like the amplification of the gene leads to constitutive activation. Amplification of ALK has been detected in neuroblastoma [126] breast cancer, anaplastic large cell lymphoma and pulmonary sarcomatoid carcinoma [127]. On the contrary, point mutations are the most common causes of resistance to ALK TKI treatment. Known resistance mutations are G1269A, C1156Y, L1196M and several other point mutations [128, 129].

The 2p chromosomal region is sensitive to genotoxic effects, favoring the breakage of the ALK gene, with the result of gene fusions and increased expression of the kinase domain of the ALK protein. In 1994, a t [2, 5] translocation was first described in anaplastic large cell lymphoma, resulting in an NPM (nucleophosmin)-ALK fusion gene, an event that is detected in 60%–80% of ALCL [130]. It has subsequently been described in additional tumors: e.g., inflammatory myofibroblastic tumor [131], colorectal and breast cancer, and esophageal squamous cell carcinoma [132]. Further fusion partners were later identified. The setup of the rearrangements is common in that the breakpoint leaves the entire ALK tyrosine kinase domain intact, while the promoter region always comes from the fusion partner. The fusion partner also contains an oligomerization domain, the presence of which allows ligand-independent constitutive activation of the receptor protein.

In 2007, Soda and colleagues detected the EML4 (echinoderm microtubule associated protein-like 4)-ALK gene rearrangement in NSCLC. This gene rearrangement is caused by an inversion of the chromosome region 2p21-23. The extracellular and transmembrane regions of ALK are replaced by EML4. There are various EML4 breakpoints and therefore, several variants of the fusion gene are known [133]. The EML4-ALK gene rearrangement results in constitutive activation of ALK RTK, an oncogenic pathway in NSCLC. The resulting EML4-ALK fusion gene product represents a novel molecular target for the treatment of non-small cell lung cancer. ALK gene rearrangement occurs in 3%–6% of all NSCLC. It is typically associated with adenocarcinoma morphology (including papillary, mucinous, and squamous cell variants) [134]. Detected mainly in non-smokers or light smokers and typically in young patients. ALK gene rearrangement, EGFR and KRAS mutation are mutually exclusive events [135].

The demonstration of EML4-ALK gene rearrangements was challenging due different variants of the fusion, requiring multiplex testing in the PCR era. FISH-based detection could be adopted with satisfying efficacy since the 3′and 5′ends of the ALK gene get separated due to the rearrangement, and their labelling with separate fluorescent probes result in the characteristic split signal. Currently, the FISH test is the gold standard for the detection of ALK rearrangements in clinical samples, requiring specific probes, fluorescence equipment and properly experienced pathologist.

Because of the above drawbacks, an immunohistochemical alternative for the detection of ALK gene involvement has been attempted. This assumes that ALK protein is not expressed in normal lung tissue, but gene fusion and ALK gene activation result in moderately increased expression of ALK protein. However, the detection of the protein underwent an evolution. A “traditional” diagnostic antibody (ALK1) previously used for anaplastic lymphoma was not sufficiently sensitive (sensitivity 67%, specificity 97%). However, the release of new antibody clones promptly followed [5A4, D5F3, anti-ALK (1A4)] and the use of highly sensitive amplification systems allowed to achieve adequate sensitivity and specificity. The advantage of IHC testing is its low cost, wide availability and rapid turnaround time. In immunohistochemistry, the ALK fusion protein shows granular cytoplasmic staining. In signet ring cells (a morphology often seen in ALK-positive adenocarcinoma), staining is present along the membrane in a thin rim that can be difficult to distinguish from background staining. Several studies have demonstrated that the use of properly validated antibody and immunohistochemical platform, together with an external control (e.g., appendix with intense ALK positivity in the ganglion cells of the wall), provides a highly reproducible result. The study of Mino-Kenudson et al. in 2010 (n = 153, sensitivity 100%, specificity 99.0%) using clone D5F3 [136] and that of Paik et al. in 2011 (n = 735, sensitivity 100%, specificity 96.2%) using clone 5A4 both showed high concordance between Ventana IHC and FISH results [137].

The high sensitivity of IHC to detect ALK aberrations is today generally accepted. Despite the rare IHC negative but FISH positive cases published in the literature, the current recommendations accept the use of IHC methodology without FISH confirmatory testing in histological specimens [138] as well as in cell block specimens prepared from malignant pleural effusions [139]. However, the IHC methodology is neither perfectly applicable nor validated in large series on bronchoscopic brush cytology specimens. For cytology preparations the FISH break apart test should primarily be chosen.

ROS1 rearrangements

The ROS1 gene is located on chromosome 6 in the 6q22 region. The gene was originally discovered in the 1980s during the study of avian sarcoma viruses. The human ROS gene is homologous to the v-ros proto-oncogene of the UR2 sarcoma virus [140, 141]. The protein contains an extracellular and an intracellular domain, the latter having tyrosine kinase activity

with structural similarity to the ALK protein. The physiological role of the ROS1 gene and protein is poorly understood, but it is thought to be involved in differentiation signaling pathways of various epithelial tissues during embryonic development [142]. ROS1 protein expression is observed in the kidney, the cerebellum, the peripheral nerves, various parts of the alimentary canal, but is not expressed in lung tissue under normal conditions [143]. The physiological ligand of the receptor is still debated. The structure of the extracellular domain suggests that cell adhesion plays a role in its activation [142] The intracellular signaling pathways activated by ROS1 are also not well understood, but induction of MAPK and PI3K pathways is hypothesized [144]. Its oncogenic relevance was first demonstrated in the early 2000s in glioblastoma [145]. In lung tumors, rearrangements affecting ROS1 gene were first described in 2007 [146]. ROS1 involvement was observed in 1%–2% of NSCLC [147]. As a result of the rearrangement, various breakpoints in the ROS1 gene may evolve, including exons 32, 34-36, or introns 31 or 33. The fusion gene commonly contains the tyrosine kinase domain of ROS. There are 9 different fusion partners known in lung cancer, such as FIG, CD74, SLC34A2 and SDC4, EZR and the list is growing [148, 149]. The oncogenic mechanism of gene rearrangement is not understood. ROS1 rearrangements typically occur in lung adenocarcinomas, rarely in adenosquamous carcinoma. These adenocarcinomas generally show a solid pattern, frequently of signet ring cell type. Younger patients and non-smokers are more frequently affected [150]. It is often detected at an advanced stage and brain metastasis is common [151]. Interestingly, lung carcinoma patients with ROS1 gene rearrangements have a higher incidence of paraneoplastic thromboembolic events [152].

Due to the larger set of fusion variants the ROS1 rearrangement can be most effectively demonstrated by FISH analysis, using ROS1 specific DNA-probes and the detection strategy of the ALK testing. Sequencing by different NGS platforms is also applicable if tumor tissue and appropriate amounts of DNA or RNA are provided. Rearrangement of the gene is associated with overexpression of the ROS-protein, allowing the use of ROS1-specific antibodies. For IHC-based diagnostic testing, the use of the D4D6 ROS1 antibody clones is recommended. In positive cases, fine granular cytoplasmic staining is observed. Fusion variants show a different staining pattern, which may be the result of the intracellular function and localization of the fusion partner. For CD74-ROS1, a globular pattern with randomly arranged cytoplasmic granules of 6–8 mm diameter and weaker background cytoplasmic staining was described, explained by the fact that CD74 is associated with intracellular membrane systems. Membranous staining was seen in the presence of EZR-ROS1 fusion, presumably due to the ezrin protein binding to plasma membrane and actin cytoskeleton [153] A uniform scoring system for the ROS1 IHC reaction is still missing, most studies use the H-score calculated from the staining intensity and the proportion of positive tumor cells. With appropriate preanalytical and analytical standards, this antibody can achieve high sensitivity (95%–100%) but relatively poor specificity (63%–90%) [154–157]. The low specificity may potentially originate from a moderate ROS1 expression by macrophages, reactive alveolar epithelium, or even by tumors without ROS rearrangement. A higher cut-off value results in a higher specificity. Overall, the IHC test has a high negative predictive value and is therefore suitable as a screening test, with a negative IHC result virtually ruling out the presence of ROS1 fusion. A positive IHC result requires confirmation by genetic means, such as FISH or NGS technology [158, 159]. Since ROS1 rearrangements are rare, the relatively simple IHC staining is highly effective and avoids the mass need for expensive molecular testing [138, 160].

RET rearrangements

The RET oncogene was identified in the 1980s by transfection of DNA extracted from a human T-cell lymphoma cell line [161]. The RET gene is located on chromosome 10 at position 10q11.22 [162] and encodes a transmembrane tyrosine kinase growth factor receptor. Its extracellular domain contains 4 cadherin-like structures [163]. Its ligand is glial cell line-derived neurotrophic factor [164, 165]. Ligand binding results in dimerization and activation of the receptor, which then activates several intracellular signaling pathways such as PI3K/AKT, RAS/RAF/MEK/ERK or JAK2/STAT3. RET activity is important for kidney and nervous system development, gene expression is precisely regulated in space and time during embryogenesis [165]. RET is required for the proper development of the enteric nervous system, in particular for the migration of neural crest cells and enteric neurons into the wall of the alimentary canal. The absence of RET expression or activity is associated with the development of Hirschsprung disease (segmental aganglionosis of the colon) [166].

Various genetic defects within the RET gene are also related with carcinogenesis. CCDC6-RET fusions have been detected in thyroid cancer as early as in 1990 [167]. Further to oncogenic fusions, activating point mutations of the RET gene are also known. They are involved in the development of medullary thyroid carcinoma and MEN2A syndrome, among others [168, 169]. In NSCLC, RET gene rearrangement was first reported in a Korean non-smoking male patient in 2012 [170–173]. Several large studies have reported that RET rearrangement is present in 1%–2% of NSCLC (Takeuchi 0.9% in 1482 NSCLC, Qiu 1.4% in 1587 NSCLC) [149, 173]. Following the summary of data from 4857 NSCLC patients from previous studies, the prevalence of RET rearrangement proved to be 1.4%, while in the adenocarcinoma group of 3,576 patients 1.8% had RET fusions [174]. KIF5B-RET fusion was observed with the highest prevalence (52%), this type was typical for women, while

CCDC6-RET was mostly observed in men with a prevalence of 26% in total. In addition, several rare fusion partners have been described (MIR392, ZBTB41, ITGA8, SLC39A8 [149]. Along with the RET rearrangements, several other genetic events may be detected; [175]. It is known that RET fusions are responsible for acquired therapy resistance during EGFR TKI inhibitor treatment in 1%–2% of the cases, mainly involving CCDC6 as the fusion partner; [176]. In most RET rearrangements, the transmembrane domain is lost, resulting in a chimeric cytosolic protein that exerts its oncogenic effect through constitutive activation of the kinase domain [161] The majority of RET rearrangements, like ALK and ROS1 fusions, occur in young, non-smoker or mild smoker women with lung adenocarcinoma diagnosis; [173, 177]. This patient group has a significantly higher incidence of brain metastases both at diagnosis (27%) and overall during the course of the disease than the RET wild-type group [178].

The demonstration of RET gene fusions in clinical samples is of great importance, as there are several FDA-approved small-molecule inhibitors promising effective treatment. Initially, non-specific multikinase inhibitors were used, more recently followed by RET-selective inhibitors [179–182].

RET rearrangements can be demonstrated by several alternative methodologies. Although immunohistochemistry is a widely available method and has proven useful in detecting ALK and ROS1 rearrangements, its value in detecting RET fusions is unfortunately limited. The IHC methodology using RET specific antibodies had low sensitivity and specificity, with a false positive rate of 62% and a false negative rate of 46%, in other words, RET rearranged samples could not be equivocally identified. These results have been unanimously confirmed by several studies, and therefore the use of IHC is not recommended for the diagnosis of RET gene rearrangement [183, 184].

On the contrary, the FISH methodology has been shown to be successful in detecting a substantial amount of gene rearrangements. Using RET gene region specific probes FISH had a high sensitivity of 100% for the chimeric proteins KIF5B and CCDC6 but less than 100% for the other partners. On the other hand, a surprisingly poor specificity of 45%–60% was measured, therefore, the currently available DNA probes have not been recommended for routine diagnostics of RET gene fusions [184, 185]. Considering all these issues NGS remains the optimal tool for general RET testing. DNA-based NGS showed a sensitivity of 87.2%–100% for detecting RET fusions, while its specificity was also highly satisfying (98.1%–100%) [186].

NTRK fusions

In humans, three neurotrophic tyrosine receptor kinase (NTRK) genes are known, encoding the transmembrane neurotrophin receptors TrkA, TrkB and TrkC. These Trk receptors are involved in embryonic development of the central and peripheral nervous system [187]. In adults, they are expressed only in neural tissue and skeletal muscle [188]. Ligand-dependent activation of Trk receptors activate several biochemical pathways, including MAPK and PI3K signaling [189]. Chromosomal rearrangements of NTRK genes result in increased expression and/or activation of Trk receptors [190]. The occurrence of NTRK gene fusions is characteristic for some rare tumor types, such as mammary analogue secretory carcinoma of the salivary gland or congenital infantile fibrosarcoma. ETV6-NTRK3 rearrangements are detected in 90% of these cases [191, 192]. Although NTRK gene rearrangements are generally rare, they have been detected in a broad range of common solid tumor types. NSCLC, colorectal carcinoma, GIST, papillary thyroid carcinoma, melanoma, pancreatic adenocarcinoma and gliomas were reported with very rare NTRK involvement of less than 1% of cases [193]. In NSCLC, the incidence rate was only 0.1%–0.3%, an order of magnitude lower than the frequency of ALK or ROS1 gene rearrangements [194]. In two very large NSCLC case-control studies, the rates of NTrk fusions were 0.1% (Gatalica, 4,073 lung adenocarcinomas [195]) and 0.23% (Solomon, 3,993 lung adenocarcinomas [196]). NTRK1 fusion could be demonstrated most (68%), followed by NTRK3 (24%) and NTRK3 fusion as the least common change. Because of the rarity of occurrence, even the largest studies had limitations defining detailed clinical characteristics of patients with NTRK fusions. They suggest equal distribution in both women and men, with a wide age range. The majority of those carrying the fusion are non-smokers, but heavy smokers were not excluded. Most NTRK-positive tumors proved to be adenocarcinomas with mucinous or poorly differentiated morphology, but fusions have also been detected in neuroendocrine carcinoma and even squamous cell carcinoma [197].

Although rare, the identifications of these tumors opens the way for NTRK-targeted TKI therapy, that is available in the last couple of years promising a favorable therapeutic response in patients with NTRK gene fusion [198, 199]. In a 2023 study, 51 patients with advanced NSCLC harboring an NTRK fusion had an ORR of 62.7% following entrectinib treatment, while the PFS and OS was 28.0 and 41.5 months, respectively [200]. Predictive testing of NTRK gene fusion-due to the altogether 3 TrK genes and numerous fusion partner genes-is quite cumbersome, even by the classic FISH arrangement. Due to the 3 independent NTRK gene regions potentially involved, three FISH assays and tests would be required. However, while the detection of fusions involving the NTRK3 gene by FISH had good sensitivity, too many false negative cases were reported for NTRK1 fusion detection. This may be due to intrachromosomal rearrangements involving a short segment, allowing only limited signal separation in the break-apart probe assay, causing interpretation difficulties. Another problem appeared as FISH probes could not detect rearrangements with some fusion partners. Because of these drawbacks, NTRK

FISH were not recommended for routine diagnostics [201]. As an alternative, RNA-based massively parallel sequencing (MPS) is considered as a favorable methodology. However, this technique is not widely available, not mentioning the turnaround time and costs of the test.

Taking everything together, immunohistochemical detection of Trk proteins as a screening test should be considered. Immunohistochemistry is available in virtually all pathology departments, is relatively rapid and inexpensive and sufficiently works with small amounts of tumor tissue. Commercially available diagnostic antibodies detect all Trk proteins. The staining pattern is variable: membrane, cytoplasmic and nuclear positivity can all be present. Unfortunately, a validated scoring system is not available at this time. A positive tumor is defined as one with at least 1% of tumor cell positivity, any kind of positivity more intense than background should be satisfactory, regardless of the staining pattern. Confirmatory testing of positive cases by MPS seems to be necessary for proper interpretation.

According to the literature the sensitivity of the IHC method ranges from 75% to 97% and the specificity is remarkably high, reaching 98%–100% [202–204]. Previous large studies have shown that the sensitivity of IHC is not uniform for the three NTRK gene fusions, 96.2%, 100% and 79.4% sensitivity rates were measured for NTRK1, -2, and -3, respectively, while the specificity was 81.1%. More specifically for the lung adenocarcinoma patient group, IHC sensitivity was 87.5% and specificity 100% [196]. The above large studies all used the Abcam EPR17341 antibody clone. However, in a more recent study, 133 (14.8%) out of 1068 NSCLC cases were panTrk IHC positive, but only 2 cases could be confirmed by RNA-based testing, resulting a positive predictive value of 1.5% for the IHC test applied [205]. Unlike in previous studies, in this work the C17F1 antibody clone was used and any staining was accepted as positive. In conclusion, predictive IHC testing of NTRK involvement also should be performed with care. Sensitivity and specificity rates may be strongly influenced by the type of the diagnostic antibody used. However, sensitivity of IHC supposed to be relatively lower for NTRK3 fusions, the reason of which is not known in detail. If uncovered, low sensitivity of the IHC screening could drop out patients of an effective treatment opportunity.

Novel driver gene defects in NSCLC

MET alterations. Met exon 14 skipping mutations

The mesenchymal epithelial transition (MET) proto-oncogene is located in t4.1he chromosomal region 7q21-q31 and encodes a transmembrane receptor tyrosine kinase protein [206, 207]. The MET protein is expressed in diverse cells of epithelial origin, and is further expressed in liver cells, endothelial cells and neurons. The ligand for this receptor is hepatocyte growth factor (HGF), which is mainly produced by mesenchymal cells, such as fibroblasts [208]. The extracellular part of the receptor protein is responsible for ligand binding and includes domains like the semaphoring and the immunoglobulin-plexin transcription factor domain. The intracellular part is composed of the juxtamembrane domain and the catalytic domain [209, 210]. Ligand binding activates the protein by causing homodimerization, which then leads to autophosphorylation of tyrosine residues in the catalytic domain. Activated MET induces several intracellular activation pathways through MAPK, PI3K, Nf-kB and signal transducer and activator of transcription3 (STAT) signaling. HGF/MET activation plays a key role in epithelial-to-mesenchymal transitions (EMT) by regulating extracellular matrix adhesion and cytoskeletal changes [211, 212]. The deactivation mechanism of the activated signaling pathway deserves attention, as changes in this process play a key role in the carcinogenic effect of MET [213]. After ligand binding, homodimerisation and intracellular signaling the active receptor protein is internalized by clathrin-mediated endocytosis, it is partially degraded but recycling and return to the cell membrane is possible. This process is controlled by ubiquitin ligase casitas B-lineage lymphoma (CBL), which recognizes the Tyr1003 residue encoded in exon 14 of the MET gene and the ubiquitinated MET is degraded by the endosome system [214, 215].

Genetic events may affect MET protein function resulting in oncogenic effects. MET gene amplification results in increased expression and constitutive activation of the kinase protein. This mechanism is supposed to be responsible for acquired resistance during EGFR TKI treatment in 3%–4% of [42, 216, 217]. Various point mutations have also been detected in several tumours, including lung carcinoma, but their oncogenic role remains unclear [218]. Moreover, some gene fusions have also been described, such as KIF5B-MET, which have potential oncogenic effects and serving as therapeutic targets [219].

Exon 14 mutations are the best known MET alterations with pathogenetic and apparently, clinical significance. This specific mutation type is a result of a two base pair insertion in intron 13. The insertion represents an alternative mRNA splicing site with the consequence of the “skipping” of the entire exon 14 during translation for protein synthesis. Therefore, the functional molecule lacks the juxtamembrane segment containing the Tyr1003 residue responsible for the internalisation of the activated receptor. Thus, the exon 14 skipping mutation enhances the stability of activated MET on the surface of the cell, resulting in a prolonged activity of the receptor signaling [220, 221]. According to one of the first large case-control studies, this mutation is present in about 3% (131/4,402) of NSCLC cases [222]. According to a recent large meta-analysis, exon14 skipping mutations can be detected at a rate of 2% in NSCLC, no significant geographical differences are reported. The

prevalence proved to be 12% in non-smokers and 2% in smokers, with a similar overall prevalence of MET14 skipping mutation positive patients with a history of smoking and non-smoking. It is more common in women and at older age (average is 73 years). It is noteworthy that while the histological type of adenocarcinoma has a prevalence of 2.4%, it is detected in 12% of sarcomatoid carcinomas [223]. Association with major driver mutations (EGFR, KRAS, BRAF and ALK, ROS1 gene fusions) appears to be rare, but some other genetic events, such as MET, EGFR amplification, or PI3K mutations may co-occur [222]. Data to date suggest that MET exon 14 mutations in NSCLC are associated with a poor prognosis.

The therapeutic targeting of MET kinase is relevant with multiple TKI drugs (e.g., tepotinib, capmatinib, savolitinib) resulting in good therapeutic response [224, 225]. This is true for the exon-skipping alteration but also for the Y1003N mutation of the juxtamembrane domain, which also inhibits CBL-mediated degradation [222].

Due to the heterogeneity of Met exon 14 aberrations, their detection is another challenge for diagnostic pathology laboratories. Attempts have also been made to detect MET alterations by immunohistochemistry. While overexpression of MET protein can be detected in many tumor types in many cases, MET overexpression can be detected in 35%–72% of NSCLC by immunohistochemistry [226, 227]. Unfortunately, in the majority of IHC positive cases, there is no Met exon 14 skipping or MET amplification. As shown in a recent study, 71 tumors in 181 NSCLC cases had MET overexpression detectable, but only 1% of IHC positive cases had amplification and 3% had Met exon 14 skipping. These results also show that IHC-based detection is not a suitable screening test for MET alterations [228]. RNA-based assays have the highest sensitivity and can detect exon 14 skipping independent of the underlying diverse genetic alterations by detecting fusions of exons 13 and 15 following mRNA transcription. The disadvantage of the RNA-based methodology is its sensitivity to RNA degradation [229]. Unfortunately, amplicon-based DNA NGS tests have a detection rate of only 63% [230], whereas hybrid-capture NGS methodology can achieve better results, but requires larger amounts of sample DNA, which is frequently not provided from small biopsy samples [231]. To overcome this issue circulating free DNA (liquid biopsy) should have lower sensitivity but a high positive predictive value [232].

HER2 alterations

HER2 (ERBB2) is a member of the HER growth factor receptor family. This family also includes EGFR, EGFR3 and EGFR4. The HER2 gene is located in the 17q11.2-q12 region. The encoded receptor protein has an extracellular ligand-binding, a transmembrane and an intracellular tyrosine kinase domain, like other growth factor receptors of the family [233]. It is unique compared to other members of the HER family in that it has no known ligand but has an intrinsic tyrosine kinase activity and is specifically prone to homo- or heterodimerization, which results in its activation. A frequent heterodimerization partner is HER3. Activated HER2 can activate several intracellular signaling pathways such as MAPK, PI3K, and STAT [234].

Several alterations may occur in the HER2 gene, which have oncogenic effects. Gene amplification of HER2 is well known in breast cancer, one of the oldest known therapeutic targets [235], but is also common in gastric [236] and ovarian cancer. HER2 alterations can also occur in lung carcinoma, but gene amplification is relatively rare. However, HER2 amplification in NSCLC may be a potential cause of acquired resistance during EGFR TKI treatment [42]. HER2 amplification is most easily detected by FISH, which is analogous to the common testing practice in breast carcinoma, with a HER2/CEP17 hybridization signal ratio greater than 2. Importantly, HER2 overexpression is often detected by immunohistochemistry, which is usually due to a balanced increase of copy number (polysomy). In this case the HER2/CEP17 ratio does not exceed 2 determined the FISH analysis [237]. Various mutations in the HER2 gene may also occur in the coding regions of all three domains with a rate of 2%–4% of NSCLC. In the first study, published in 2004, HER2 mutations were presented in 10% of the lung adenocarcinomas [238] Subsequently, several studies have reported higher case numbers, with lower frequencies (1.6% testing 671 NSCLC cases) [239]. The most common types of mutations proved to be in-frame insertions in the kinase domain coding region in exon 20. These mutations change the protein conformation and increase kinase activity, thereby activating intracellular signaling [240]. HER2 exon 20 insertions are like exon 20 insertions detected in the EGFR gene [241]. Many of these insertions have been described, the most common being the YVMA insertion, which was detected in 68% of the 98 HER2 mutant tumors detected in a study of altogether 2,788 patients [242]. HER2 mutations occur mainly in women, non-smokers, are associated with adenocarcinoma histology and brain metastasis is common in these patients [243]. Another large study reported HER2 mutations in 24 of 920 patients (3%), 71% non-smokers, 58% women, mean age was 62 years [244]. The co-occurrence of HER2 mutations with other classical driver mutations is virtually excluded [239, 245]. However, some HER2 mutations develop in about 1% of cases of acquired resistance to EGFR TKI treatment [246]. Neither immunohistochemistry nor FISH is suitable for HER2 mutation detection. Since many types of these mutations are known, NGS sequencing methodology is the only effective way of testing [247].

Immune-checkpoint alterations

Modulation of the anti-tumor immune response as another option of anti-tumor therapy becoming part of everyday oncological care. Tumor-related antigens can be recognised by immune cells through the complex process of antigen-presentation and T-cell activation. Ideally, T-cells migrate to the tumor, where they recognise and destroy tumor cells, the efficacy of which is regulated by several receptors and ligands triggering co-stimulatory and inhibitory signals (immune checkpoints). Tumour neoantigens play an important role in the activation of the immune response enabling the separation of tumor from normal cells. The accumulation of mutations in genomically instable cancers is associated with the generation of neo-antigens showing marked immunogenicity. More specifically, the mutational burden is higher in cancers associated with prolonged exposure to environmental carcinogens. Examples include UV radiation in melanoma development, and respiratory carcinogens, primarily polycyclic aromatic compounds in the tobacco smoke, in relation with lung cancer, both small cell and non-small cell type. Increased mutation frequency is a result of insufficient DNA repair mechanisms, e.g., the loss of mismatch repair (MMR) gene functions [248].

The immune checkpoint regulation can be modified by tumor cells, an important immune resistance mechanism. Programmed cell death protein 1 (PD1) is a cell surface immune checkpoint receptor in activated T- and B-lymphocytes, with inhibitory impact on effector cell functions [249]. The PD1 gene is located on chromosome 2 and belongs to the immunoglobulin gene family [250]. The PD1 protein has an extracellular domain consistent with immunoglobulin variable IgV domain, and is featured with an intracellular segment including the immunoreceptor inhibitory tyrosine-based switching motif (ITSM), responsible for inhibition of T-cell activation. The ligand for the PD1 receptor is PD-L1, a cell surface protein containing IgV and IgC domains [251]. PD-L1 can be expressed by T and B cells, macrophages, dendritic cells, and many non-haemopoietic cells. Cells expressing PD-L1 are tolerated by the immune system. Antigen-presenting cells expressing PD-L1 can inhibit T lymphocytes. Thus, the physiological function of PD-L1 is to provide immune-tolerance by inhibiting the adaptive immune response [252]. Unfortunately, tumor cells may also acquire PD-L1 expression in an adaptive or constitutional manner. Adaptive PD-L1 expression occurs in response to interferon-gamma, secreted by T-cells activated by tumor antigens and is mainly observed in the tumor-immune contact zone. Constitutive expression results from activation of various signaling pathways and is uniformly distributed throughout the tumor. Upregulation of PD-L1 ligand by tumor cells inhibits the antitumor immune response in the tumor microenvironment. In many tumor types, including NSCLC, PD-L1 expression is observed in tumor cells, especially in poorly differentiated tumors, and several large meta-analyses have shown that increased PD-L1 expression has a negative prognostic impact [253, 254].

Consequently, the therapeutic blockade of the PD1-PD-L1 relationship represents a promising therapeutic modality in oncology which was outlined by a surprising response in several tumor types from the earliest stage of clinical studies [255]. By now, therapeutic monoclonal antibodies with immune checkpoint inhibitory effect have been introduced for the treatment of a variety of tumor types. However, only about 20% of patients developed an objective response, the rest either having no meaningful effect or developing resistance to treatment. Although ICI treatment does not have serious side effects compared to chemotherapy, characteristic adverse effects may occur, which are often also serious. Therefore, predictive biomarkers for patient selection of PD-L1-PD1 inhibitor treatment would be of particular importance [256]. Unfortunately, there is currently no really good universal predictive biomarker to select patients who could potentially benefit from ICI treatment. In theory, the potential predictive role of factors influencing the tumor-host immune system relationship could be all be considered, including the tumor mutational burden (TMB), the tumor infiltrating lymphocyte (TIL) count, DNA repair systems, in particular mismatch repair and finally the expression of PD-L1. Unfortunately, the predictive clinical value of these factors varies significantly between tumor types.

In NSCLC, the assessment of PD-L1 expression in tumor tissue has been shown to have the strongest predictive value. Early studies have already indicated that the efficacy of PD1 blockade is highly dependent on PD-L1 expression in tumor cells. However, a confusing situation has developed in the field of predictive PD-L1 testing. In a short period of time, four PD1-PD-L1 inhibitor drugs (nivolumab, pembrolizumab, atezolizumab, durvalumab and more recently cemiplimab) have been launched. In parallel, several diagnostic antibodies have been released for the detection of PD-L1 expression. Large clinical studies to test the therapeutic efficacy of their agents applied different antibodies and used different immunohistochemistry platforms. Currently, there are four FDA-approved PD-L1 diagnostic antibodies run on two different IHC platforms: clones 22C3 and 28-8 are determined for the Dako link48 platform (Agilent), and clones SP263 and SP142 for the Ventana (Roche) platform. In addition, various scoring systems for PD-L1 expression have been established. Briefly, the tumor proportion score (TPS) gives the proportion of tumor cells with membrane expression, the combined proportion score (CPS) to assesses the expression of immune cells in the surrounding area in addition to tumor cells, and the IC score to determine the expression of immune cells. Moreover, different cut-off values have been set for the same active substance, depending on whether it is defined for a first-line or a multi-

line treatment. For some of the drugs, national medicines authorities insist on the use of companion testing, e.g., the 22C3 antibody Dako link48 for pembrolizumab or SP263 for durvalumab treatment. In other indications the use of PD-L1 testing is complementary, helping to select patients for a more pronounced therapeutic response, thus providing a more accurate assessment of the risk/benefit ratio. Such a complementary test should be used, for example, for atezolizumab with SP142 or for nivolumab with 28-8 antibody clones. There are some indications and drugs where predictive marker testing is not justified under the current pharmacopoeian standards [257]. The results of the KEYNOTE-001 clinical drug trial have shown a strong correlation between the efficacy of pembrolizumab and the level of PD-L1 expression as determined by the 22C3 antibody [256, 258]. The results suggested the utility of 50% TPS as a cut-off, a value which was confirmed by subsequent studies (Keynote-010 and 024). Interestingly, PD-L1 expression as determined by 28-8 antibodies in the Checkmate 017 study was not found to be predictive for nivolumab response. However, the Checkmate 057 study concluded, that PD-L1 expression and clinical response significantly correlate, although only a modest ORR increase was observed as expression increased. Thus, the FDA has accepted PD-L1 detection for this drug as a complementary test [259]. PD-L1 detection for atezolizumab has also been accepted as a complementary test, based on data from the POPLAR and OAK studies [260]. Most recently, cemiplimab has received FDA approval for use, based on the results of the EMPOWER-lung-1 study, and the use of this drug was also linked to greater than 50% PD-L1 expression, determined by SP263 and the Ventana platform as a companion test [261, 262]. The alternatives of specific ICI therapies and PD-L1 predictive testing techniques resulted in differences in the current NCCN and ESMO recommendations, and these are also reflected in the various national medicines regulatory specifications [120, 263].

Taken together the above, a never seen complexity of a biomarker determination can be stated. The variety of different diagnostic PD-L1 antibodies and development platforms and different evaluation systems as well as the growing number of ICI drugs and relevant national recommendations required the comparison of PD-L1 detection methodologies. The results of PD-L1 determination with four commonly available anti-PD-L1 antibodies (22C3, 28-8, SP142 and EIL3N) were compared in a multi-institutional study [264]. In addition to IASCL, the relevant pharmaceutical and diagnostic companies were also involved in the design and conduction of the Blueprint 1 and 2 studies. The very detailed results indicated that the evaluated tests were not always interchangeable. The 22C3, 28-8 and SP263 antibodies and their elicitation systems have been shown to be highly concordant, in terms of sensitivity and specificity, for the determination of TPS. In contrast, SP142 showed a consistently lower TPS value, while 73-10, tested in the Blueprint 2 study, showed a much more intense staining reaction [265, 266].

A major limitation in PD-L1 testing is that it can only be reliably done on embedded tissue samples. Although some studies have reported results of PD-L1 detection on cytological smears (bronchial brush smear, lymph node EBUS guided FNA smear) [267], IHC on this sample type is difficult to standardize and results show a large variability. The large variability of preanalytical characteristics in cytology samples is well known. As indicated in previous studies, fixation is a key pre-analytical factor, e.g., alcohol-based fixatives strongly reduce the feasibility of IHC reactions. Thus, the PD-L1 IHC reaction on smears should be validated in every laboratory. The determination of IC and CPS in cytology is also problematic as the assessment of tumor cell-immune cell relations in direct smears is almost impossible. Larger cell clusters, 3-dimensional clusters, blood contamination hamper the evaluation. In addition, instead of the membranous staining seen in tissue sections, there may be diffuse surface staining on direct smears, mimicking a cytoplasmic reaction [268–270]. As a result, there is a high interpretation and interobserver variability in the assessment of PD-L1 detection when cytology smears are used [268]. For these reasons, the manufacturers of FDA-approved diagnostic antibodies do not recommend the use of cytology smears and users are advised to favor cell blocks. Cytology samples processed in cell block format have been shown to be suitable for PD-L1 detection. This methodology is optimized for and is analytically similar to IHC and allows standardization criteria of the IHC reaction. Several studies have shown satisfactory concordance between the results of PD-L1 detection on cell blocks and tissue samples [271]. In special cases, when the sampling from the tumor tissue (both histology or cytology) fails, it is possible to determine PD-L1 expression from cell blocks of malignant pleural effusion (MPE) samples. Relatively few studies have been performed on this sample type, with small case numbers. The results to date have shown good concordance (85.1%, kappa 0.774) with PD-L1 expression detected in paired primary tumor tissue samples, using three TPS cut-off values. Interestingly, MPE cells appeared to show significantly higher PD-L1 expression (p = 0.005) [272]. Our own institute has also had positive experience, successfully using MPE cell blocks in the absence of tissue samples to assess tumor PD-L1 status (data not published) In conclusion, the formalin-fixed paraffin-embedded cell block preparation technology is the ideal alternative to test PD-L1 in cytology specimen [272–274].

The development of pioneering assays to make PD-L1 determination is highly progressive. A new potential tool to assess PD-L1 expression following immunohistochemistry is digital image analysis, with or without the support of artificial intelligence. One such system is the Aitrox AI Model [275]. These digital systems provide powerful assistance in exact

quantification and scoring of PD-L1 expression in classic histological conditions.

Determination of soluble PD-1 or PD-L1 in plasma by enzyme-linked immunosorbent assay (ELISA) is another new approach to investigate PD-L1 status. High soluble PD-L1 detected before treatment indicates unfavorable prognosis in ICI-treated lung carcinomas patients, with both PFS and OS being shorter. Changes is sPD-L1 levels after therapy could not be associated with disease outcome. A predictive role of sPD-L1 has not been confirmed to date.

A further promising blood-based assay evaluates exosomal PD-L1 and PD-L1 in circulating tumor cells. In the first series of studies there was no significant correlation between circulating tumor cell PD-L1 expression (neither pre- nor post-treatment) and OS following ICI treatment. However, the dynamic interaction between tumor and immune system was suggested as significantly shorter PFS was observed with high exoPD-L1 levels before ICI treatment, whereas longer PFS was observed with higher exoPD-L1 after treatment.

These plasma-derived PD-L1-associated assays, in addition to simple and non-invasive sampling promise the potential of PD-L1 monitoring to reflect the dynamic, temporal relationship between tumor and immune system. The actual prognostic and predictive role of these biomarkers for ICI treatment is not yet clear [276].

There is generally a negative correlation between tumor PD-L1 expression and response to immunotherapy and the presence of oncogene driver mutations, except for KRAS and partially BRAF and met exon 14 skipping mutations [277]. This correlation has been confirmed in several studies [278, 279]. Since oncogene addicted NSCLC is a rather heterogeneous group both genetically and biologically, there are differences in response to ICI therapy between tumors defined by individual gene defects [280]. Classical EGFR mutations and exon 20 insertions usually show moderate PD-L1 expression and low TMB, and are generally unresponsive to ICI treatment [281]. ALK and ROS1 gene rearrangements often show high PD-L1 expression but low TMB, and these tumours are not responsive to ICI treatment [282, 283]. So strong is this negative correlation that the presence of EGFR and ALK mutations is a treatment exclusion in most ICI recommendations. HER2 mutations are also associated with moderate levels of PD-L1 expression and low TMB detection, and ICI treatments are not effective [284]. RET rearrangements also show low TMB, variable levels of PD-L1 expression and, although there are few and conflicting data, they do not suggest that ICI treatment is effective [285]. The tumours defined by the gene defects listed so far, as previously detailed, are predominantly located in the periphery of the lung and occur mostly in never-smokers or light smokers. Driver mutations play a key role in the formation of these tumours, with escape from immune mechanisms playing a minor role, so that ICI treatment is usually ineffective or results in a modest response [12]. EGFR mutations and driver gene fusions are rare in lung tumors that develop with prolonged exposure to carcinogen tobacco smoke, but KRAS mutations are common [286, 287]. In addition, the tumour mutational burden of these tumours is high. These smoking-associated tumours are markedly immunogenic, and thus tumour formation is influenced by immune escape mechanisms, such as high expression of PD-L1 by tumour cells. Not surprisingly, these smoking-associated tumours with high PD-L1 expression and high TMB generally respond well to ICI treatment [12]. However, due to genetic heterogeneity, there are also significant differences within these tumour groups. In the case of KRAS mutation, good results with ICI treatment are seen in the presence of p53 mutation [288]. Such good results are not observed for STK11 or KEAP co-mutations (KRAS mut/STK11 mut: ORR11.6%, PFS: 2.0 months, OS: 6.2 months, KRAS mut/STK11 wild type: ORR: 32.4%, PFS: 4.8 months, OS: 17.3 months, KRASmut/KEAP mut: ORR: 17.8%, PFS: 1.8 months, OS: 4.8 months, KRAS mut/KEAP wild type: ORR 29.3%, PFS: 4.6 months, OS: 18.4 months) [289]. Among BRAF mutations, a relatively good therapeutic response to ICI treatment is expected in the presence of smoking-associated class II-III non-V600E mutations. In the presence of V600e mutations, only moderate results are observed with ICI treatment [290, 291]. For tumours carrying Met exon 14 mutations, moderate therapeutic response with ICI treatment has been observed [292].

The everyday challenges of biomarker testing

There has been an explosion of knowledge on the oncogenic mechanisms of NSCLC drivers in recent years. Consequently, molecular biomarkers have become known, which are in use for predictive testing to optimize treatment of patients with advanced lung cancer. The conventional approach to biomarker testing is based on the analysis of tumour tissue samples. The extended needs on different testing platforms require increased amounts of tissue and DNA or RNA extracted. Unfortunately, only about 20% of patients are resectable, and in about 80% of cases the diagnosis is based on small biopsy and/or cytological sample [293]. Previous reports (before 2010) suggested that in up to 70% of all lung tumours, diagnosis was made on cytological specimen alone [294]. In the past decades, the differentiation of SCLC and NSCLC was sufficient, but today the accurate subtyping of NSCLC is required as the effect of targeted treatments is mostly expected in adenocarcinomas [295]. NSCLC subtyping is most reliable when tumor specimens are used but cytology smears are principally useless in specific cases, e.g., PD-L1 determination.

The predictive molecular testing recommendations for lung cancer therapy are constantly changing and in the light of new scientific findings and evolving technologies. Further, there are significant differences between the current NCCN, CAP, ESMO

TABLE 1 Most common diagnostic antibodies used to test for driver gene alterations or PD-L1 expression.

Driver gene alteration	Antibody clone	Vendor
EGFR L858R [48–52]	43B2 SP125	Cell Signaling Ventana
EGFR del 19 (E746-A750) [48–52]	6B6 SP111	Cell Signaling Ventana
Alk [136, 137]	D5F3 5A4	Cell Signaling Novocastra
ROS1 [153, 155]	D4D6	Cell Signaling
RET [185]	EPR2871	Abcam
NTRK [196, 204, 205]	EPR17341 C17F1	Abcam Cell Signaling
BRAF [115, 117]	VE1	SpringBio
MET [228, 231]	SP44	Ventana
PD-L1 [265, 266]	22C3 28-8 SP142 SP263 73-10	Dako Dako Ventana Ventana Dako

and Pan-Asian NSCLC recommendations [120, 263, 296, 297]. At the beginning of the era only one or two biomarkers needed to be tested, starting with the EGFR mutational status. According to 2023 ESMO recommendations, all advanced non-squamous NSCLC cases should be tested for ALK, ROS1, NTRK, RET fusions, MET exon14 skipping mutations, BRAF, KRAS G12C and HER2 mutations in addition to EGFR mutations. Molecular testing is only justified for squamous cell carcinoma in special circumstances: young age (below 50 years), non-smoker, ex-moderate smoker or long-time non-smoker status. At this complexity the use an NGS testing platform is recommended, if available. Due to the expansion of gene fusions of interest, RNA-based NGS appears to be the best option. In addition, liquid-based cfDNA testing is also acceptable, but in case of negative results, tissue sampling is required [297]. It is reasonable to determine PD-L1 expression by IHC testing for both advanced squamous and non-squamous NSCLC cases [263]. As already detailed above, the available diagnostic platforms and scoring should be carefully applied: testing with 22C3, 28-8, and SP263 antibody clones show a high concordance, whereas the clone SP142 results in a lower TPS [265, 266]. All these factors require a carefully standardized planning of the daily diagnostic practice.

National guidelines based on international recommendations tend to develop, tailored to the national healthcare system and financial resources. Accordingly, there might be significant differences in the national recommendations. While the reflex testing of biopsy specimens from all patients with advanced NSCLC is generally recommended, on-demand testing is preferred in some countries to save costs. Unfortunately, according to a 2018 study, access to molecular testing is more limited in several Central European countries due to limited resources, and in many countries, on-demand testing is preferred to reflex testing [298]. Since the publication of the aforementioned study, there have been several encouraging developments in these countries [299]. The question arises if it is worth to expend resources on quasi-useless testing for patients with poor performance status, ECOG4, who are not suitable for active oncological care. The hierarchy of testing methods should also be considered for the routine detection of rare genetic events. As an example, it is cost-effective to screen for rare NTRK rearrangements by immunohistochemistry and then to confirm only positive cases by sequencing. The most commonly used diagnostic antibodies currently commercially available are summarised in Table 1.

Individual tests, like series of immunohistochemistry, FISH, and PCR can lead to sample exhaustion, and thus, inconclusive, or false negative results in small biopsies and/or samples with low tumour cell counts by providing insufficient amounts of extracted nucleic acid [300]. Thus, biomarker testing practice is increasingly moving towards multigene technologies, such as the NGS [301].

Based on cost-effectiveness calculations, NGS is already preferable to single-gene tests when testing more than 4 targets simultaneously. In addition, this approach also realizes life-year gains, as calculated in several states [302, 303].

A not negligible aspect of predictive biomarker testing for NSCLC is turnaround time (TAT). Time consuming testing will result in delays in patient treatment, which may even fail due to patient deterioration. International recommendations suggest a TAT of 10 days from receipt of the sample to the communication of the result. The molecular test optimally should be performed in nearby laboratory. However, molecular testing is frequently centralized, which may prolong the TAT for logistical reasons (sample transport). Reflex testing also shortens the TAT, and supports optimal sample usage. In contrast, on-demand testing is more appropriate to avoid unnecessary tests, at the expense of the TAT [304]. It is important mentioning that the 10-day TAT recommendations is difficult to achieve with NGS in general. The sequencing approach is usually designed for 8 samples run simultaneously and the biochemistry is followed by a bioinformatic session of various complexity [304]. Any molecular techniques have their pros and cons laboratories should consider for their specific aims and needs. The choice of sequencing chemistry, device and software solutions also defines the acquisition of specific targets, the reagent requirements, the rate of the testing and turnaround times.

In lung cancer patients the tumour is frequently irresectable and/or the patient, or the tumor is unsuitable for bronchoscopic and/or transthoracic sampling. Small biopsy samples may often be not representative. In many of these cases, malignant pleural fluid is an alternative diagnostic specimen. MPE is present in about 15%–25% of lung tumours at diagnosis and occurs in 50%–

60% of lung cancer patients overall during the disease [305–307]. However, the fluid cytology samples are sometimes also difficult to evaluate. It is recommended to prepare a cell block from the MPE specimen instead of the conventional cytospin smear. In general, cell blocks offer several advantages: the sample specimen can be examined as a tissue sample, it allows safe separation of activated mesothelial and tumour cells, and it provides accurate tumour typing. The cell block has higher diagnostic specificity and sensitivity than the cytospin smear, depending on tumor type (25, 53, 78% and 95% for squamous cell carcinoma, SCLC, adenocarcinoma and ovarian carcinoma, respectively [306, 308]. As a major benefit, cell blocks are suitable for immunohistochemical and molecular studies, including the investigation of all common predictive biomarkers for NSCLC therapy (EGFR, KRAS and ALK, ROS1, PD-L1 state).

Conclusion

As described in our study, over the last 20 years, the increasing understanding of the molecular background of NSCLC has led to the identification of new therapeutic targets. Year after year, molecularly targeted treatment options are giving a growing group of patients with advanced NSCLC longer survival and better quality of life, without the life-threatening serious side effects of cytotoxic treatments. At the same time, patient selection has created unprecedented challenges for the diagnostic professions, particularly pathology departments. Further persistent work is needed to identify new molecular aberrations in addition to the current therapeutic targets, which will allow the use of more effective treatments for patients without the already identified driver mutation.

Author contributions

LT: conceptualization, methodology, data curation, writing-original draft, visualisation. AM: writing-review and editing, supervision. GM: writing-reviewing and editing, project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Decreasing incidence and mortality of lung cancer in Hungary between 2011 and 2021 revealed by robust estimates reconciling multiple data sources

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Objective: Hungary has repeatedly been shown to have the highest cancer-related mortality and incidence in Europe. Despite lung cancer being the most abundant malignant diagnosis in Hungary, numerous concerns have been raised recently regarding the bias inherent to reported incidence estimates. Re-analysis of reimbursement claims has been suggested previously by our group as an alternative approach, offering revised figures of lung cancer incidence between 2011 and 2016. Leveraging on this methodology, we aimed at updating Hungarian lung cancer incidence estimates with an additional 5 years (2017–2021), including years affected by the COVID-19 pandemic. Additionally, we also attempted to improve the robustness of estimates by taking additional characteristics of the patient pathway into account.

Methods: Lung cancer patients between 2011 and 2021 were identified based on reimbursement-associated ICD-10 codes, histology codes and time patterns.

Multiple query architectures were tested for sensitivity and compared to official estimates of the Hungarian National Cancer Registry (HNCR). Epidemiological trends were estimated by Poisson-regression, corrected for age and sex.

Results: A total of 89,948 lung cancer patients diagnosed in Hungary between 2011 and 2021 have been identified by our study. In 2019 alone, 7,887 patients were diagnosed according to our optimized query. ESP2013 standardized rate was estimated between 92.5/100,000 (2011) and 78.4/100,000 (2019). In 2019, standardized incidence was 106.8/100,000 for men and 59.7/100,000 for women. Up until the COVID-19 pandemic, lung cancer incidence was decreasing by 3.18% (2.1%–4.3%) yearly in men, while there was no significant decrease in women. Young age groups (40–49 and 50–59) featured the largest improvement, but women aged 60–79 are at an increasing risk for developing lung cancer. The COVID-19 pandemic resulted in a statistically significant decrease in lung cancer incidence, especially in the 50–59 age group (both sexes).

Conclusion: Our results show that using an optimized approach, re-analysis of reimbursement claims yields robust estimates of lung cancer incidence. According to this approach, the incidence rate of male lung cancer is declining in Hungary, in concordance with the trend observed for lung cancer mortality. Among women aged 60–79, the incidence of lung cancer has risen, requiring more attention in the near future.

KEYWORDS

lung cancer, incidence, mortality, COVID-19, Hungary

Introduction

Current understanding of lung cancer epidemiology in Hungary

It is widely accepted that compared to most European countries, Hungary suffers from a relatively high malignant disease incidence and mortality. This has been shown for example by a series of papers comparing 40 European countries [1–3], for both overall cancer epidemiology and individual tumor types. It is important to stress that for Hungary, these epidemiological studies estimate cancer incidence indirectly, based on statistical models relying on reported deaths and incidence in neighboring countries [4]. This indirect approach might already add some uncertainty to the estimates, while other reports have suggested mortality reported for Hungary to be exaggerated recently [5].

Furthermore, the reliability of tumor classification in reports has also been questioned by a recent amendment [6, 7] of the Hungarian National Cancer Registry (HNCR). One of the tumor types most frequently misclassified was lung cancer, possibly since lung is also a common site of metastases. Lung cancer on the other hand, is the most abundant cancer type in men, accounting for more than 20% of all cases and the top cause of cancer mortality in both sexes. Given its share, an inflated number of reported lung cancer cases can already impact the assessment of the total number of cancer patients, while the described bias might also affect other cancer types (common metastatic sites, for example), adding further uncertainty to epidemiological observations. A potential solution would be manual curation of individual records before reporting case numbers, as it was done by the HNCR with the amendment of lung cancer cases for 2018. This was found to be extremely demanding and not routinely feasible for each tumor type and year given the current resources.

Approaches to refine incidence estimates

An alternative approach to manual curation of hospital records for the estimation of cancer incidence could be the utilization of alternative sources of information. The National Health Insurance Fund of Hungary (NHIF) is a valuable resource capturing detailed information on patient pathways via reimbursement claims. Previous publications from our research group [4, 8] have already demonstrated that epidemiological indicators can be estimated efficiently using this data source. Additionally, patient-level details captured in reimbursement claims have also provided valuable insights on the high number of *post mortem* diagnosed lung cancer cases as a potential confounder of reported case numbers [7].

Even if incidence estimated by any of the above described methods cannot be 100% accurate, reproducibility of querying the NHIF database and the possibility to finetune stringency of the query provides information on the range where the actual value lies and what biases one should look out for. This deeper understanding of epidemiological indicators is essential in the timely allocation of

resources to meet emerging challenges as well as in the assessment of updates to healthcare policies. Lung cancer being the most frequent malignant disease in Hungary, it is the first choice of cancer type for modelling the robustness of epidemiological indicators and trends.

Aims

The present research was carried out as part of the Hungarian Evaluation of Lung cancer Patient Pathway Project (HELP3) research project describing lung cancer epidemiology and patient pathways in Hungary. The primary aim of the current study is to update lung cancer incidence and mortality figures in Hungary. Recent advances uncovering potential confounders offer an excellent opportunity to refine epidemiological trends incorporating data based on the NHIF, using an improved query structure. The impact of query parameter optimization is assessed based on the robustness of trends calculated from results yielded by these queries. The impact of the COVID-19 pandemic, caused by the SARS-CoV-2 virus, on lung cancer epidemiology in Hungary can be traced in the risk ratio of pre-COVID and COVID years 2019 and 2020.

Materials and methods

Data sources

Yearly incidence of lung cancer was estimated based on healthcare reimbursement claims in the NHIF database, as described by our group previously [4, 8, 9]. Briefly, patients named in claims with the ICD-10 code of C34 were regarded as lung cancer patients. The year of the first occurrence of C34 was accepted as the year of diagnosis. Patients newly diagnosed between the 1st of January 2011 and the 31st of December 2021 were studied, with an additional screening period of 2009-2010 to account for patients already diagnosed outside the study period.

To further improve the specificity of our definitions, additional constraints were added to the query, specifying the number of C34 codes in an individual's history and the minimum (30 days) as well as the maximum (180 or 365 days) amount of time accepted between two C34 codes. Patients who have died within 60 days of the first diagnosis were not required to feature multiple C34 codes in their history. This multi-tier, optimized query is referred to as "Optimized," in sensitivity analyses (Supplementary Figure S1). When comparing the optimized query, the alternative requiring only one single mention of C33 or C34 will be referred to as *1.1*, while the query requiring 2 mentions is *1.2A*. A minimum of two mentions within a year is referred to as *1.2B*, while 2 mentions within 180 days is *1.2C*. Three mentions within 180 days is *1.3*. Estimates provided by the HNCR (stat.nrr.hu, accessed 20/10/23) were used as a reference. Please note that the optimized query is very similar to the 1.2B alternative, with the additional constraint of at least 30 days between the two claims and a small relaxation regarding patients deceased within 60 days.

Number of deaths associated with lung cancer are based on reports of the Hungarian Central Statistical Office (HCSO). Both incidence and mortality were standardized using the European Standard Population (ESP) of 2013 and standardized rates are given using that reference, unless otherwise indicated. For the sake of comparison with estimates of Ferlay's workgroup, rates standardized to the ESP 1976 population are also shown in this context. To facilitate reproducibility of our results, patient numbers yielded by NHIF queries, retrieved from the HNCR or the HCSO are provided in a tidy long format as Supplementary Table S1, as well as mid-year population retrieved from the HCSO (Supplementary Table S2)

Statistical analysis

All calculations were carried out in the open source software environment for statistical computing R (v4.2.1). Average Yearly Change was estimated by Poisson-regression, correcting the model for population size, age, and sex. The logarithmic value of the number of individuals in a given age group was used as offset, while age, sex and year were explanatory variables. Statistical significance was inferred from the *p*-values of Wald-tests implemented by the *Testing Linear Regression Models* (lmtest v0.9) package and considered significant in case it was $p \leq 0.05$. Confidence intervals (95% CI) were estimated using the packages lmtest and *Robust Covariance Matrix Estimators* (sandwich v3.1) variance estimation [10] to correct for non-independent nature of the data. A normal distribution was assumed when assigning (95% CI) of direct standardized rates. Risk ratio was calculated using the *Epidemiology Tools* (epitools v0.5) package.

Results

Annual number of patients

The number of yearly diagnosed lung cancer patients in Hungary ranged between 8,752 and 7,003. For 2019, the last year before the COVID-19 pandemic, our query after optimizations has identified 7,887 new lung cancer patients (Supplementary Figure S1). As a comparison, the least stringent case definition 1.1 returned 9,600 hits, while changing the stringency by requiring multiple visits in the patient history (filters 1.2A, 1.2B, 1.2C) increased the number of candidates to 8,498, 8,144 and 8,240, respectively. Requiring 3 visits, without further restrictions (filter 1.3C) picked up only 7,121 potential lung cancer cases.

The highest number following each approach was observed for 2011 and a steady decrease in patient numbers could be observed until 2020, featuring the lowest number of new patients during the studied period. For each year, a male predominance in the number of patients could be observed. For the initial year 2011, 5,517 men and 3,235 women were found to be diagnosed with lung cancer. By

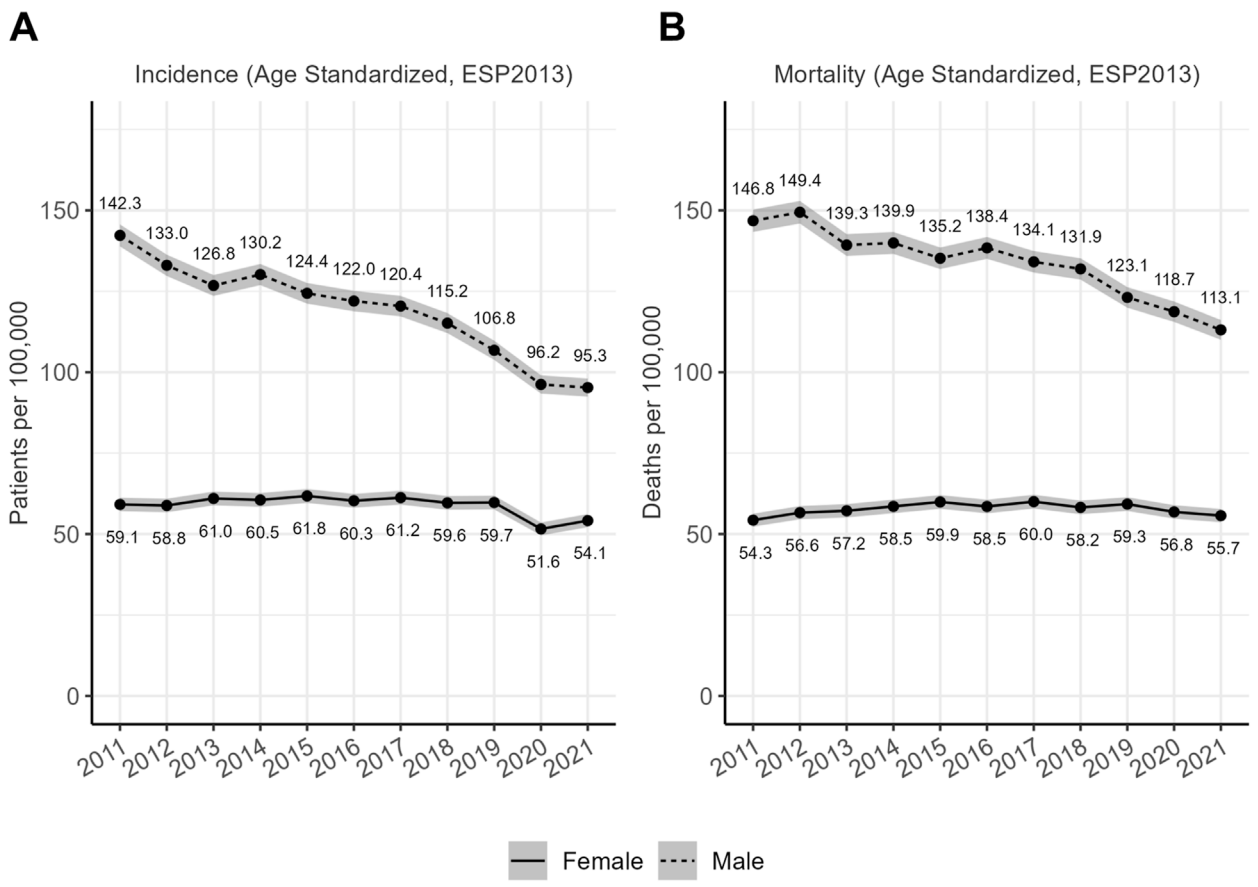

FIGURE 1
(A) Standardized incidence rates of lung cancer in Hungary between 2011 and 2021. Direct standardized rates using the ESP2013 population weights. Grey ribbon around the lines represents the 95% confidence interval. **(B)** Standardized mortality rates of lung cancer in Hungary between 2011 and 2021 (ESP2013 standardized rates). Direct standardized rates using the ESP2013 population weights. Grey ribbon around the lines represents the 95% confidence interval.

the end of the study period, this difference was smaller (4,424 and 3,436, respectively, in 2019), but still visible.

Age standardized rates

In 2011, according to the results of the optimized, consensus query, there were 142.3/100,000 (ESP2013) new male lung cancer patients identified, while only 59.1/100,000 (ESP2013) female patients (Figure 1A). Standardized incidence has decreased to 106.8/100,000 in men by 2019, while it remained at the same level with 59.7/100,000 for women. As a comparison, standardized mortality due to lung cancer was reported to be 146.8/100,000 in men and 54.3/100,000 for women (Figure 1B) in 2011, which has changed to 123.1 in men and 59.3 in women. A detailed report of standardized incidence (Supplementary Table S3) and mortality (Supplementary Table S4) rates for all studied years and both sexes can be found in the Supplementary Material.

Lung cancer had the highest incidence in the 60–69 (women) and 70–79 (men) age groups (Figure 2). Age distribution of lung cancer patients has been shifting towards stronger representation of older age groups, especially in women. Age distribution of patients died of lung cancer shows a similar pattern, with an even more pronounced decrease in the share of the 40–49 age group in both sexes.

Observed trends

Lung cancer incidence in the general population was found to decrease between 2011 and 2019 by 1.76% on an annual basis (95% CI: 0.5%–3%). For men only, the decrease was 3.18% (95% CI: 2.1%–4.3%), while for women, the estimated 0.33% increase (95% CI: −0.1%–1.6%) did not indicate a significant change (Figure 3; Supplementary Table S7). Young age groups (40–49 ad 50–59) featured the largest decrease in lung cancer incidence for both women and men. Conversely, the incidence was less likely to decrease in the 60–69 and the 70–79 age

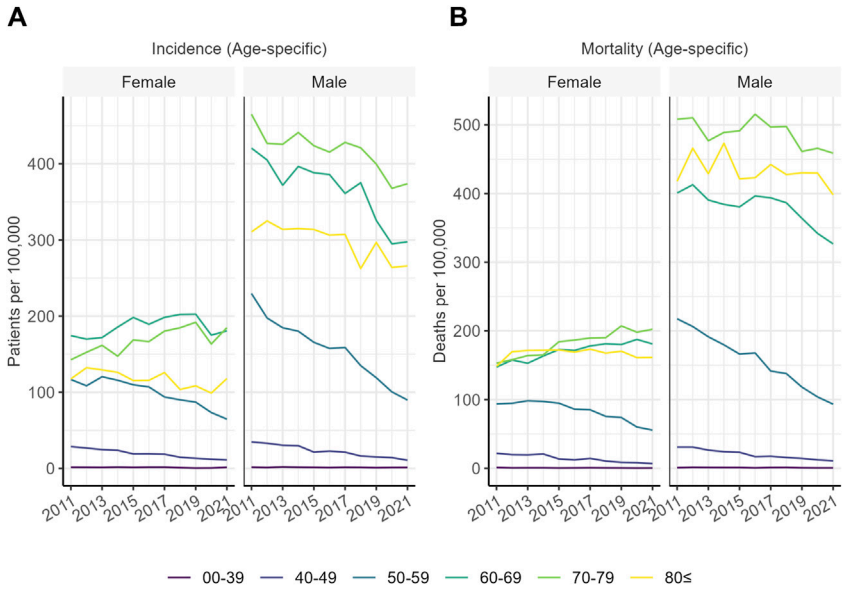

FIGURE 2
(A) Age-specific incidence of lung cancer in Hungary in the studied age groups. Values indicate the number of patients diagnosed in a given year out of 100,000 individuals of the age group. **(B)** Age-specific mortality of lung cancer in Hungary in the studied age groups. Values indicate the number of patients deceased in a given year out of 100,000 individuals of the age group.

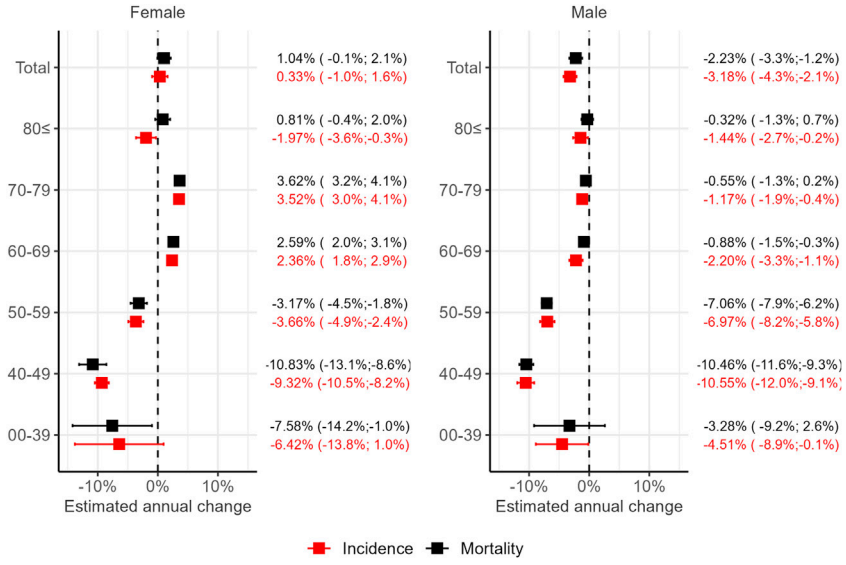

FIGURE 3
The average annual change in lung cancer incidence and mortality in Hungary between 2011 and 2019 for the total population, as well as by age groups. Estimated using Poisson regression and robust confidence intervals (95% CI) with the sandwich method.

groups. Lung cancer incidence in elderly women has even increased slightly during the study period.

A similar pattern of lung cancer mortality (Figure 3; Supplementary Table S8) could be observed between 2011 and 2019, with an overall decrease of 0.99% (95% CI: −2.0%–0.1% change). While the overall trend was not found to be statistically significant, there was a significant, 2.23% decrease (95% CI: −1.2%–3.3%) change when taking only men into account. The 1.04% yearly increase (95% CI: −0.1%–2.1%) estimated for women was not statistically significant either. An improvement in

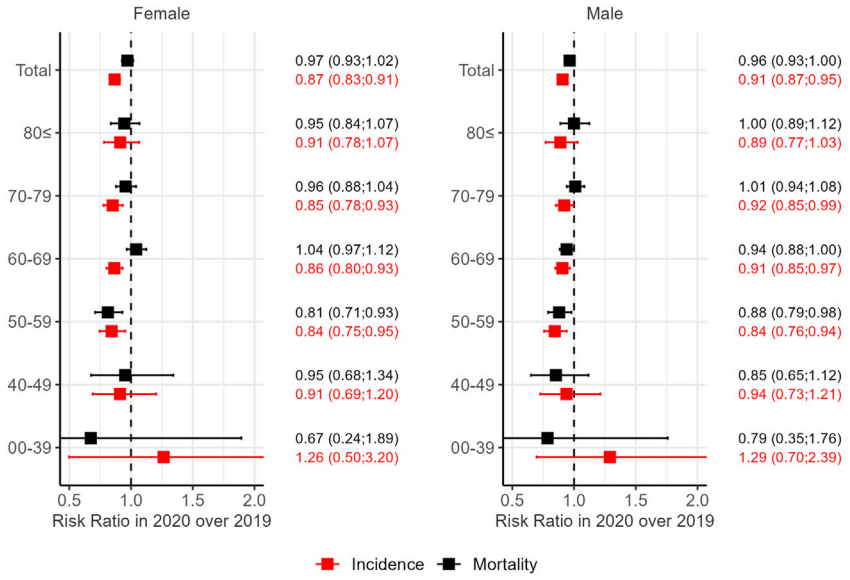

FIGURE 4
Change in lung cancer incidence and mortality in Hungary during the COVID year 2020 expressed as the risk ratio (RR) of 2020 vs. 2019. Confidence intervals (95% CI) of RR are given in brackets.

mortality could be confirmed in the 40–49 and 50–59 age groups of both sexes, while mortality has increased among women aged 60–69 and 70–79.

COVID-19 impact

To assess the impact of the COVID-19 pandemic on lung cancer incidence and mortality in Hungary, the pandemic year 2020 was compared to the previous year, 2019 (Figure 4). The risk of lung cancer diagnosis was significantly lower for both women and men in 2020 than in 2019 (Risk Ratio (RR) = 0.87 and 0.91, respectively), while the risk of lung cancer specific death did not decrease significantly (RR = 0.97 and RR = 0.96, respectively). The difference in terms of patient numbers means that 891 less patients were diagnosed with lung cancer in 2020 than in 2019 (6,970 and 7,861, respectively). The decrease in incidence compared to 2019 was driven mainly by the 50–59, 60–69, and 70–79 age groups in both sexes. Lung cancer mortality, however, showed a similar decrease only in the 50–59 age group (RR = 0.81, women; RR = 0.88, men). The calculated RR values for incidence (Supplementary Table S9) and mortality (Supplementary Table S10) are also available in the Supplementary Material.

Sensitivity analysis

Standardized lung cancer incidence estimated using different case definitions resulted in a range around 133–179/100,000 for men and a narrower range of 55–79/100,000 for women in 2011 (Supplementary Figure S2). Estimate using the less stringent query (1.1) was close to the number reported by the HNCR and restricting the definition by requiring more than one record featuring the ICD-10 code of lung cancer (1.2A) already yielded results similar to the final estimate. Restrictions regarding the minimum or maximum amount of time between visits (1.2B or 1.2C) had a much smaller impact on the results. Capturing the raw numbers behind these rates, a total of 8,191 to 11,261 new lung cancer patients were found for the reference year 2012 (Supplementary Figure S1). The least stringent case definition aligns well with the 11,000 patients reported by the HNCR. Using different case definitions had hardly any effect on the time trend analysis (Supplementary Figure S3).

European context

Comparison of previously reported standardized incidence rates of Hungary to neighboring countries also suggests a possible bias, resulting in the overestimation of Hungarian lung cancer incidence. In a comprehensive study including 40 European countries, Ferlay's workgroup has reported a 109.3/100,000 male lung cancer incidence rate (ESP1976) for Hungary in 2012 [3] and 111.6/100,000 in 2018 [2]. These rates would be extremely high in Europe and this extent of outlying alone would be alarming. On the other hand, the 133 per 100,000 Person Years estimated based on the ESP2013 standards for 2012 and reported above (Figure 1.), is equivalent to 95.2/100,000 calculated using the ESP1976 weights. Similarly, the ESP1976-eqivalent incidence rate for 2018 was 80.5/100,

000 based on our data. These estimates are much closer to the (Central) European average (Supplementary Figures S4, S5) than the ones reported previously for Hungary. The ESP1976 standardized rates for all studied years are provided for multiple incidence estimates (Supplementary Table S5) as well as for mortality reported by the HCSO (Supplementary Table S6) in the Supplementary Material.

Discussion

Updated lung cancer incidence in Hungary

By extending the studied period to more than 10 years, the current study provides updates to epidemiological indicators of lung cancer in Hungary with an even more refined approach, but also largely building on our previous reports [4, 7, 9]. This update confirms the inconsistency between lung cancer incidence rates reported previously for Hungary and the rates estimated by our approach. Differences in the numerical values of lung cancer estimates reported for Hungary are partially due to differences in standardization methods as they have been evolving through time. Values given relative to the ESP2013 population can be converted to reflect the rate in the ESP1976 population, as also done in this study to compare with historical data. Even after conversion, however, conflicting rates have been for Hungary [2, 3]. We claim that this remaining difference originates at least partially from differences between statistical models used to estimate incidence. One can even trace the changes that occur to the model used by one single research group over time, changing the input slightly to rely on different countries [2, 3], to improve confidence of the estimate.

Appreciating that the increasing weight of input from the national cancer registry of the Czech Republic in the reported Hungarian estimate is in agreement with our observations regarding the similarity of healthcare indicators in Hungary to other Eastern European countries [7], we would like to advocate for granting greater importance to local datasets, when developing these models. Since the availability of observational data for Hungary is limited currently, we would like to provide such empirical data, hoping to contribute to closing the gap between lung cancer incidence estimates in the literature.

While a critical revision of standard incidence contributes to a realistic assessment of current disease burden, the trends described by these figures are even more crucial when planning future preventive actions. Acknowledging that limitations to our approach prevent us from identifying some patients, thus our data can be regarded as close estimates only, we argue that the trends identified in these figures are robust enough to identify emerging needs or give positive feedback on improvement. Differences in the dynamics of the epidemiology of lung cancer between women and men has been shown multiple times, including our publications [4], and our current results recapitulate this pattern nicely. Furthermore, an increasing lung cancer incidence among women between 60 and 79 already points out a population that needs more attention regarding prevention, early detection, or care.

Improving trend in 2011–2019

Our data suggests that lung cancer incidence has started to decline during the last decade (2011–2019) in men, similar to observations for Germany [11]. This observation is in line with our previous reports on lung cancer, describing improvements over previous periods [4, 8], in contrast to interpretations forecasting an alarming number of new lung cancer cases [12]. As smoking contributes to about 85%–90% of lung cancers [13], based on the slowly decreasing smoking prevalence in almost every European country [14], a consequent decrease in lung cancer suggested by our results seems logical.

An increase in lung cancer incidence within the population would only be expected if other risk factors were emerging, or in certain subpopulations. While air pollution could be such an emerging risk factor, showing a well-established association with lung cancer etiology [15], no major rise in Particulate Matter <2.5 μm (PM2.5) or PM10 levels has been reported for Hungary during the study period. Nevertheless, PM2.5 levels, a recently re-confirmed etiological factor in the pathogenesis of adenocarcinoma [16], have been constantly high in Hungary for decades [17]. This prolonged exposition might contribute to the increased risk among women. Occupational risk factors, such as asbestos have been shown to contribute little to population-level lung cancer risk in Central Eastern Europe [18]. Inherited genetic variations generally contribute to lung cancer etiology indirectly via susceptibility to environmental exposure [13]. Even though positive family history of lung cancer has been shown to increase lung cancer risk in the Eastern European population [19], contribution of genetic causes can still be considered minor compared to the risk of smoking in patients above 50.

While the observed improvement at the population level is logical considering the decrease in smoking prevalence, the lack of improvement among women is in certain age groups is somewhat surprising. As described in our previous revision of lung cancer epidemiology in Hungary [4], however, smoking prevalence is not homogenous in the population and even gender imbalance has dynamically changed over time. In contrast to men, where the number of ever-smokers has decreased steadily in every birth cohort, an increase in smoking prevalence has preceded the recent decrease in women. This difference in smoking patterns has already been shown to affect lung cancer incidence in European populations [20] and explains the heterogenous trends observed in our data stratified by age groups.

It is also important to note that our study focuses on changes regarding the risk of an individual, captured in standardized

incidence and mortality rates. Standardization is carried out precisely to compensate for the confounding effect of the population's aging. The increasing number of elderly people in a population inevitably leads to an increase in age-related diseases, like lung cancer. This might lead to a paradoxical observation of increasing patient numbers (and burden on the healthcare system) even when the individual's risk is decreasing. Although both indicators (patient numbers and risk) carry important information, we decided to focus on risk as this better describes the effect of preventive measures or the improvement in quality of care. Nonetheless, this is the measure that can be compared among countries or regions.

The impact of COVID-19

The COVID-19 pandemic declared by the World Health Organization (WHO) in 2020 has resulted in a sudden, dramatic change in accessibility. The impact of these restrictions on lung cancer detection has been shown for example by a direct comparison of the number of lung cancer cases in the UK during lockdowns in 2020 and the same period of 2019 [21], reporting a 26% decrease during the pandemic. A 14.4% decrease in lung cancer incidence has also been observed for Hungary, together with decrease in breast and colorectal cancer patient numbers [22]. Thus, the incidence during the COVID-19 pandemic was not specific to lung cancer, only the extent of the decrease varied between tumor types. In addition to confirming the decreased breast cancer incidence [23], we have also described that breast cancer mortality did not increase significantly. This suggests that care of patients already diagnosed was not affected by restrictions or bottlenecks in resources, but rather the detection of cancer cases was delayed. Screening program participation, for example, has also reduced dramatically due to the pandemic: 25.8% less centrally organized mammography examinations were conducted in 2020 than in the previous year [24]. The reduction in diagnostic capacities was probably even greater in the case of cancer types where an organized screening program is not available, like lung cancer.

Reduced incidence was not associated with a change in mortality in our study population of Hungarian lung cancer patients, similarly to what has been described for breast cancer earlier [23]. Delayed diagnosis is, however, expected to cause an increase in lung cancer mortality of around 4.8%–5.3% during the next years, as suggested by a modeling study based on the UK population [25]. Another model based on the Australian population warns about a potential mid-term reversal of positive epidemiological trends (decreasing cancer incidence and mortality) caused by the aftermath of the restrictions in 2020 [26].

Patients not identified in 2020 are also likely to increase lung cancer incidence or at least mortality in the post-pandemic era. According to our data, as many as 891 lung cancer patients might have been missed in 2020. Even if the decreasing trend is considered, ~800 lung cancer patients will receive a delayed diagnosis due to the COVID-19 pandemic. These patients are expected to either show up in the healthcare system as an excess number of new patients in 2021, 2022 or might never actually be identified if they also suffered from COVID and the outcome was fatal. It is not yet clear from currently available data, which scenario is true, but these considerations should be taken into account, when assessing lung cancer epidemiology of post-pandemic years.

Lung cancer incidence has decreased during the COVID-19 pandemic primarily in the 50–59, 60–69, and 70–79 age groups, both sexes. The sex-related pattern of lung cancer epidemiology is not reflected in the changes attributable to the COVID-19 pandemic. Interestingly, no decrease was seen among elderly patients, above 80. This might be due to the simultaneous prevalence of multiple diseases in this age group and consequently, frequent hospitalizations. There are sporadic reports on incidental findings on chest CTs requested to confirm COVID turned out to be early lung cancer [27] and these non-targeted diagnostic procedures might have been carried out at a higher rate in elderly, multimorbid patients presenting with symptoms suggestive of COVID. Even if part of the population at risk for lung cancer might have profited from the preventive measures during the pandemic, the chance of recognizing lung cancer was hindered in the general population.

The only age group featuring a significant change in mortality is patients between 50 and 59. Our data is not sufficient yet to decide if this age group profited from the alertness of the healthcare system during the pandemic or this decrease is independent of the COVID-19 pandemic. The strongly decreasing trend in both incidence and mortality of lung cancer in this age group already before the COVID-19 pandemic suggests an idiosyncratic effect.

Robustness of data and limitations

A major limitation to our claims-data-based approach is that detailed clinical history is not available in this database, thus it is not possible to medically validate the records. In fact, this confirmation would also not be feasible at this scale even if all health records were available and accessible. In a similar attempt, the HNCR carried out manual validation of the diagnoses associated with every individual reported as lung cancer patients in 2018 [28]. Reviewing the patients from one single year has already proven to be a tremendous effort, far beyond resources available for epidemiological studies. The amended patient number (9,541 patients), however, is an important reference to benchmark any alternative approach, such as the one followed in this study.

The present approach has identified 8,252 patients in 2018 that is lower than the reference reported by the HNCR. Identifying this subset of claimed patients with a high probability of a valid disease classification might offer an opportunity on the other hand to associate a confidence level to patients registered by the HNCR. Despite still not being able to identify some lung cancer patients, performance of the current approach features remarkable improvements even over the method used in a previous study based on the same source [4]. This improvement was enabled by a combinatorial optimization of the query parameters, better adapting to common patient pathway scenarios. One such scenario would be the common case, when a late-stage patient is not able to go through the diagnostic procedure to arrive at a definite diagnosis. By requiring less stringent conditions for patients deceased within 60 days of the first claim featuring a lung cancer related ICD-10 code, reduces the risk of missing these patients. Using patient pathway-related criteria to exclude patients coded as lung cancer by mistake is a reproducible, high-throughput approach that is feasible to be carried out even in case of large patient cohorts. Thereby we suggest that, while not being able to achieve data quality offered by manual curation, it is able to flag potentially miscoded patients; the error type identified most frequently during manual curation [28].

Another potential issue identified by researchers at the HNCR, could be the inconsistent reporting of lung metastases from other sites [5] and this is related to a third source of uncertainty, identified by our research group recently: the considerable number of lung cancer patients diagnosed *post mortem* only [7]. As a comparison, ~10% of lung cancer cases in Germany are recognized only during autopsy [11]. Patients presenting at a very advanced stage might be in such a severe condition already that diagnostic procedures cannot be carried out and a definite diagnosis will never be made. Although some of these patients might appear in the claims dataset under ICD-10 codes D38 or R91H0, it is very hard to assess the number of the patients recognized at this stage and we cannot describe the change in this patient segment over the studied period using our method. A change in coding preference of the hospitals during the period might also confound our observations. Both lung metastases and *post mortem* diagnosed patients add a further level of complexity to obtaining a realistic estimate of lung cancer incidence, but a consistent, reproducible methodology described here can still provide valuable insights on epidemiological indicators.

Bias related to *post mortem* diagnosis, or even unspecified metastases could also impact on cause-specific mortality reported by the HCSO. It has already been proposed by studies conducted within the HNCR that a slight overestimation of patient numbers is inherent to many cancer registries [5] and perhaps, even mortality reports. According to our previous observations described in the HULC study [7], the number of *post mortem* diagnosed lung cancer cases is comparable to the total number of lung cancer patients. Based on the proportion of these cases, it seems logical that individuals never diagnosed with lung cancer, but reported after an autopsy to have died of the disease, contribute to an overestimation of lung cancer mortality, as suggested also by the HNCR. A further argument supporting this possibility is that the mortality-to-incidence ratio (MIR) calculated from our revised incidence rates is close to 1, while globally it has been around 0.7 [29, 30]. This problem does not affect every cancer type equally. Lung cancer is likely among the sites more severely impacted by this bias due to the high probability of metastases from other sites developing in the lung. Similarly, not every country is affected equally, possibly due to the disparate autopsy rates among countries, as discussed earlier [7]. While revising the accuracy of diagnosis for patients registered in the HNCR has been carried out recently [28], such a revision regarding cause-specific mortality reported by the HCSO was not in the scope of the current study.

Regardless of the number of lung cancer patients missed by our approach, it is important that the error rate is constant across years, rendering trend estimations based on our data robust and reproducible. As shown in Supplementary Figure S3, the stringency of our case definition did not significantly change the trend estimated by our models. As an alternative validation approach, incidence trends estimated by our approach were also compared to mortality trends. Only one age group (women above 80) featured a significant difference that confirms the expected parallel change of incidence and mortality in most age groups. Notably, trend estimated based of HNCR data is also not parallel with mortality in this single age group. These observations support the validity of epidemiological trends calculated using our approach and corroborates evidence supporting recent improvements in Hungarian lung cancer epidemiology.

Conclusion

Our results provide further evidence challenging currently reported case numbers and offers an alternative approach to estimate lung cancer incidence, yielding more robust estimates. Better alignment of the resulting figures with numbers reported from neighboring countries, as well as with the dynamics of cause-specific mortality suggest that the more conservative estimates are closer to the actual number of new patients. Without denying the burden imposed by lung cancer on Hungary, we would like to advocate for a more realistic picture, where the dynamics of lung cancer in Hungary is very similar to other Central European countries.

A more realistic assessment of lung cancer epidemiology in Hungary offers a better opportunity to identify emerging challenges, allocate resources and confirm success. Furthermore, our data suggests that the described trends are

even more robust than the individual yearly estimates. Identifying differences in the dynamics of the epidemiology between women and men offers opportunities for intervention. One such emerging challenge identified by our study is the increasing risk of lung cancer in women between 60 and 79 despite improvements in the general population. Robust estimation of lung cancer incidence also contributes to a more precise assessment of the impact of the COVID-19 pandemic on delaying lung cancer diagnosis and highlight potential consequences.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Central Ethical Committee of Hungary (IV/3940-3/2021/EKU). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

GG: conceptualization, methodology, writing–original draft, GS: data analysis, visualization, writing–original draft, LT: supervision, writing–review editing, VM: conceptualization, supervision, writing–review editing, JM: conceptualization, supervision, writing–review editing, VS: supervision, AK-F: supervision, writing–review editing, TK: supervision, validation, EC: supervision, validation, ZP-S: supervision, validation, ZS: supervision, validation, ZtK: supervision, validation, GH: methodology, supervision, validation, ZaK: validation, ÉB: validation, KK: supervision, validation, MD: methodology, writing–review editing, data curation, VB: methodology, writing–review editing, data curation, GR: methodology, writing–review editing, data curation, data acquisition, supervision, ZA-T: data analysis, data acquisition, visualization, validation, ZnK: conceptualization, methodology, investigation, visualization, supervision, writing–original draft, ZV: conceptualization, methodology, writing–original draft, KB: conceptualization, methodology, supervision, validation, writing–original draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

GS, GH, ÉB, and KK are employees of MSD Pharma Hungary Ltd. ZV is an employee of Semmelweis University where his contribution to this project was financially compensated. ZKis is also an employee of MSD Pharma Hungary Ltd. and has an affiliation at the Second Department of Medicine and Nephrology-Diabetes Center, University of Pécs Medical School, Pécs, Hungary. GR and ZA-T are employees of RxTarget Ltd. where their contribution to this project was financially compensated. The project was financed by MSD Pharma Hungary Ltd. VM has received consultation fees from AstraZeneca, Boehringer Ingelheim, Roche, Berlin-Chemie, Chiesi, BMS, Novartis, Actelion, Gilead, Pfizer, Richter, Lilly, Orion Pharma and Ipsen and served as PI for over 10 LC studies. LT is an employee of Semmelweis University.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.por-journal.com/articles/10.3389/pore.2024.1611754/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Comparison of patient numbers estimated via different approaches. The consensus estimate of the present study is compared to the raw number of incident patients reported by the National Cancer Registry (NCR) as well as to query variations (1.1–1.3C, see methods for detailed definition) featuring different levels of stringency.

SUPPLEMENTARY FIGURE S2
Sensitivity analysis of incidence estimates based on different approaches. Direct standardized rates are calculated for the ESP2013 population standard. The consensus estimate of the present study is compared to

the incidence reported by the National Cancer Registry (NCR) as well as to query variations (1.1–1.3C, see methods for detailed definition) featuring different levels of stringency.

SUPPLEMENTARY FIGURE S3
Sensitivity analysis of trend estimations. Epidemiological trends estimated based on officially reported mortality, reported new patients in the National Cancer Registry (NCR) and queries of different stringency (1.1–1.3C, see methods for detailed definition) are compared to the consensus estimated by the present study. Annual change estimated using Poisson regression; 95% confidence intervals calculated via the sandwich method. Young age cohorts with small numbers of reported cases not shown for the sake of clarity.

SUPPLEMENTARY FIGURE S4
Hungarian lung cancer incidence (women) in the European context. Female Lung cancer incidence rates standardized to the ESP1976 population by our approach for 2012 and 2018 (Hungary*) are compared to numbers reported by Ferlay et al. for Hungary, as well as other European countries.

SUPPLEMENTARY FIGURE S5
Hungarian lung cancer incidence (men) in the European context. Male Lung cancer incidence rates standardized to the ESP1976 population by our approach for 2012 and 2018 (Hungary*) are compared to numbers reported by Ferlay et al. for Hungary, as well as other European countries.

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Novel diagnostic processes and challenges in bronchoscopy

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Diagnostic bronchoscopy is a minimally invasive procedure that plays a crucial role in the diagnosis and management of various respiratory conditions. This paper explores the advancements in technology that have revolutionized the field and focuses on the new diagnostic procedures in bronchoscopy that have emerged in recent years. These innovative techniques have expanded the diagnostic capabilities of bronchoscopy, allowing for more accurate and comprehensive evaluation of respiratory conditions. This paper will also discuss the challenges in the diagnostic process with bronchoscope.

KEYWORDS

diagnostic bronchoscopy, respiratory medicine, endobronchial ultrasound, virtual bronchoscopy, autofluorescence bronchoscopy

Introduction

Diagnostic bronchoscopy is a minimally invasive procedure that allows for direct visualization and sampling of the airways, providing valuable diagnostic information in the evaluation of various respiratory conditions. Technological advancements have revolutionized the field of diagnostic bronchoscopy. Flexible bronchoscopes have replaced rigid instruments, enabling easier access to peripheral airways [1]. Endoscopic ultrasound techniques revolutionized the diagnostic work-up, due to their extreme importance we leave it to be discussed in a separate paper. Virtual bronchoscopy and navigation systems provide enhanced visualization and guidance during the procedure. Optical coherence tomography and confocal laser endomicroscopy offer real-time imaging of the airway mucosa, aiding in the detection of early neoplastic lesions. The field of diagnostic bronchoscopy continues to evolve, with ongoing research and development of advanced imaging techniques, biomarkers, and molecular testing. The integration of artificial intelligence in bronchoscopy holds promise for improving diagnostic accuracy and efficiency.

Autofluorescence bronchoscopy (AFB)

Autofluorescence bronchoscopy (AFB) is a technique that utilizes the natural fluorescence properties of bronchial tissue to detect early changes associated with pre-malignant and malignant lesions. It involves the use of a specialized bronchoscope equipped with a light source that emits specific wavelengths of light to excite the fluorophores in the tissue.

Indications and applications of AFB

AFB is primarily used for the detection and surveillance of pre-malignant and early-stage lung cancer. It can identify subtle changes in the bronchial mucosa that may not be visible under white light bronchoscopy. AFB is particularly useful in patients with a high risk of developing lung cancer, such as smokers or individuals with a history of occupational exposure to carcinogens [2].

In addition to lung cancer, AFB has shown promise in the evaluation of other respiratory conditions, such as bronchial dysplasia, chronic obstructive pulmonary disease (COPD), and interstitial lung diseases. It can aid in the early detection and characterization of these conditions, allowing for timely intervention and management.

Technique and procedure of AFB

AFB is performed using a specialized bronchoscope that emits blue or ultraviolet light to excite the fluorophores in the bronchial tissue. The emitted fluorescence is then visualized and interpreted by a bronchoscopist. Areas of abnormal fluorescence, such as loss of autofluorescence or increased fluorescence intensity, may indicate the presence of pre-malignant or malignant lesions.

The procedure is typically performed under conscious sedation or general anesthesia, depending on the patient's tolerance and the complexity of the case. AFB requires specialized training and expertise to accurately interpret the fluorescence patterns and differentiate between normal and abnormal findings.

Confocal laser endomicroscopy (CLE)

Confocal laser endomicroscopy (CLE) is a real-time imaging technique that allows for microscopic visualization of the bronchial mucosa during bronchoscopy. It involves the use of a miniaturized confocal microscope probe that can be inserted through the working channel of a standard bronchoscope [3].

Indications and applications of CLE

CLE is primarily used for the evaluation of bronchial mucosal abnormalities, such as pre-malignant lesions, inflammatory conditions, and infectious processes. It provides high-resolution images of the cellular and subcellular structures of the bronchial mucosa, allowing for detailed assessment and characterization of these abnormalities.

CLE has shown promise in the early detection and surveillance of lung cancer, as well as the evaluation of other respiratory conditions, such as asthma, COPD, and interstitial lung diseases. It can aid in the identification of specific cellular features, such as cellular atypia or inflammatory cell infiltrates, that may not be visible under white light bronchoscopy. It can be used as a guiding system to find optimal cryobiopsy location in interstitial lung diseases [4].

Technique and procedure of CLE

CLE is performed using a specialized bronchoscope equipped with a confocal microscope probe. The probe is inserted through the working channel of the bronchoscope and positioned adjacent to the target area of interest. Laser light is then emitted from the probe and focused on the bronchial mucosa, while the emitted fluorescent signals are captured and processed to generate real-time microscopic images.

The procedure is typically performed under conscious sedation or general anesthesia, depending on the patient's tolerance and the complexity of the case. CLE requires specialized training and expertise to accurately interpret the microscopic images and differentiate between normal and abnormal findings.

Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is an imaging technique that utilizes light waves to generate cross-sectional images of the bronchial mucosa. It provides high-resolution images of the tissue architecture, allowing for the assessment of cellular and structural abnormalities.

Indications and applications of OCT

OCT is primarily used for the evaluation of bronchial mucosal abnormalities, such as pre-malignant lesions, inflammatory conditions, and airway remodeling. It can aid in the early detection and surveillance of lung cancer, as well as the evaluation of other respiratory conditions, such as asthma, COPD, and bronchiectasis.

OCT has also shown potential in guiding therapeutic interventions, such as laser ablation or photodynamic therapy, by providing real-time feedback on the depth and extent of tissue involvement. It can help optimize treatment planning and improve treatment outcomes.

Technique and procedure of OCT

OCT is performed using a specialized bronchoscope equipped with an OCT imaging probe. The probe is inserted

through the working channel of the bronchoscope and positioned adjacent to the target area of interest. Low-coherence light waves are emitted from the probe and directed onto the bronchial mucosa, while the reflected light is captured and processed to generate cross-sectional images.

The procedure is typically performed under conscious sedation or general anesthesia, depending on the patient's tolerance and the complexity of the case. OCT requires specialized training and expertise to accurately interpret the cross-sectional images and differentiate between normal and abnormal findings.

New diagnostic procedures in bronchoscopy, such as autofluorescence bronchoscopy (AFB), confocal laser endomicroscopy (CLE), and optical coherence tomography (OCT), have expanded the diagnostic capabilities of bronchoscopy by providing real-time imaging and characterization of bronchial mucosal abnormalities. These techniques offer the potential for early detection, precise characterization, and targeted management of respiratory conditions, including pre-malignant and malignant lesions. Understanding the indications, techniques, and limitations of these new diagnostic procedures is essential for healthcare professionals involved in bronchoscopy and respiratory care.

Ultrathin bronchoscopy: advancements in minimally invasive diagnostic techniques

Ultrathin bronchoscopy is a minimally invasive diagnostic technique that utilizes a thin and flexible bronchoscope to visualize and access the airways. This chapter explores the advancements in ultrathin bronchoscopy, its applications, benefits, and limitations in the field of respiratory medicine [5].

Ultrathin bronchoscopy: an overview

Ultrathin bronchoscopy involves the use of a bronchoscope with a small diameter, typically ranging from 2.2 to 3.0 mm. This slim and flexible design allows for easier navigation through the airways, including the peripheral regions. The bronchoscopist can visualize the airways and perform diagnostic procedures with minimal discomfort to the patient.

Applications of ultrathin bronchoscopy

Peripheral lung lesions

One of the primary applications of ultrathin bronchoscopy is the evaluation of peripheral lung lesions. These lesions are often challenging to access using traditional bronchoscopic techniques due to their location in the smaller airways. Ultrathin bronchoscopy provides improved maneuverability and visualization in these areas, allowing for targeted biopsies and sampling of peripheral lesions [6].

Airway assessment and management

Ultrathin bronchoscopy is also useful for assessing and managing various airway conditions. It can be used to evaluate airway stenosis, granulation tissue, and other abnormalities. Additionally, it allows for the placement of stents or other therapeutic interventions in the airways, providing relief for patients with obstructive airway diseases [7].

Pediatric bronchoscopy

Ultrathin bronchoscopy is particularly valuable in pediatric patients. The small diameter of the bronchoscope reduces discomfort and the risk of complications in children. It allows for thorough evaluation of the airways and facilitates diagnostic procedures, such as bronchoalveolar lavage and transbronchial lung biopsy, in pediatric populations.

Benefits of ultrathin bronchoscopy

Minimally invasive procedure

Ultrathin bronchoscopy offers a minimally invasive alternative to traditional bronchoscopy. The small diameter of the bronchoscope reduces patient discomfort and the risk of complications, such as bleeding or pneumothorax. It allows for outpatient procedures and faster recovery times.

Improved access to peripheral lesions

The slim and flexible design of the ultrathin bronchoscope enables better access to peripheral lung lesions. It can navigate through narrow and tortuous airways, reaching areas that may be challenging to visualize and sample with larger bronchoscopes. This improves the diagnostic yield and reduces the need for more invasive procedures, such as surgical lung biopsy.

Enhanced patient tolerance

Ultrathin bronchoscopy is better tolerated by patients, especially those with sensitive airways or respiratory conditions. The small diameter and flexibility of the bronchoscope cause less irritation and discomfort during the procedure, making it more suitable for patients with compromised lung function or heightened airway sensitivity.

Limitations and challenges of ultrathin bronchoscopy

Limited instrumentation and maneuverability

The small diameter of the ultrathin bronchoscope limits the availability of specialized instruments and accessories. This may

restrict certain diagnostic and therapeutic procedures that require large instruments or tools. Additionally, the flexibility of the bronchoscope may limit its maneuverability in some cases.

Reduced visualization and image quality

Ultrathin bronchoscopes may have limitations in image quality and visualization compared to large bronchoscopes. The small diameter can result in reduced light transmission and image resolution. However, advancements in technology have led to improvements in image quality, mitigating this limitation to some extent.

Learning curve and expertise

Ultrathin bronchoscopy requires specialized training and expertise. The bronchoscopist must develop skills in navigating through the smaller airways and performing procedures with the limited instrumentation available. Adequate training and experience are crucial to ensure safe and effective use of ultrathin bronchoscopy.

Future directions and conclusion

Ultrathin bronchoscopy has emerged as a valuable tool in the field of respiratory medicine, offering a minimally invasive approach to diagnose and manage various airway conditions. Ongoing advancements in technology and instrumentation are expected to further improve the capabilities and image quality of ultrathin bronchoscopes. With continued research and training, ultrathin bronchoscopy has the potential to become a standard diagnostic technique, providing safer and more comfortable procedures for patients while maintaining diagnostic accuracy.

Electromagnetic navigation bronchoscopy (ENB)

Electromagnetic navigation bronchoscopy (ENB) is a technique that allows for the navigation and sampling of peripheral lung lesions that are not easily accessible by conventional bronchoscopy. It utilizes electromagnetic technology to create a virtual 3D map of the patient's airways, guiding the bronchoscope to the target lesion [8].

Indications and applications of ENB

ENB is primarily used for the diagnosis and staging of peripheral lung lesions, particularly when other diagnostic modalities, such as CT-guided biopsy or surgical resection, are not feasible or desirable. It allows for the sampling of small or inaccessible lesions, providing valuable information for treatment planning and prognosis.

ENB can also be used for the placement of fiducial markers for radiation therapy, as well as the delivery of therapeutic agents, such as brachytherapy or photodynamic therapy, to peripheral lung lesions. It offers a less invasive alternative to surgical resection for selected patients with early-stage lung cancer.

Technique and procedure of ENB

ENB involves the use of a specialized bronchoscope equipped with electromagnetic sensors and a working channel for biopsy instruments. Prior to the procedure, a CT scan of the patient's chest is obtained, which is then used to create a virtual 3D map of the airways and target lesion.

During the procedure, the bronchoscope is navigated through the airways using real-time electromagnetic guidance. The virtual 3D map is overlaid onto the live bronchoscopic images, allowing the operator to accurately guide the bronchoscope to the target lesion. Biopsy instruments can then be advanced through the working channel to obtain tissue samples for diagnosis [9].

Virtual bronchoscopy: a non-invasive approach to airway visualization and assessment

Virtual bronchoscopy is a non-invasive imaging technique that utilizes computed tomography (CT) or magnetic resonance imaging (MRI) data to create a three-dimensional virtual representation of the airways. This chapter explores the advancements in virtual bronchoscopy, its applications, benefits, and limitations in the field of respiratory medicine [10].

Virtual bronchoscopy: an overview

Virtual bronchoscopy involves the use of advanced imaging software to generate a virtual model of the airways based on CT or MRI scans. The virtual model allows for a detailed visualization of the airway anatomy, providing valuable information for diagnostic and therapeutic purposes [11].

Applications of virtual bronchoscopy

Airway assessment and pathology detection

Virtual bronchoscopy is primarily used for the assessment of airway anatomy and the detection of various pathologies. It allows for a comprehensive evaluation of the airways, including the detection of tumors, stenosis, strictures, and other abnormalities. Virtual bronchoscopy can aid in the diagnosis and planning of treatment for conditions such as lung cancer, bronchiectasis, and tracheobronchomalacia.

Preoperative planning and simulation

Virtual bronchoscopy is valuable in preoperative planning for airway interventions. It enables the bronchoscopist to assess the feasibility and optimal approach for procedures such as bronchial stenting, laser therapy, or endobronchial valve placement. Virtual bronchoscopy can simulate the procedure, allowing for precise planning and reducing the risk of complications.

Patient education and communication

Virtual bronchoscopy provides a visual representation of the airways that can be easily understood by patients. It can be used as a tool for patient education, allowing them to visualize their airway condition and understand the proposed treatment plan. Virtual bronchoscopy enhances communication between the healthcare provider and the patient, leading to better patient engagement and informed decision-making.

Benefits of virtual bronchoscopy

Non-invasive and radiation-free

Virtual bronchoscopy is a non-invasive imaging technique that does not require the insertion of a bronchoscope into the airways. This eliminates the need for sedation or anesthesia and reduces the risk of complications associated with invasive procedures. Additionally, virtual bronchoscopy does not involve ionizing radiation, making it a safer alternative to traditional bronchoscopy or CT scans.

Comprehensive airway visualization

Virtual bronchoscopy provides a comprehensive visualization of the airways, allowing for a detailed assessment of the anatomy and pathology. The three-dimensional virtual model offers a panoramic view of the airways, enabling the bronchoscopist to explore different angles and perspectives. This enhances the diagnostic accuracy and aids in treatment planning.

Time and cost efficiency

Virtual bronchoscopy can be performed using existing CT or MRI scans, eliminating the need for additional imaging procedures. This saves time and reduces healthcare costs associated with multiple imaging studies. Virtual bronchoscopy also allows for efficient preoperative planning, optimizing the use of resources and minimizing procedural delays.

Limitations and challenges of virtual bronchoscopy

Limited functional information

Virtual bronchoscopy provides detailed anatomical information but lacks functional data. It cannot assess dynamic airway collapse, airflow obstruction, or mucosal abnormalities that may be observed during traditional bronchoscopy. Therefore, virtual bronchoscopy should be used in conjunction with other diagnostic modalities to obtain a comprehensive evaluation of the airways.

Dependence on high-quality imaging

The accuracy and reliability of virtual bronchoscopy depend on the quality of the CT or MRI scans used to generate the virtual model. Suboptimal image quality, artifacts, or motion artifacts can affect the accuracy of the virtual model and limit its diagnostic value. Therefore, it is essential to ensure high-quality imaging for optimal results.

Operator experience and interpretation

Virtual bronchoscopy requires expertise in image interpretation and manipulation. The bronchoscopist must be familiar with the software and techniques used to generate and navigate the virtual model. Adequate training and experience are necessary to accurately interpret the virtual bronchoscopy images and make informed clinical decisions.

Future directions and conclusion

Virtual bronchoscopy has emerged as a valuable non-invasive tool in the field of respiratory medicine, providing detailed visualization of the airways and aiding in diagnosis, treatment planning, and patient communication. Ongoing advancements in imaging technology and software algorithms are expected to further enhance the capabilities of virtual bronchoscopy. With continued research and development, virtual bronchoscopy has the potential to become an integral part of the diagnostic and therapeutic armamentarium, improving patient care and outcomes in respiratory medicine.

Cone beam CT in bronchoscopy: advancements in imaging and navigation

Cone Beam CT (CBCT) is a three-dimensional imaging technique that has revolutionized the field of bronchoscopy. This chapter explores the advancements in CBCT technology, its applications, benefits, and limitations in the context of bronchoscopy [12].

Cone beam CT: an overview

Cone Beam CT is a specialized imaging technique that utilizes a cone-shaped X-ray beam and a flat-panel detector to capture high-resolution, three-dimensional images of the airways. Unlike traditional CT scans, CBCT provides real-time imaging during

bronchoscopy procedures, allowing for improved navigation and accurate localization of lesions.

Applications of cone beam CT in bronchoscopy

Localization of peripheral lung lesions

One of the primary applications of CBCT in bronchoscopy is the localization of peripheral lung lesions. CBCT provides real-time imaging of the airways and allows the bronchoscopist to precisely locate and target lesions that may be difficult to visualize with traditional bronchoscopic techniques. This improves the accuracy of diagnostic procedures and reduces the need for additional invasive interventions.

Guidance for biopsy and sampling

CBCT can guide the bronchoscopist during biopsy and sampling procedures. The real-time imaging provided by CBCT helps in accurately positioning the bronchoscope and instruments, ensuring optimal sampling of the target lesion. This improves the diagnostic yield and reduces the risk of complications associated with blind biopsies.

Assessment of airway anatomy and pathology

CBCT allows for detailed assessment of airway anatomy and pathology. It provides high-resolution images that can reveal structural abnormalities, such as stenosis, strictures, or tumors, which may not be easily visible with traditional bronchoscopic techniques. This aids in pre-procedural planning and enhances the bronchoscopist's understanding of the patient's airway condition.

Benefits of cone beam CT in bronchoscopy

Real-time imaging and navigation

The real-time imaging capability of CBCT provides immediate feedback to the bronchoscopist during the procedure. This allows for precise navigation through the airways, reducing the risk of complications and improving the efficiency of the procedure. The bronchoscopist can visualize the position of the bronchoscope and instruments in relation to the target lesion, ensuring accurate sampling and minimizing damage to healthy tissue.

Improved lesion localization

CBCT enables accurate localization of peripheral lung lesions, even in challenging anatomical locations. The three-dimensional imaging provides a clear visualization of the lesion's position relative to the airway, facilitating targeted biopsies and reducing the need for additional procedures.

Reduced radiation exposure

Compared to traditional CT scans, CBCT in bronchoscopy typically involves lower radiation doses. The cone-shaped X-ray beam used in CBCT focuses on the area of interest, minimizing radiation exposure to surrounding tissues. This is particularly beneficial for patients who require multiple imaging procedures or have a higher risk of radiation-related complications.

Limitations and challenges of cone beam CT in bronchoscopy

Equipment and infrastructure requirements

CBCT requires specialized equipment and infrastructure, including a dedicated CBCT system and a flat-panel detector. These requirements may limit the availability of CBCT in certain healthcare settings, particularly smaller clinics or facilities with limited resources.

Learning curve and interpretation

The interpretation of CBCT images requires specialized training and expertise. Bronchoscopists need to develop skills in navigating and interpreting three-dimensional images to effectively utilize CBCT during procedures. Adequate training and experience are essential to ensure accurate interpretation and optimal utilization of CBCT in bronchoscopy.

Cost considerations

The cost of CBCT equipment and maintenance can be a limiting factor for widespread adoption. The initial investment and ongoing expenses associated with CBCT may pose challenges for healthcare institutions, particularly those with limited budgets.

Future directions and conclusion

Cone Beam CT has emerged as a valuable tool in bronchoscopy, providing real-time imaging and navigation capabilities. Ongoing advancements in CBCT technology, such as improved image quality and reduced radiation doses, are expected to further enhance its utility in the field. With continued research and development, CBCT has the potential to become a standard imaging modality in bronchoscopy, enabling more accurate diagnoses, targeted interventions, and improved patient outcomes.

Robotic bronchoscopy: advancements in diagnostic techniques

Robotic bronchoscopy is an emerging technology that combines the precision of robotics with the diagnostic capabilities of bronchoscopy. This chapter explores the

advancements in robotic bronchoscopy, its applications, benefits, and limitations in the field of respiratory medicine. Robotic bronchoscopy involves the use of a robotic system to navigate and manipulate a bronchoscope within the airways. The robotic system consists of a robotic arm, a control console, and specialized instruments. The bronchoscopist operates the system from the console, which provides a 3D visualization of the airways and precise control of the robotic arm [13].

Applications of robotic bronchoscopy

Peripheral lung lesions

One of the primary applications of robotic bronchoscopy is the diagnosis and management of peripheral lung lesions. These lesions are often challenging to access using traditional bronchoscopic techniques. Robotic bronchoscopy allows for improved navigation and maneuverability within the peripheral airways, enabling the bronchoscopist to reach and sample these lesions more effectively [14, 15].

Mediastinal lymph node sampling

Robotic bronchoscopy also offers advantages in mediastinal lymph node sampling. The robotic system provides enhanced visualization and precise control, allowing for accurate targeting and sampling of lymph nodes. This is particularly beneficial in cases where lymph nodes are in difficult-to-reach areas or when there is a need for precise sampling for staging of lung cancer.

Interventional procedures

In addition to diagnostic purposes, robotic bronchoscopy can be used for interventional procedures. The robotic system enables the bronchoscopist to perform procedures such as endobronchial biopsy, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), and laser ablation with increased precision and control. This opens new possibilities for minimally invasive treatment options [16].

Benefits of robotic bronchoscopy

Improved access and navigation

Robotic bronchoscopy provides improved access to peripheral lung lesions and challenging anatomical locations. The robotic arm can navigate through narrow and tortuous airways with greater precision, reducing the risk of complications and improving the diagnostic yield.

Enhanced visualization

The 3D visualization provided by the robotic system enhances the bronchoscopist's ability to visualize the airways and target lesions. This improved visualization allows for more accurate targeting and sampling, leading to better diagnostic outcomes.

Increased precision and control

The robotic system offers increased precision and control during bronchoscopy procedures. The robotic arm can perform precise movements and manipulations, reducing the risk of tissue damage and improving the safety of the procedure.

Limitations and challenges of robotic bronchoscopy

Cost and availability

One of the main limitations of robotic bronchoscopy is its cost and availability. The robotic systems and associated instruments are expensive, making them less accessible in certain healthcare settings. Additionally, the expertise required to operate the robotic system may be limited to specialized centers with trained personnel.

Learning curve

Robotic bronchoscopy requires specialized training and expertise. The learning curve for mastering the robotic system and its associated techniques can be steep. Adequate training and proctoring are essential to ensure safe and effective use of the technology.

Technical limitations

While robotic bronchoscopy offers many advantages, it also has some technical limitations. The size of the robotic arm and instruments may limit access to certain areas of the airways. Additionally, the robotic system may not be suitable for all patients, such as those with severe airway abnormalities or significant comorbidities.

Future directions and conclusion

Robotic bronchoscopy is a promising advancement in the field of respiratory medicine. As technology continues to evolve, we can expect further improvements in robotic systems, including miniaturization of instruments and enhanced capabilities. With ongoing research and development, robotic bronchoscopy has the potential to revolutionize the diagnostic and interventional management of respiratory conditions, providing safer and more precise procedures for patients.

Challenges in the diagnostic process of bronchoscopy

Bronchoscopy is a valuable diagnostic tool for evaluating respiratory conditions. However, the diagnostic process of bronchoscopy is not without challenges. We will discuss some of the common challenges encountered during bronchoscopy and strategies to overcome them, ensuring a successful diagnostic outcome.

Technical challenges

Limited access to peripheral lesions

One of the major challenges in bronchoscopy is accessing peripheral lung lesions. These lesions are located deep within the lung tissue and may be difficult to reach using a standard bronchoscope. Traditional bronchoscopic techniques, such as transbronchial biopsy, may have limited success in obtaining adequate tissue samples from these lesions.

To overcome this challenge, various advanced techniques have been developed, including electromagnetic navigation bronchoscopy (ENB) and radial endobronchial ultrasound (EBUS). These techniques provide real-time imaging guidance, allowing for accurate navigation to peripheral lesions and increasing the diagnostic yield.

Inadequate visualization

Another challenge in bronchoscopy is inadequate visualization of the airway and target lesions. Factors such as excessive secretions, blood, or poor lighting can hinder the bronchoscopist's ability to clearly visualize the airway and obtain accurate diagnostic information.

To address this challenge, proper airway preparation is essential. Pre-bronchoscopy measures, such as bronchial hygiene techniques and administration of mucolytic agents, can help reduce secretions and improve visualization. Adequate suctioning and irrigation during the procedure can also help clear the airway and improve visualization.

Diagnostic challenges

Sampling error

Sampling error is a common challenge in bronchoscopy, particularly when obtaining tissue samples for histopathological examination. The bronchoscopist must ensure that the biopsy samples are representative of the target lesion to obtain an accurate diagnosis.

To minimize sampling error, it is important to carefully select the biopsy site based on radiological findings and bronchoscopic assessment. Techniques such as transbronchial needle aspiration (TBNA) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) can provide more targeted sampling of mediastinal lymph nodes and peripheral lesions, improving the diagnostic yield.

False-negative results

Obtaining false-negative results is another challenge in bronchoscopy. In some cases, the bronchoscopist may not be able to visualize or sample the lesion adequately, leading to a negative diagnostic outcome despite the presence of pathology.

To address this challenge, a multidisciplinary approach is crucial. Collaboration with radiologists, pathologists, and other specialists can help correlate clinical, radiological, and pathological findings to ensure a comprehensive diagnostic evaluation. Repeat bronchoscopy or alternative diagnostic procedures may be considered if there is a high suspicion of pathology despite initial negative results.

Safety challenges

Complications and adverse events

Bronchoscopy, like any invasive procedure, carries the risk of complications and adverse events. These can range from minor complications such as bleeding or pneumothorax to more serious events such as respiratory distress or cardiac arrhythmias.

To mitigate these risks, it is important to adhere to strict safety protocols and guidelines. Proper patient selection, thorough pre-procedure assessment, and appropriate monitoring during and after the procedure are essential. Adequate training and expertise in bronchoscopy, as well as prompt recognition and management of complications, are crucial for ensuring patient safety.

Infection control

Infection control is a significant challenge in bronchoscopy due to the potential for cross-contamination and transmission of infectious agents. The bronchoscope and associated accessories can harbor bacteria or other pathogens, posing a risk of infection to both patients and healthcare providers.

To address this challenge, strict adherence to infection control practices is essential. This includes proper cleaning and disinfection or sterilization of bronchoscopes and accessories, adherence to hand hygiene protocols, and use of personal protective equipment. Regular monitoring and auditing of infection control practices can help identify and address any gaps or deficiencies.

Conclusion

The diagnostic process of bronchoscopy is not without challenges. Technical challenges, such as limited access to peripheral lesions and inadequate visualization, can impact the diagnostic yield. Diagnostic challenges, including sampling error and false-negative results, require a multidisciplinary approach and careful consideration of alternative diagnostic procedures. Safety challenges, such as complications and infection control, necessitate adherence to

strict protocols and guidelines. By recognizing and addressing these challenges, healthcare professionals can optimize the diagnostic process of bronchoscopy and improve patient outcomes.

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Advances in combined neuroendocrine carcinoma of lung cancer

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Lung cancer incidence and mortality rates are increasing worldwide, posing a significant public health challenge and an immense burden to affected families. Lung cancer encompasses distinct subtypes, namely, non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). In clinical investigations, researchers have observed that neuroendocrine tumors can be classified into four types: typical carcinoid, atypical carcinoid, small-cell carcinoma, and large-cell neuroendocrine carcinoma based on their unique features. However, there exist combined forms of neuroendocrine cancer. This study focuses specifically on combined pulmonary carcinomas with a neuroendocrine component. In this comprehensive review article, the authors provide an overview of combined lung cancers and present two pathological images to visually depict these distinctive subtypes.

KEYWORDS

small cell neuroendocrine carcinoma, large cell neuroendocrine carcinoma, adenocarcinoma, squamous cell carcinoma, lung cancer

Introduction

Globally, the morbidity and mortality rates of lung cancer are increasing [1], making it a significant public health concern and burden for families [2]. Approximately 75% of the 2.20 million newly diagnosed lung cancer patients will succumb to the disease within 5 years [3–5]. Lung cancer comprises distinct subtypes, such as non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). The mortality rate of NSCLC has been shown to improve from 2013 to 2016 following diagnosis [6], due to advances in screening, early patient management, immunotherapy, and other interventions [7]. However, SCLC remains challenging due to its propensity for relapse and higher mortality rates accounting for up to 15% of all lung cancers [8]. Despite decades of research focused on targeted treatments based on biomarker selection and immunotherapy, SCLC continues to be one of the most difficult-to-treat tumorigenic diseases [9].

In 2021, the WHO classified the lung tumors since 2015 [10] into five categories: 1) Small cell carcinoma, 2) Large cell neuroendocrine carcinoma, 3) Adenosquamous carcinoma (if both components ≥10%), 4) Adenocarcinoma, squamous cell

carcinoma, adenosquamous carcinoma, or large cell carcinoma with unclear immunohistochemical features, 5) Pleomorphic, spindle cell, and/or giant cell carcinoma for the resection specimens. However, in clinical work, researchers have observed an additional subtype. In pathology studies, neuroendocrine tumors can be further divided into four types: typical carcinoid tumor, atypical carcinoid tumor, small cell carcinoma, and large cell neuroendocrine carcinoma based on their combined features [11]. This study focuses specifically on combined pulmonary carcinoma with a neuroendocrine component.

Combined small cell neuroendocrine carcinoma with adenocarcinoma

The current consensus is that small-cell lung cancer is transferred from adenocarcinoma following treatment with EGFR tyrosine kinase inhibitors [12]. This phenomenon was initially reported in a female patient diagnosed with adenocarcinoma who received erlotinib in 2007. After prolonged treatment, a biopsy of the same site revealed SCLC based on the exon 19 mutation of the epidermal growth factor receptor (EGFR), which was consistent with primary NSCLC [13]. Another case involved a 38-year-old patient with EGFR exon 21 L8585R lung adenocarcinoma who developed SCLC transformation after receiving regular erlotinib treatment for 18 months [14]. Two hypotheses have been proposed to elucidate the pathogenesis of SCLC transformation [15]. The majority of researchers posit that this transformation arises from resistance to tyrosine kinase inhibitors (TKIs) [16] targeting EGFR, ALK, and ROS1, or immunotherapies [17]. Five resistant tumors were found to harbor mechanisms such as the EGFR T790M mutation, MET gene amplification, EGFR amplification, mutations in the PIK3CA gene and others associated with the epithelial-to-mesenchymal transition. These transformed tumors from NSCLC to SCLC showed sensitivity to standard SCLC treatments. However, due to significant heterogeneity in resistance mechanisms and different prognoses among cases [18, 19], individualized therapeutic strategies are required. The transformation can be detected by mutations in biomarkers such as EGFR, tumor protein p53 (TP53), RB transcriptional corepressor 1 (RB1), and SRY-box transcription factor 2 (SOX2) before and after transformation [20].

However, it should be noted that this transformation may also be pseudo. In a study conducted by Rui Li et al. in 2021 involving 11 cases previously diagnosed as SCLC, only one sample did not exhibit any SCLC elements within the primary adenocarcinoma sections. This case was defined as true small-cell transformation (SCT) [21]. In other words, there were instances where SCLC components coexisted within the adenocarcinoma but were considered pseudo-SCT. The observation of RB1 deletion and mutant TP53 overexpression in either pseudo-SCT or true SCT cannot exclude the possibility of combined SCLC with adenocarcinoma.

Meanwhile, 34 cases of combined high-grade neuroendocrine carcinoma (HGNEC) were reported with 48% of subjects with combined HGNEC and adenocarcinoma having a lepidic adenocarcinoma component suggesting that HGNEC can develop in association with pre-existing adenocarcinoma which is often retained [22]. In the same year, a case based on combined SCLC with non-small cell carcinoma component was reported, in which two distinct neoplastic components were found. One consisted of small-sized cells without giant cell carcinoma shown in the biopsy specimen while the other was verified as combined SCLC with a giant cell carcinoma through histopathological examination of the lobectomy specimen [23]. These findings indicated that SCLC and adenocarcinoma can coexist in the same patients. The results cannot be solely attributed to the transformation from adenocarcinoma, and it is imperative not to overlook the significance of combined SCLC.

The origin of two different tumor components may be from the same pluripotent epithelial precursor cell due to loss of heterozygosity (LOH) in the different tumor areas [24]. Additionally, IL-16 rs859 was found to have a statistically significant susceptibility to lung small-cell carcinoma and adenocarcinoma [25].

Expression of stem cell transcription factors (scTF) has been detected in both small cell carcinoma and non-adenocarcinoma in prostate cancer, influencing the transformation from adenocarcinoma [26]. Inhibition of exportin 1 has been suggested as a potential therapeutic target for the prevention or treatment of neuroendocrine transformation of lung and prostate adenocarcinomas [27]. Nowadays, a platinum plus etoposide chemotherapy regimen is preferred to treat patients with SCLC transformation based on EGFR mutation. However, new strategies, such as immune checkpoint inhibitors are being explored [28]. The DLL3-directed antibody-drug conjugate rovalpituzumab tesirine [29] and its application have been considered for the unique EGFR mutant SCLC transformation cancer [30]. Serum neuron-specific enolase (NSE) may serve as a novel marker for predicting neuroendocrine tumor transformation [31].

Combined large cell neuroendocrine carcinoma with adenocarcinoma

The incidence of large cell neuroendocrine carcinoma (LCNC) in lung cancer is only 3% [32], and it has a poor prognosis due to its rarity, aggressiveness, and distinct treatment approach [33]. In 2009, E Cakir et al. identified different combinations of histological subtypes in lung cancer, such as adenosquamous carcinoma, combined neuroendocrine tumors, and biphasic tumors. Combined neuroendocrine carcinoma consists of SCLC + nonneuroendocrine carcinoma (NNEC), SCLC + LCNC, and LCNC + NNEC, it has been revealed that patients with

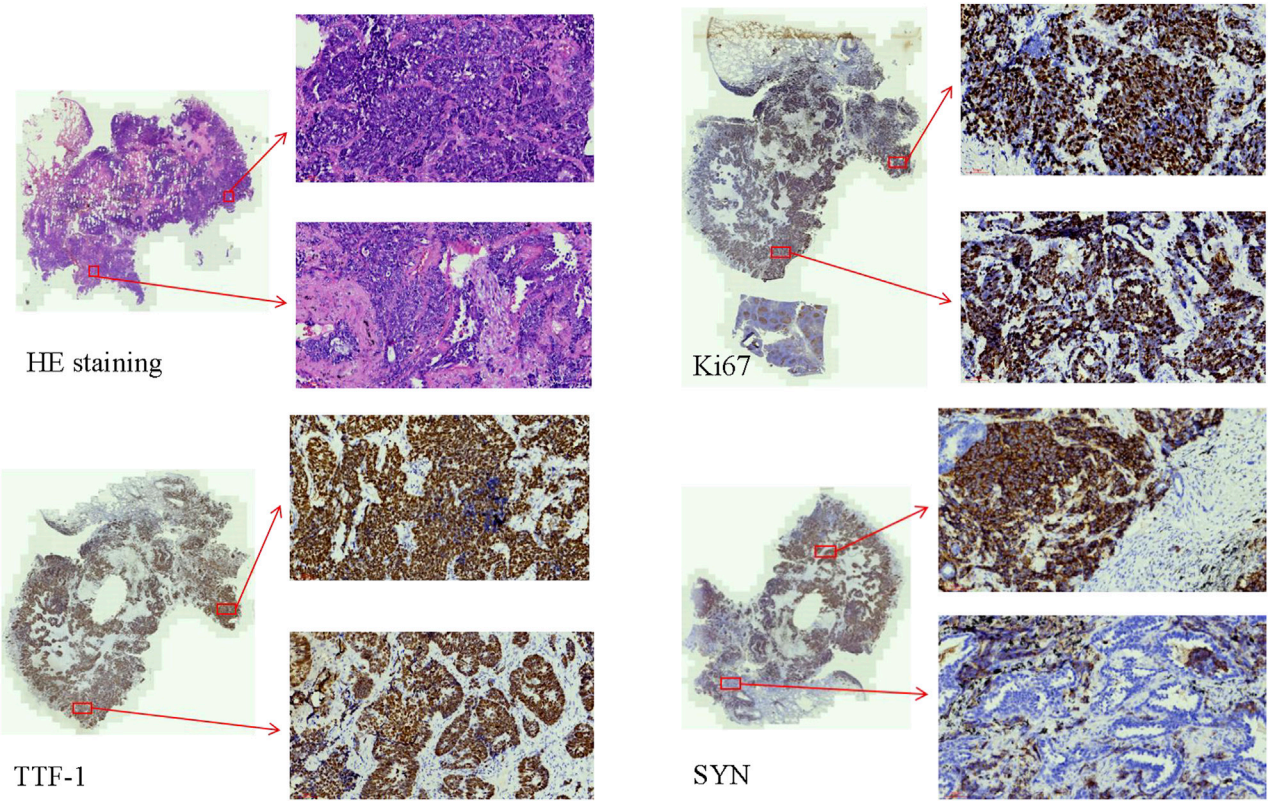

FIGURE 1
The HE staining and IHC of the combined LCNC with adenocarcinoma (x20). The Ki67 index was approximately 85%. TTF-1 and SYN were positive.

combined neuroendocrine tumors had more advanced stages and vascular invasion compared to those with single histology types, their 2-year survival rate was only 25% [34]. Moreover, accurate differentiation of LCNC from atypical carcinoids is challenging with the limited tissue samples obtained through lung biopsy [35].

These findings highlight the existence of evidence of combined large-cell neuroendocrine carcinoma. The diagnosis of LCNC relies on histopathological examination while immunohistochemical (IHC) features provide precise and accurate identification. Neuroendocrine components strongly express markers such as Chromogranin A (CgA), Synaptophysin (Syn), and neural cell adhesion molecule 56 (CD56). On the other hand, thyroid transcription factor 1 (TTF-1) is associated with EGFR mutations, while NapsinA is highly specific for lung adenocarcinoma [36]. P40 serves as an excellent marker for distinguishing between squamous cell carcinoma and lung adenocarcinoma [37], similar to P63. Among combined LCNC cases, adenocarcinoma is the most common combination, accounting for approximately 70% of cases [38]. A retrospective study of surgical resection of combined LCNC included 96 patients, 71 of whom were diagnosed as having LCNC combined with adenocarcinoma.

During clinical work, the authors encountered a case of LCNC combined with adenocarcinoma that could be diagnosed by IHC. This is a resection specimen. In this particular case, positive markers for TTF-1, SYN, CD56, CK, and P63 were observed, along with partial positivity for NapsinA. However, CgA and P40 showed negative results. The Ki67 index was approximately 85% (Figure 1). Metastasis was observed in 22 of 33 lymphatic nodes.

Adjuvant chemotherapy, particularly etoposide-based chemotherapy, proved to be a beneficial option [39]. Furthermore, it is crucial to confirm the importance of adjuvant chemotherapy (especially using the small cell carcinoma regimen) to improve patients' outcomes [40]. Nevertheless, immunotherapy rarely provides benefits when combined with LCNC treatment. Further research should be done [41].

Combined large cell neuroendocrine carcinoma with squamous cell carcinoma

Squamous cell carcinoma is a subtype of non-small cell carcinoma, which also constitutes the composition in

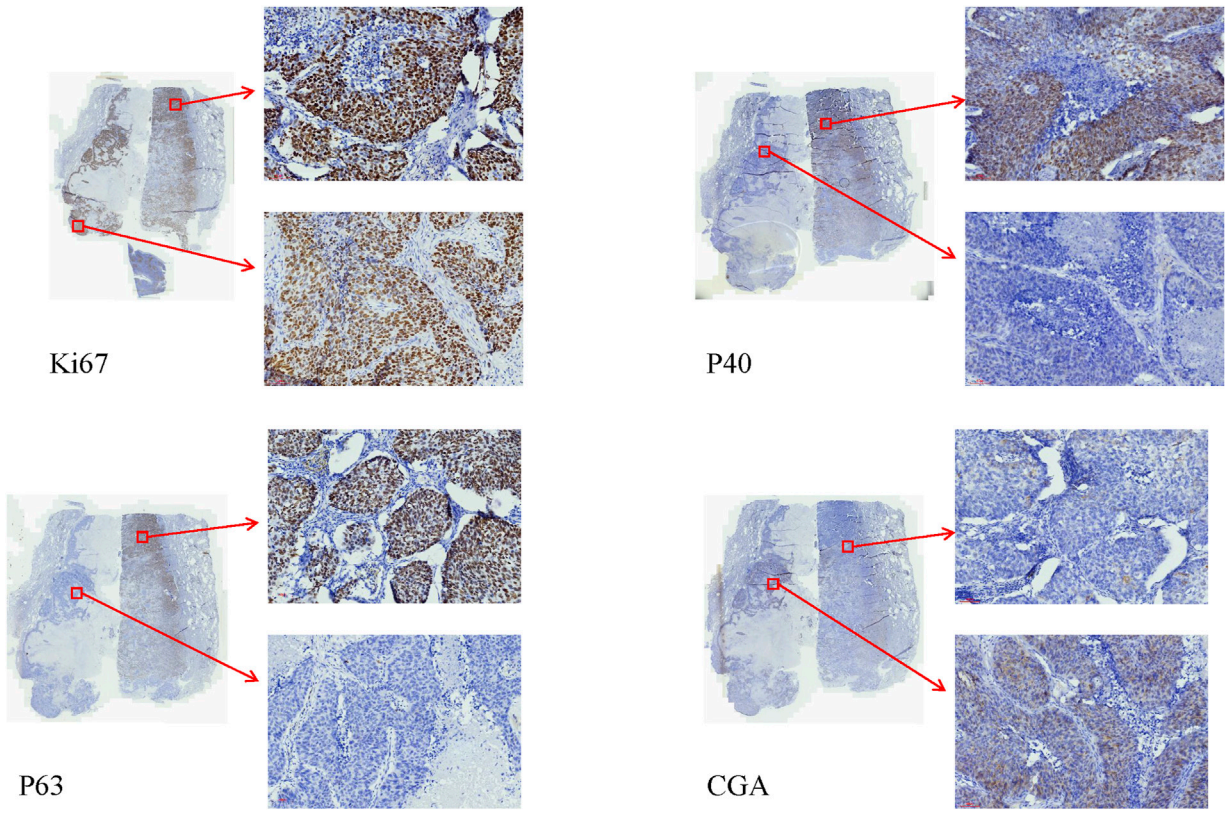

FIGURE 2
The IHC of the combined LCNC with squamous cell carcinoma (×20). The Ki67 index was approximately 80%. The sections of the P40, and P63 of squamous cell carcinoma were positive. The sections of the P40, and P63 of LCNC were negative. The sections of CgA of LCNC were positive. The sections of CgA of the squamous cell carcinoma were negative.

combined LCNC. A case of LCNC of the lung with carcinoid syndrome was reported involving a 76-year-old woman who underwent computed tomography that revealed a liver mass originating from the lung as diagnosed by biopsy. The pathology analysis of the lung biopsy demonstrated combined LCNC and squamous cell carcinoma. The tumor area tested negative for TTF-1 but positive for cytokeratin14 (CK14) and P40 [42]. Despite receiving chemotherapy following the diagnosis, the patient died 50 days after hospital admission due to her deteriorating physical condition. In 2004, another case was reported in which a patient diagnosed with combined LCNC as part of squamous cell carcinoma [pT4 (pm) N2M0] on postoperative histological tissue remained in good health for 9 months until the article was published [43]. In addition, other similar cases have been documented [44].

The authors encountered a case of combined LCNC with squamous cell carcinoma that was diagnosed using IHC and pathological features. In this particular case, the IHC markers: CK, and CK17 were found to be positive. Additionally, the tumor sections were positive for CgA, P40, and P63. However, the markers CK7, TTF-1, NapsinA, SYN, CD56, and CD117 were negative. The Ki67 index was approximately 80%. Notably, organoid and palisading patterns were observed in the majority of the lung tumor cells within the tumor areas. Furthermore, another section revealed a prominent presence of atypical cells exhibiting keratinization (Figure 2). Importantly, no lymphatic node metastasis was detected.

According to the IHC, the patient was diagnosed with combined LCNC and squamous cell carcinoma due to the presence of two distinct elements. Additionally, molecular testing could be used to satisfy the criteria of precision medicine [45].

Conversely, miR-31 is found to be upregulated in adenocarcinoma, squamous cell carcinoma, and large-cell neuroendocrine carcinoma of the lung, while it is not overexpressed in small-cell carcinoma or carcinoids. MiR-31 has been identified as a potential therapeutic target that promotes tumor growth in mice of xenografted human adenocarcinoma and squamous cell carcinoma cell lines but not in large- or small-cell carcinoma lines [46]. The Ki-67 proliferation index cutoff of 55% could predict the prognosis of LCNC and combined LCNC, with combined LCNC patients

having longer overall survival (OS) when diagnosed with adenocarcinoma compared to those diagnosed with squamous cell carcinoma [47].

Notably, there was a case report demonstrating the coexistence of LCNC with adenocarcinoma, and squamous cell carcinoma. Hematoxylin—Eosin staining revealed that the tumor consisted of 40% acinar adenocarcinoma, 10% mucous adenocarcinoma, 40% LCNC, and 10% poorly differentiated squamous cell carcinoma. The markers TTF-1, SYN, and P40 exhibited positive expression in the correlative tumor [48]. The researcher indicated that surgical resection along with adjuvant chemotherapy using SCLC regimen may improve Disease Free Survival (DFS) and OS.

Treatment of combined LCNC with squamous cell carcinoma could include immune checkpoint inhibitors after multimodality therapy incorporating cytotoxic anticancer drugs and radiotherapy. A 60-year-old man diagnosed with LCNEC combined with squamous cell carcinoma and staged as T2aN0M0 stage IB through histopathology showed a favorable response to treatment and achieved a survival period exceeding 5 years [49].

The identical phenomenon of merging two or more compounds has also been observed in other parts of the body, such as the head and neck region [50] and the uterine cervix [51].

In this comprehensive review, the authors meticulously summarize the various types of combined lung cancers, including the concurrence of combined small cell neuroendocrine carcinoma with adenocarcinoma, combined LCNC with adenocarcinoma, and combined LCNC with squamous cell carcinoma.

Interestingly, there seems to be no literature available on the coexistence of small-cell neuroendocrine carcinoma and squamous cell carcinoma in the lung. This particular type of lung cancer has a significantly poorer prognosis, and thus, research concerning its treatment is currently underway. The review also highlights the importance of understanding the different combinations of lung cancer subtypes, as this knowledge can greatly contribute to more tailored and effective treatment strategies.

Author contributions

HZ, HM, XC, and FL conducted the experiments, FW supplied critical pathologic immunohistochemical markers, ZH and FY wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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HUNCHEST projects—advancing low-dose CT lung cancer screening in Hungary

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Lung cancer, the leading cause of malignancy-related deaths worldwide, demands proactive measures to mitigate its impact. Low-dose computer tomography (LDCT) has emerged as a promising tool for secondary prevention through lung cancer screening (LCS). The HUNCHEST study, inspired by the success of international trials, including the National Lung Cancer Screening Trial and the Dutch NELSON study, embarked on the first LDCT-based LCS program in Hungary. The initiative assessed the screening efficiency, incorporating lung function tests and exploring the interplay between lung cancer and chronic obstructive pulmonary disease (COPD). Building upon this foundation, an implementation trial involving 18 Hungarian centers supported by the Ministry of Human Capacities demonstrated the feasibility of LCS within a multicentric framework. These centers, equipped with radiology capabilities, collaborated with multidisciplinary oncology teams, ensuring optimal patient pathways. However, a critical challenge remained the patient recruitment. To address this, the HUNCHEST 3 project, initiated in 2023, seeks to engage general practitioners (GPs) to reach out to eligible patients within a municipality collective of 60 thousand inhabitants. The project's ultimate success is contingent upon the willingness of eligible individuals to undergo LDCT scans. In conclusion, the HUNCHEST program represents a crucial step in advancing lung cancer screening in Hungary. With a focus on efficiency, multidisciplinary collaboration, and innovative patient recruitment strategies, it endeavors to contribute to the reduction of lung cancer mortality and serve as a blueprint for potential nationwide LCS programs.

KEYWORDS

lung cancer, screening, LDCT, pulmonary nodules, implementation

Abbreviations: COPD, chronic obstructive pulmonary disorder; CXR, Chest X-ray; HC1, HUNCHEST I; HC2, HUNCHEST II; LCS, Lung cancer screening; LDCT, low dose computer tomography; MDT, multidisciplinary team; NELSON, Nederlands–Leuvens Longkanker Screenings Onderzoek (Dutch–Belgian lung-cancer screening trial); OKPI, Országos Korányi Pulmonológiai Intézet (National Korányi Institute for Pulmonolgy); NLST, National Lung Screening Trial; SOLACE, Strengthening the screening of Lung Cancer in Europe; VDT, Volume doubling time.

Introduction

The year 1912 marked the beginning of formal documentation of lung cancer cases, with Isaac Adler publishing a review that identified 374 documented instances [1]. Fast forward to the present, and lung cancer annually claims the lives of 1.7 million people globally, with Hungary alone witnessing about 10,000 new cases each year [2].

Regrettably, by the time lung cancer becomes symptomatic, it often presents in an advanced or metastatic stage. Presently, surgery remains the sole curative option, but without early detection, only 15%–25% of cases are operable [3].

The disease has witnessed significant progress in the realm of early detection and prevention. This article focuses on the role of low-dose computer tomography (LDCT) in identifying the disease in its earliest, most treatable stages. While primary prevention through smoking cessation programs is essential to reduce new cases, secondary prevention, in the form of screening, plays a vital role in reducing mortality rates.

Early screening efforts initially centred on conventional radiography, utilizing chest X-rays (CXR) since they were widely accessible. In the 1960 s, several controlled trials were conducted, such as the Czechoslovakian and the Mayo Lung Project, which used chest X-rays, and the Johns Hopkins trial, which employed sputum cytology [4–6]. The final significant trial employing chest X-rays was the Prostate, Lung, Colorectal, and Ovarian trial, which followed over 150,000 patients for 13 years, but failed to show a reduction in mortality [7]. In Hungary, lung CXRs were a part of the fight against tuberculosis—the mandatory nature was later revoked, but still a large proportion of adults view CXRs as part of a health check [8]. Nearly 1,000 lung cancer cases are still detected this way in a population of 10 million. These patients have approximately twice the number of resectable LCS than their symptomatically detected counterparts [9].

The true breakthrough came with advancements in medical imaging technology. In 1992, the Early Lung Cancer Action Project (ELCAP) was launched in the United States of Amerika, by Claudia Henschke and her team, with a focus on LDCT screening. Over 31,000 asymptomatic individuals were screened, resulting in the diagnosis of 484 lung cancers, 85% of which were at Stage I. This was the first large-scale trial to demonstrate the potential of LDCT screening in lung cancer [10].

In the United States, the National Lung Cancer Screening Trial (NLST), initiated in 2002, proved to be a game-changer. It was a control-armed, prospective trial, involving 53,454 high-risk individuals. The results announced in 2013 revealed a 20% mortality reduction in the LDCT screening arm [11]. This compelling data led to the United States Preventive Services Task Force (USPSTF) in 2013 recommending LDCT lung cancer screening for individuals between 55–80 years of age with a smoking history of at least 30 pack-years who are active smokers or quit within the last 15 years—based on clinical data. This was modified in 2021 to include individuals as young as 50 years of age with a 20 pack-year history [12]. Medicare coverage was provided for at-risk individuals, although uptake remained low [13].

In Europe, the Dutch-Belgian Randomized Lung Cancer Screening Trial (NELSON) is the largest concluded lung cancer screening study to date. Data presented in 2018 showed a 26% reduction in mortality for high-risk males, and even more significant benefits for women. The introduction of an "indeterminate for cancer" category reduced the number of false positives in comparison to the NLST [14].

In 2015, the European Respiratory Society and the European Society of Radiologists published a joint statement followed by the European position statement on lung cancer screening in 2017 [15, 16]. These documents emphasize the importance of risk stratification, patient education, quality assurance, and a clear pathway for managing screen-detected nodules.

In Hungary, the first prospective LDCT lung cancer screening project started as early as in 2013. In this article the authors present a brief review of the results of the finished screening projects, and introduce the ongoing LDCT-LCS projects.

HUNCHEST I

The HUNCHEST (Hungarian Chest Screening) pilot initiative, spearheaded by the National Korányi Institute for Pulmonology in Budapest, sought to evaluate the efficacy of LDCT in detecting lung cancer in asymptomatic individuals, regardless of established risk factors [17].

Initially conceived as a single-center study, the program aimed to establish screening protocols, reporting mechanisms, and ensure comprehensive patient follow-up. In 2015, additional thoracic centers specializing in lung cancer imaging joined the initiative, bringing the total to six active centers contributing to screening efforts. Each center employed adaptable recruitment strategies, leveraging media campaigns, websites, posters, newspaper advertisements, and informational leaflets to encourage voluntary participation.

The study encompassed individuals undergoing the first screening round between October 2013 and January 2020. Inclusion criteria targeted asymptomatic individuals aged between 50 and 79 years of age, irrespective of known risk factors. Participants with a history of smoking received smoking cessation counseling at recruitment. Exclusion criteria, in line with the NELSON trial and study protocol, excluded individuals with specific health conditions, self-reported moderate or poor health, permanent oxygen therapy needs, body weight of 140 kg or more, a history of cancer within the past 5 years, previous lung surgery, or chest CT examinations within the last 2 years. Written informed consent was

mandatory, and those unable to provide it were excluded. Participants were categorized based on smoking habits and comorbidities.

The HUNCHEST program included lung function tests (spirometry) for all applicants to identify undiagnosed chronic obstructive pulmonary disease (COPD), with specific criteria for diagnosis and severity assessment.

LDCT protocols were tailored to the scanner model, with scans conducted during suspended maximal inspiration in a single breath-hold, covering the entire lungs. Radiation exposure was controlled, and imaging conditions were standardized across sites. Two independent radiologists read all scans with semiautomatic segmentation of nodules and manual measurements conducted as needed. The Siemens SyngoVia MM Oncology Lung Computer-Aided Detection (CAD) software played a crucial role in matching previously detected nodules and calculating the volume doubling time (VDT) for nodule growth assessment.

Nodules were categorized based on their VDT into likely benign (VDT >600 days), suspicious (VDT <400 days), inflammatory (VDT <40 days), and indeterminate (VDT between 400 and 600 days), necessitating further evaluation.

The number of screen-detected malignancies and positive predictive values in the study aligned with internationally published studies. Similarly to the NELSON protocol, the study incorporated not only positive/negative categories, but also an indeterminate category, optimizing nodule management. A web-based structured reporting platform facilitated clear pathways post a positive screen, enabling cost-effectiveness calculations and providing vital data for endorsing a nationwide risk group-based screening program.

Notably, the trial included never-smokers in its cohort, both with and without COPD as a comorbidity. This pioneering aspect positioned the initiative among the first to comprehensively evaluate Caucasian never-smoker participants concerning COPD within the context of a low-dose CT screening project. Despite acknowledged limitations, including the absence of a detailed evaluation on why non-smokers were sensitized for screening, the trial's outcomes offered a unique vantage point for assessing the cost-effectiveness within this specific subgroup. Participants who tested positive during screening were referred to specialized pulmonologists. These experts assessed the necessity for further diagnostic measures or treatments based on available guidelines. These measures included full-dose contrast-enhanced chest or comprehensive staging CT scans, PET-CT scans, bronchoscopy, transthoracic needle biopsy (TNB), or video-assisted thoracoscopic surgery (VATS). The study meticulously documented the diagnosis, stage, pathology, and treatment plan for each case of lung cancer.

In conclusion, 1.5% of participants were diagnosed with histologically proven lung cancer, a percentage consistent with international data within the study population, ranging between 0.8%–2.2%.

The HUNCHEST study provided answers to health economy questions, revealing the annual costs of both screened and unscreened populations [18]. In the initial year, lung cancer screening with LDCT incurs an additional annual cost of approximately 3.3 billion HUF. By the 5th year, there is a yearly surplus cost of 1.9 billion HUF, considering a 10% participation rate of the affected population. The direct additional costs associated with screening amount to roughly 2.6 billion HUF per year. In the first 3 years of screening, the therapy for newly detected patients is more expensive than for those without screening. However, in the 4th and 5th years, the cost of treating later-stage, more expensive, and less effectively managed patients in the unscreened group surpasses the therapeutic cost of screened patients. By year ten screening is not only cost effective, but cost-saving.

HUNCHEST-II

The HUNCHEST II extended implementation study model examination was launched in 2019 with the proposal and support of the State Secretariat for Healthcare of the Ministry of Human Resources. The study included 18 centers, following a uniform protocol, applying the same patient follow-up scheme after positive screening results. The goal was to shed light on how a LDCT lung cancer screening program could be expanded nationwide. The key question during the study was whether it could be proven that lung cancer is more likely to be detected in symptom-free, early stages among 50–74-year-olds who are current or former heavy smokers participating in the program. Another aim was to conduct a cost-effectiveness and budgetary impact analysis based on the real-life data obtained during patient care in HUNCHEST II [19].

A cornerstone of the study was the uniform nodule tracking protocol, with the expectation of minimizing regional healthcare disparities. The recommendation and implementation of smoking cessation support for active smokers was carried out according to the specified professional guidelines using the methods outlined for smoking cessation support. The task of expediting the examination of highlighted patients fell under the responsibility of the territorial pulmonary department. Special diagnostic teams dedicated to handling the diagnostic pathway of nodules detected during lung cancer screening had to be established at the examination centers. Initially, these teams were closely associated with the oncology multi-disciplinary team (MDT) and in cases of confirmed lung cancer diagnosis, the routine MDT consultation decided on the patient's further course. (Internationally, it is recommended to establish a MDT for discussing solitary pulmonary nodules—approximately 70% of the cases identified are ultimately non-tumorous, and unnecessary invasive investigations can be reduced through MDT discussions).

During the examination, the designated radiologist at each center evaluated LDCT images on-site or through remote reporting. In addition, a central core Computer-Aided Detection (CAD) system provided by Aidence, the Veye Lung software provides the necessary secondary reporting. The images are automatically sent from the examination center's PACS system to CoreCAD for central processing, and almost in real-time (expectedly within 5–10 min), CoreCAD provides a diagnosis established by the computer. The radiologist had this data available by the time she started the reporting process -this also replaced the need for the resource-intensive dual radiologist reporting.

AI tools have become a necessity in LCS programs—the correct volume measurements require computer assistance, and the correct assessment of VDT also relies on CAD system. These cannot however be applied as first readers, as it suggests in the name these "aid the diagnosis." Deep learning (DL) systems are also developed in the field of LCS—in a recent metaanalysis, their specificity was 0.63 and sensitivity 0.93. The biggest question behind these systems that it is unclear how the machine calculates these results, so while promising, they are not yet accepted as part of non-study based screening projects [20].

Each center reported screenings to the National Korányi Institute for Pulmonology through an online data submission platform designed for this purpose, in compliance with GDPR regulations. The online interface is based on the tuberculosis surveillance system recorded by the National Korányi Institute for Pulmonology (OKPI) Methodology Department but is separate from it. Not only did it monitor the completion and results of controls in indeterminate screenings, but also, in the case of a positive screen it recorded the results of all necessary investigations. In the event of a lung cancer diagnosis, the histological type, stage, and the therapy suggested by the MDT was also documented. In the event of an alternative diagnosis, diagnosis and a brief description of the diagnostic pathway leading to it (bronchoscopy, PET/CT, biopsy, surgical intervention) was also noted.

In the clinical trial, data from more than 4,000 individuals were analyzed, with an average age of around 61 at the time of enrollment. Among the participants, the baseline LDCT examination result was negative in nearly 75% of all cases, and positive in 4%. The remaining group required LDCT follow-up, predominantly resulting in negative findings. In cases with positive results, every individual underwent a pulmonary specialist examination. Those with suspected tumors were appropriately referred to the local MDT for further assessment according to the protocol. Ultimately, 61 individuals were confirmed to have malignant lung tumors based on histopathology and/or clinical and radiological images.

Comparing the stage-wise distribution of new lung cancer patients participating in the HUNCHEST II program with those treated in the National Korányi Institute for Pulmonology (same time frame, same age range, confirmed smokers), it became evident that the HUNCHEST II study more frequently succeeded in detecting lung cancer in early stages. According to OKPI data, nearly 70% of patients presenting with symptoms were inoperable, while in HUNCHEST II, this was only the case for 20% of screen-detected tumor patients (Table 1).

Comparing the statistics between HUNCHEST I and HUNCHEST II reveals several differences in participant characteristics during the 1st round of screening. The average age in HUNCHEST I was 63.2, slightly higher than the average age of 61.3 in HUNCHEST II. The female percentage among current smokers was similar in both studies. The number of former or never smokers was comparable between the two studies, with slight differences in age and gender distribution. The prevalence of COPD as a comorbidity was higher in HUNCHEST I (18.6%) compared to HUNCHEST II (13.2%) This was due to the proactive screening for COPD in the first study with standard lung function testing, whereas in HUNCHEST II self-reporting of the disease was noted. In summary, HUNCHEST II involved a larger and slightly younger cohort, with a lower prevalence of COPD, as a comorbidity. The gender distribution varied slightly, and HUNCHEST II had a higher number of participants with a positive screen in the 1st round compared to HUNCHEST I.

The examination of lung cancer histological subtypes within the screening programs HUNCHEST I (HC1) and HUNCHEST II (HC2), alongside data from OKPI, unveils intriguing variations. In the screening-focused HC1, adenocarcinomas prevailed at 62.1%, contrasting with HC2 at 56.2%. OKPI reported 50% in 2022 and 47% in 2019. HC2 exhibited a higher frequency of squamous cell carcinomas (31.2%) compared to HC1 (24.1%), closely mirroring OKPI's 23% in 2022 and 24% in 2019—the lower incidence of this subtype in HUNCHEST I is possibly due to the fact that this program included never smokers, where squamous cell carcinomas are not common. For small cell carcinomas, HC1 was at 6.9%, HC2 at 6.2%, OKPI 2022 at 13%, and OKPI 2019 at 14%—the number of small cell carcinomas are usually lower in screening programs than in real life data, due to its more aggressive nature—the tumor grows much faster, thus making screening for it difficult. Other subtypes (including large cell tumors and carcinoids) constituted 6.9% in HC1, 10.4% in HC2, 5% in OKPI 2022, and 6% in OKPI 2019. These observations underscore the nuanced prevalence of lung cancer subtypes in screening programs, emphasizing the importance of considering diverse datasets in clinical and research contexts, particularly in the context of screening efforts (Table 2).

Lung cancer screening projects in central Europe

In the past decade more and more European initiatives have started, most of them pilots—including Italy (MILD) and France (CASCADE), to name a few [21]. In the UK, regional programs have

TABLE 1 Comparison of histological subtypes of screen detected and incidental lung cancer.

	All[d]	Adenocc	Squamousc.cc	Small cell Cc	Other[e]
HC1[a]	29	18 (62.1%)	7 (24.1%)	2 (6.9%)	2 (6.9%)
HC2[b]	48	27 (56.2%)	15 (31.2%)	3 (6.2%)	5 (10.4%)
OKPI[c] data 2022	NA	50%	23%	13%	5%
OKPI[c] data 2019	NA	47%	24%	14%	6%

[a]HUNCHEST I.
[b]HUNCHEST II.
[c]National Korányi Institute for Pulmonology.
[d]In case of HC2 26 patients withdrew from follow-up, exact histological data cannot be collected, therefore excluded.
[e]In both 2019 and 2022 9% of OKPI patients had no exact histological classification.

TABLE 2 Comparison of characteristics of participants in 1st round of screening in HUNCHEST I and II.

	All participants		People who currently smoke		Former or never smokers		Positive screen in 1st round	
	HC1[a]	HC2[b]	HC1	HC2	HC1	HC2	HC1	HC2
Number of participants	1890	4,215	870	3,284	1,020	931	70	174
Age	63.2	61.3	64.5	60.6	62.1	63.1		
Females	1,071 (56.7%)	2,254 (53.5%)	0,564 (55.3%)	1811 (55.1%)	507 (58.3%)	443 (47.6%)	38	97
COPD[c] as comorbidity	351 (18.6%)	556 (13.2%)	258 (24.3%)	439 (13.4%)	103 (11.8%)	117 (12.6%)	19	34

[a]HUNCHEST I.
[b]HUNCHEST II.
[c]Chronic obstructive pulmonary disease.

developed in such an extent, that today the Targeted Lung Health Checks are covering England by 2024 [22]. In 2020 Croatia was the first European country to roll out a nationwide screening project, with enrollment standing at over 29 thousand as the end of 2023 [23]. Historically Poland has a long standing history with LCS starting in 2008—today Poland has also started a nationwide project, based on the voivodeship system [24]. In the Czech Republic the nationwide system is based on pulmonologist, they refer patients in case of existing risk factors to the radiology departments [25]. These efforts reflect a comprehensive approach to lung cancer prevention and early detection across Europe. In Austria, Slovakia, Slovenia, Romania and Serbia no nationwide pilots were rolled out as of date, smaller studies such as the Vojvodina project in Serbia have been established, or in case of Slovakia, a comprehensive white paper has been formulated. Many of these countries, however, are part of the SOLACE project, thus implementation might start in these countries too [26–29].

Ongoing programs

HUNCHEST-III

In anticipation of a potential nationwide screening program, further studies are still necessary. The HUNCHEST I and II programs have provided compelling evidence supporting the cost-effectiveness of LDCT lung cancer screening within the appropriate risk group in Hungary. Notably, these initiatives were characterized by voluntary and opportunistic screening methodologies, focusing on modeling patient pathways post-screening rather than elucidating the routes leading to screening. Recognizing the significance of clarifying pre-screening patient journeys, we recommend a more nuanced approach by modeling primary care patient selection within a more confined population.

The primary dilemma facing lung cancer screening programs is their departure from age-specific screening, unlike other public health screenings, adopting a risk-based approach instead. Currently, there is available literature data regarding the effectiveness of screening individuals aged 50 (55)–75 (80) years with a significant smoking history (25–30 pack-years). Given this, the initiation of screening for this group is imperative. Unfortunately, obtaining precise smoking history is not readily available in most places, making the traditional invitation system based on residency records, as used in other screenings (e.g., breast cancer screening), unsuitable for lung cancer screening.

Illustrating the challenges faced in real-world, non-trial screenings, Kinsinger et al. conducted screening in Veterans Health Administration hospitals from 2013 to 2015, utilizing

NLST criteria. Initially identifying 93,033 individuals meeting the initial screening criteria (age between 55–80 years, no serious comorbidities, and life expectancy of more than 6 months), nurses reviewed their histories, seeking individuals with at least a 30 pack-year smoking history, either current smokers or those who quit within the last 15 years. Due to missing data, 36,555 individuals were excluded, and an additional 38,395 lacked a smoking history suitable for screening. Among the remaining 18,083 individuals, doctors did not assess 13,084 cases, while 789 were deemed unsuitable for LDCT screening. Out of the remaining 4,246 patients, 1,794 declined screening, and eventually, 2,106 screenings were completed within the timeframe. Of these, 59.7% had a positive result based on NLST criteria, detecting suspicious lesions in 73 patients, ultimately confirming lung cancer in 31 cases. The false-positive rate was remarkably high at 97.5%. Additionally, 40.7% of patients had incidental findings, most commonly emphysema and coronary atherosclerosis [30].

Among those who participated in the screening, 1,120 individuals, according to NLST criteria, were expected to attend a follow-up examination after 1 year. Despite repeated invitations via written and telephone communication, only 870 individuals attended. This was less than 78%, significantly lower than the 95% recall success rate assumed in NLST's public health calculations. These challenges underscore the complexities faced in implementing effective lung cancer screening programs, particularly in identifying and engaging the target population.

Thus, the aim of the final HUNCHEST project is to test prescreening pathways. To that effect a smaller, well described population based study was called for. Demographic overview encompassing the settlements affiliated with the "Budakörnyéki egészségprogram" Peri-Budapest Healthcare Program—Biatorbágy, Budajenő, Budakeszi, Herceghalom, Nagykovácsi, Páty, Perbál, Pilisjászfalu, Remeteszőlős, Telki, Tinnye, Tök collectively house approximately 60–65,000 residents. Within this demographic, an estimated 12,000 individuals fall within the 50–75 age bracket, with an anticipated 3,500–4,000 individuals exhibiting a substantial history of tobacco use. The overarching objective is to meticulously map the smoking history of all individuals within the specified age group, facilitating the identification and subsequent invitation of those at risk for LDCT screening.

The success of the screening program hinges upon the active involvement of general practitioners and their assistants. Their pivotal role involves assessing the smoking history of individuals aged 50–75 in their respective areas and discerning those deemed suitable for screening. Furthermore, at this juncture, a targeted smoking minimal intervention is administered. Following this initial phase, coordination with the Comprehensive Cancer Center's coordinator ensues, whereby the collected information is meticulously recorded within the HUNCHEST platform.

We anticipate that 20% of screened patients will be recalled for a 3-month follow-up assessment based on our previous pilots. Based on the HUNCHEST program, the lung cancer identification rate is projected to range between 1.5%–2% in Hungary. This implies the potential detection of approximately 80 cases of lung cancer, with a substantial majority—around 70%—being identified in the early stages. This comprehensive approach holds the promise that approximately 65 patients may receive a genuine opportunity for long-term survival.

The HUNCHEST-III project is currently in the active recruitment phase, screening started in September 2023, preliminary results are excepted in early 2025.

SOLACE

In 2022, the EU4Health project introduced a groundbreaking initiative aligned with Europe's Beating Cancer Plan—the Strengthening the screening of Lung Cancer in Europe (SOLACE) project [31]. This innovative endeavor aims to streamline the implementation of lung cancer screening programs across Europe, ensuring equitable access for individuals from diverse social and economic backgrounds. Representing a significant stride in comprehensive lung cancer screening, SOLACE is dedicated to developing, testing, and disseminating tools that address identified obstacles and health inequalities in various European countries.

The primary goal of SOLACE is to provide a versatile toolbox for personalized approaches to lung cancer screening, applicable on both national and regional scales. The project specifically focuses on facilitating and supporting the structured implementation of LDCT lung cancer screening programs throughout Europe. By doing so, SOLACE aims to enhance the overall quality of lung cancer screening practices, while also improving accessibility, benefit-harm balance, and cost-effectiveness.

A key feature of SOLACE involves unprecedented collaboration among key stakeholders essential for designing, planning, and implementing sustainable lung cancer screening programs in member states. To ensure the lasting impact of the project, the proposal includes the establishment of the European Lung Cancer Screening Alliance (ELCSA).

Hungary actively participates in SOLACE with three centers, notably including the OKPI. Over the next 18 months, the project will test various recruitment strategies to measure their effectiveness in targeting different groups, with a special focus on the socio-economically deprived and on those with preexisting pulmonary comorbidities. Notably, the project places a particular emphasis on women, recognizing the insufficient data on lung cancer screening strategies in the female population.

TABLE 3 Comparison of the different HUNCHEST studies.

	HUNCHEST I	HUNCHEST II	HUNCHEST III
aim	Demonstrating the feasibility of LCS in Hungary	Establishing patient pathways	Modelling population wide screening (estimated 4,000 at risk—determining efficacy of invitation methods)
date	2013-20	2019-22	2023-ongoing
criteria	50–79 both smokers and neversmokers	50–75 20 + pys	50–75 20 + pys
participants	1,890	4,215	TBA
Lung cancers found	1.5%	1.8%	TBA

Conclusion

With the 3 HUNCHEST projects, we have modeled the three pillars of screening: the technical implementation of screening, the design of patient pathways post-screening, and the identification and invitation of high-risk patients, as seen in Table 3. The next step is to determine the feasibility of a potential public health screening. In the meantime, it may be advisable for the health administration to create a means allowing high-risk individuals to participate in LDCT screening once a year.

Considering the future of large-scale LDCT screenings, key questions arise regarding the application of artificial intelligence (AI) and deep learning models to address human resource challenges. Furthermore, there is a need to determine additional biomarkers for individuals currently not in high-risk groups, such as non-smokers or young individuals, in order to develop screening in potentially identifiable risk groups, while adhering a thorough cost/benefit analysis.

The integration of AI and deep learning models in LDCT screenings presents a promising avenue for enhancing efficiency and accuracy in diagnosis. This technological advancement can alleviate human resource constraints by automating the analysis of LDCT results, and enabling quicker and more precise identification of potential tumors. Adequate training for healthcare professionals in collaboration with AI systems will be crucial to optimize this integration.

Identifying biomarkers beyond the current high-risk groups is essential. Research efforts should focus on exploring additional biomarkers that can aid in identifying low-risk groups more accurately. Factors such as genetic predispositions, environmental exposures, and other elements should be considered to refine the screening criteria and ensure a more targeted approach.

Conducting comprehensive cost/benefit analyses is imperative for shaping effective screening programs. Evaluating the costs against potential savings and improvements in patients' quality of life will provide insights into the economic viability of such programs. Considering the long-term health and economic impacts is crucial in making informed decisions.

Author contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript, AK-F was responsible for writing the article, with KB editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Applied models and molecular characteristics of small cell lung cancer

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Small cell lung cancer (SCLC) is a highly aggressive type of cancer frequently diagnosed with metastatic spread, rendering it surgically unresectable for the majority of patients. Although initial responses to platinum-based therapies are often observed, SCLC invariably relapses within months, frequently developing drug-resistance ultimately contributing to short overall survival rates. Recently, SCLC research aimed to elucidate the dynamic changes in the genetic and epigenetic landscape. These have revealed distinct subtypes of SCLC, each characterized by unique molecular signatures. The recent understanding of the molecular heterogeneity of SCLC has opened up potential avenues for precision medicine, enabling the development of targeted therapeutic strategies. In this review, we delve into the applied models and computational approaches that have been instrumental in the identification of promising drug candidates. We also explore the emerging molecular diagnostic tools that hold the potential to transform clinical practice and patient care.

KEYWORDS

SCLC, drug response, ctDNA, liquid biopsy, databases

Introduction

Small Cell Lung Cancer (SCLC) is an extremely aggressive form of cancer, accounting for about 15% of all lung cancer cases. Due to its aggressive nature, over 60% of SCLC cases already show metastasis at the time of diagnosis, despite regular imaging [1]. Consequently, surgical resection is rarely an option, leaving chemotherapy, radiation, and in some instances, immunotherapy, as the main treatment methods. This situation adversely affects SCLC research and the development of new molecular diagnostic tools as well, as tumor samples are rarely available. Since no major improvements have been achieved in SCLC treatment in over three decades, which is paired with short life expectancy, the National Cancer Institute to categorizes this disease as a "recalcitrant" cancer. Therefore, there is an urgent need for a more profound understanding of SCLC's

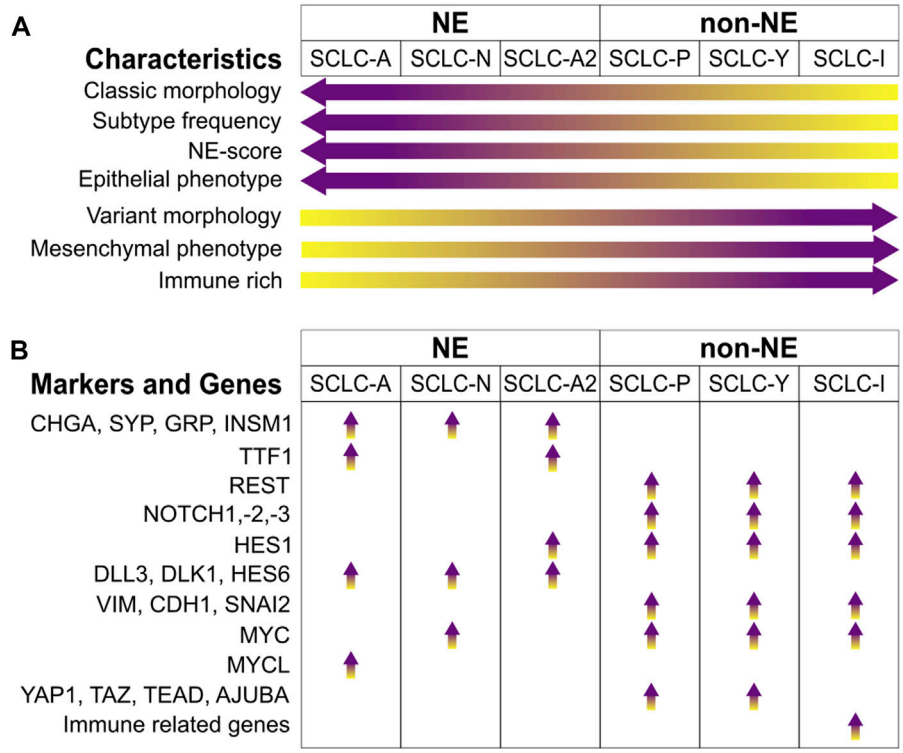

FIGURE 1
SCLC subtypes. **(A)** Signature enrichment of subtypes. **(B)** Key markers and genes enriched in the different SCLC subtypes. Abbreviations: CHGA, Chromogranin A; SYP, Synaptophysin; GRP, Gastrin-Releasing Peptide; INSM1, Insulinoma-Associated Protein 1; TTF1, Thyroid-Transcription Factor 1; REST, RE1-Silencing Transcription Factor; NOTCH1,-2,-3, Neurogenic Locus Notch Homolog Protein 1, -2, -3; HES1, Hes Family BHLH Transcription Factor 1; DLL3, Delta Like Canonical Notch Ligand 3; DLK1, Protein Delta Homologue 1; HES6, Hes Family BHLH Transcription Factor 6; VIM, Vimentin; CDH1, Cadherin 1; SNAI2, Snail Family Transcriptional Repressor 2; MYC, MYC Proto-Oncogene, BHLH Transcription Factor; MYCL, MYCL Proto-Oncogene, BHLH Transcription Factor; YAP1, Yes1 Associated Transcriptional Regulator; TAZ, Transcriptional Coactivator With A PDZ-Binding Domain; TEAD, TEA Domain Transcription Factors; AJUBA, LIM Domain-Containing Protein Ajuba.

development and progression, the creation of more accurate models, and the development of new molecular diagnostic tools that can overcome the challenges presented by this complex disease.

Subtypes of SCLC

A decade ago, SCLC was predominantly viewed as a uniform type of pulmonary neuroendocrine cancer. The World Health Organization (WHO) and the National Comprehensive Cancer Network (NCCN) still classify SCLC into two subtypes: small cell carcinoma (previously known as oat cell carcinoma) and combined-SCLC, characterized by features of both small and non-small cell carcinoma [2].[1] When SCLC cell lines were first developed approximately 30 years ago, they revealed two distinct morphological subtypes: classic and variant subtypes. Classic cell lines formed non-adherent aggregates or spheroid cells, while variant cell lines exhibited either loosely adhering aggregates or formed tightly adhering monolayers [3].

The homogeneity of SCLC is exhausted by the prevalent TP53 and RB1 inactivation [4–7], from which new characterizations have been developed over the years. A critical finding was that SCLCs could be categorized based on their neuroendocrine (NE) characteristics, into NE (with high NE scores) and non-NE (with low NE scores) types by IHC staining for neuroendocrine markers such as SYP (Synaptophysin) or CHGA (Chromogranin A) [8]. Transcriptomic profiling of these cell lines has led to the identification of further subtypes based on the expression of transcription factors, a classification also supported by tumor sample analysis [7, 9–11], which we summarized in Figure 1.

The most prevalent subtype is characterized by elevated expression of Achaete-scute homologue 1 (ASCL1), termed SCLC-A, which is crucial in regulating neuroendocrine differentiation [12–14]. NEUROD1 (Neuronal Differentiation

1 https://www.nccn.org/

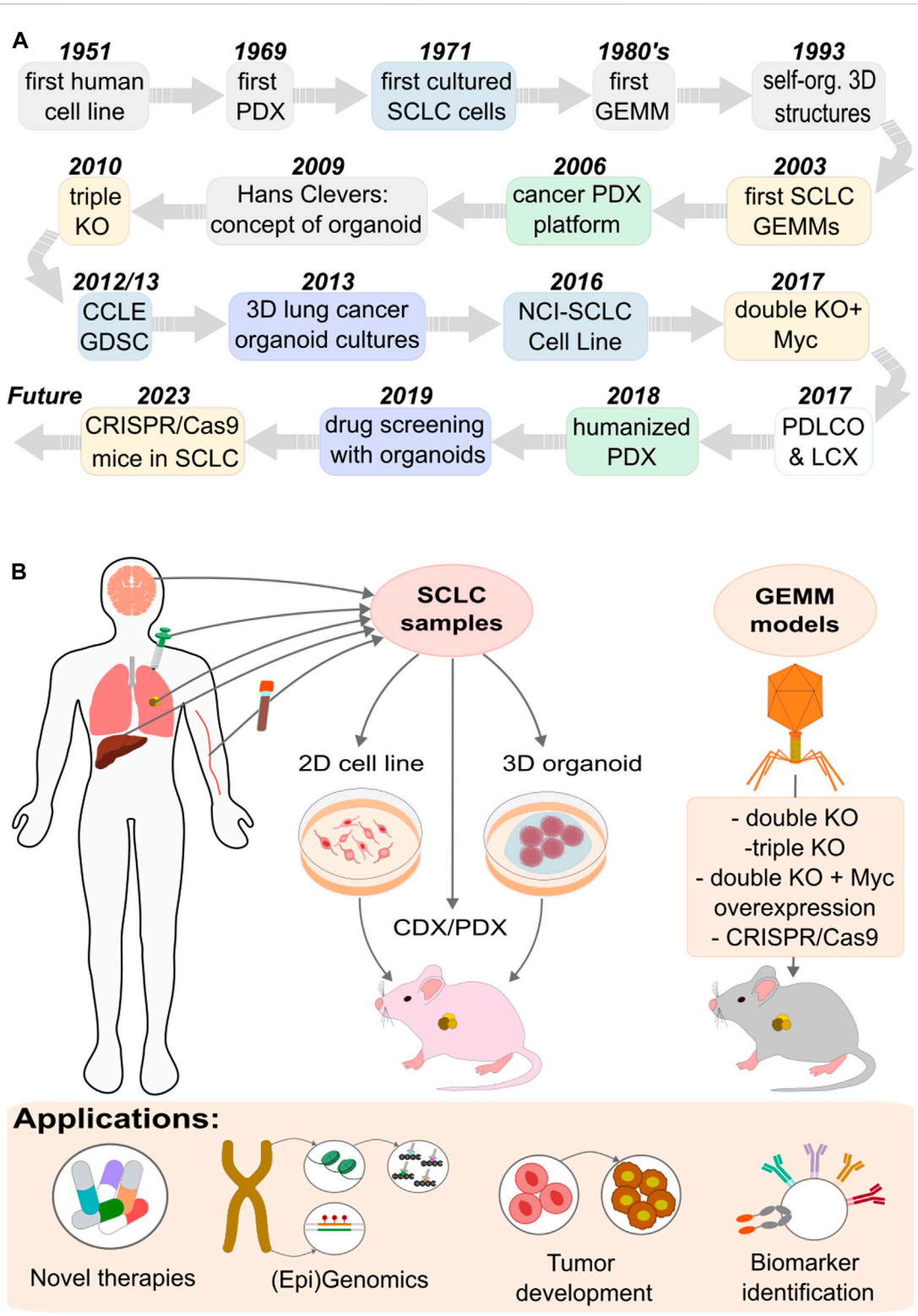

FIGURE 2
Experimental model systems used to explore SCLC. **(A)** Timeline of the developed models. **(B)** Approaches and applications of the different models.

1), another marker for the NE subtype, often co-exists with ASCL1. NEUROD1, enriched in the SCLC-N subtype, also influences NE differentiation and contributes to the progression of cancer, [14]. A less common group, which is negative for both ASCL1 and NEUROD1, falls into the non-NE category. These tumors and cell lines sometimes express ASCL2, suggesting its role as an alternative transcription driver [12]. The main non-NE subtypes are distinguished by the expression of POU2F3 (SCLC-P) and YAP1 (SCLC-Y). POU2F3 (POU Class 2 Homeobox 3), a key transcription factor in chemosensory tuft cells, is expressed in SCLC variants that share a similar expression profile with these cells, indicating a possible origin from this cell lineage [15]. YAP1 (Yes1 Associated Transcriptional Regulator) and TAZ (Transcriptional Coactivator with a PDZ-Binding Domain) are involved in the Hippo pathway as transcriptional coactivators and effector proteins, leading to tissue overgrowth and oncogenesis [16]. SCLCs expressing YAP1 represent a relatively rare subgroup [7, 11, 17].

The (NE) and non-NE subtypes of SCLC show distinct transcriptional signatures. Zhang et al. identified a set of 50 genes that are differentially expressed in SCLC tumors, cell lines, and genetically engineered mouse models (GEMMs) [12]. This set includes 25 genes closely associated with NE SCLCs and another 25 linked with non-NE SCLCs. These genes were used to generate an NE scoring system to help patient stratification. An ASCL1 subset that expresses HES1 (Hes Family BHLH Transcription Factor 1) was also identified and was termed as SCLC-A2 or NEv2 [18–20], which was often associated to liver metastases [19].

The CHGA and SYP genes are widely recognized NE markers, showing high expression levels in both SCLC-A and SCLC-N subtypes [9, 12]. Insulinoma-Associated Protein 1 (INSM1), a zinc-finger transcription factor found in developing neuroendocrine tissues [21], is indicative of SCLC and associated with NE characteristics [22, 23]. Additional genes linked with NE subtypes include Gastrin-Releasing Peptide (GRP) [7], Protein Delta Homologue 1 (DLK1) [24], and BEX1 (Brain Expressed, X-Linked 1) [25]. NKX2-1, the gene for Thyroid-Transcription Factor 1 (TTF1), is a transcriptional target of ASCL1, making its expression specific to the SCLC-A subtype [9, 26].

Notch signaling is known to facilitate a transition from NE to a chemoresistant non-NE phenotype in SCLC [20]. It activates the expression of REST (RE1-Silencing Transcription Factor), which suppresses the expression of NE markers such as ASCL1, SYP, or CHGA. Similarly, YAP1 has been found to support this Notch-induced shift to a non-NE phenotype [27]. Additionally, HES1 (Hes Family BHLH Transcription Factor 1) has a negative correlation with NE scoring [12], as its expression is governed by NOTCH1, which hinders the transcription of REST and YAP1 [27, 28]. While Notch signaling fosters non-NE differentiation, some genes, such as DLL3, DLK1 and HES6, which activate this pathway, have been found to correlate with the NE subtype, thereby acting in a pro-tumorigenic manner towards NE cells [12, 19, 29, 30].

Genes in the TGFβ pathway have been found to be expressed in non-NE cases of SCLC, acting as suppressors of ASCL1 [12, 19]. There is a crosstalk between the Notch and the Hippo pathway [31, 32]. YAP1 and TAZ are overexpressed in SCLC-Y, as well as the TEAD genes (TEAD2 and TEAD3) and AJUBA (Ajuba LIM Protein), negative regulators of the pathway [12, 19]. Additionally, the transition from NE to non-NE phenotype can be influenced by Notch and TGFβ signaling, which is linked with the process of epithelial to mesenchymal transition (EMT). EMT is known to promote metastasis and resistance to treatment in cancer cells [33]. The expression of the intermediate filament vimentin (VIM), and SNAI2 (Snail Family Transcriptional Repressor 2), a repressor of E-cadherin (CDH1), has been observed to negatively correlate with the NE state of SCLC [12, 20]. The MYC proto-oncogene paralogues (BHLH Transcription Factors), are differentially expressed in SCLC. MYCL being the target of ASCL1 is expressed in SCLC-A [19], while MYC, a target of NEUROD1, which drives to a non-NE phenotype is elevated in SCLC-N [34, 35] and in non-NE SCLC-Y [7] subtypes.

DNA replication stress is a key biological feature of SCLC [36]. The near universal loss of p53 and RB1 tumor suppressors is one reason of replication stress as they play roles in cell cycle progression [37]. Another reason could be the overexpression of MYC family of oncogenes, which promote heightened replication initiation, which lead to defects in replication [38, 39]. Replication stress is higher in NE tumors, presenting a specific gene expression pattern with genes to deal with the elevated replication rates, to hinder the DNA damage, DNA repair, and cell cycle related genes [19, 40]. The elevated replication stress observed in NE tumors may be a reason why many tumors have exceptional initial response [36].

A new subtype of small cell lung cancer (SCLC) has been recently identified, which does not fully align with the previous four subtypes based on transcription factor expression, although SCLC-Y is commonly found. Instead, this subtype, termed SCLC-inflamed or SCLC-I, is characterized by the expression of immune-related genes. SCLC-I tumors exhibit the highest levels of $CD8^+$ T-cell and overall immune infiltration among all the subtypes. Additionally, SCLC-I is marked by high levels of immune checkpoint molecules (such as PD-L1, PDCD1, CTLA4, CD38, IDO1, TIGIT, VISTA, ICOS, LAG3), T cell attractant chemokines (CCL5, CXCL10), and MHC genes (HLA-DRB1, HLA-DQA1, MICA). This suggests they may be more responsive to checkpoint inhibitors compared to other subtypes. Gay et al., in a reanalysis of the *IMpower133* data, noted that SCLC-I tumors tend to respond favorably to carboplatin/etoposide/atezolizumab treatment [9, 41].

This highlights the heterogeneity of SCLC and underscores the importance of adopting these proposed subtype classifications. Continued subtype-specific research is essential

TABLE 1 Summary of advantages and disadvantages of each model.

Characteristics	SCLC models			
	Cell line	CDX/ PDX	PDO	GEMM
Cost	–	+	+	+
Time consuming	–	+	·	+
Difficult to generate	–	+	·	+
Rapid expansion	+	–	+	–
Reproducibility	+	–	–	·
Tumor heterogenity	–	+	+	·
Original tumor biology	·	·	+	+
Primary disease	+	·	+	+
Metastasis	·	+	+	·
Biomarker discovery	·	+	+	·
Drugscreening	+	+	+	+
Translational research	–	+	+	·

to understand their distinct pathophysiologies and to identify optimal treatment strategies.

Experimental models of SCLC

There are several model systems used to examine cancer and SCLC (Figure 2). Each system has advantages and disadvantages, covering a wide range of application, such as drug testing, performing omics studies, or to characterize SCLC development (Table 1).

Patient derived cell lines (PDC)

The basic growth properties of SCLC were first defined using panels of cell lines developed from 1971 through the early 1990s [42–45] (Figure 2A). Primarily derived from metastatic SCLC tumors, these cell lines have been instrumental models for understanding gene functions and testing potential drug candidates.

During the early 1990s, the National Cancer Institute (NCI, Bethesda, MD), pioneered a new method for drug screening focused on specific diseases. This method involved using a collection of 60 human cancer cell lines from nine different cancer types [46, 47]. Originally, SCLC was not part of the NCI-60, which made drug predictions for this cancer type unfeasible. However, the advent of high-throughput technologies and improvements in characterizing cell lines have led to the development of more extensive cell line collections that now include SCLC. These databases contain detailed information on gene mutations, structural alterations, and changes in copy numbers, as well as mRNA expression profiles. This allows for comparative analyses across different cell lines and cancer types. For example, the Cancer Cell Line Encyclopedia (CCLE) [48] resource utilized data from massively parallel sequencing and microarray expression profiles from 947 human cancer cell lines, alongside the responses to 24 anticancer drugs in 479 of these lines. Additionally, the Genomics of Drug Sensitivity in Cancer (GDSC) has become a major public source for data on cancer cell drug sensitivity and molecular indicators of drug response [49]. The GDSC database includes information from nearly 75,000 experiments, covering responses to 138 anticancer drugs across approximately 700 cancer cell lines. Recent studies enable the exploration of the role of microRNAs as potential biomarkers in SCLC. By the early 1990s, investigations had already been conducted on 126 SCLC cell lines, providing insights into the response of these cell lines to anticancer drugs, and a library of investigational agents complemented by exon and microRNA arrays [50].

Despite their affordability and suitability for high throughput screening, these cells do not fully capture the complex nature of the tumor environment [51]. For this reason, such cancer models have roughly a 10% success rate in advancing anti-cancer drugs to clinical trial stages [52]. In addition, even the promising drug candidates usually failed at preventing recurrence in pre-clinical and clinical trials [52]. Nonetheless, they are still useful model organisms that can be used to better characterize and study what genetic and epigenetic factors affect SCLC growth and development, providing quick and easy tools for drug and CRISPR based screens.

Patient derived organoids (PDO)

In 2009, Hans Clevers laid the foundation for organoid research, demonstrating new methods for organoid culture [53, 54] (Figure 2A), significantly boosting the development of patient-derived organoids (PDOs). The first lung cancer PDOs were generated by Inoue and coworkers [55, 56]. Compared to traditional cancer cell lines and patient-derived xenograft (PDX) models, lung cancer PDOs offer several advantages [57, 58]. PDO is a 3D structure culture formed from enriched patient cancer cells. It exhibits genetic stability, self-renewal capabilities, drug sensitivity, and high degrees of similarity to human organs in both structure and function [59].

A key attribute of PDOs is their faithful retention of the parental tumor's genomic changes, yet they allow for faster modeling and some degree of gene editing [57, 60–62]. Through whole-exome sequencing, whole-genome sequencing, and RNA-seq Kim and their colleagues found that short-term cultured lung organoids retained 92.7% and 77% of the driver mutations found in the primary tissue, respectively [63, 64]. They developed 80 lung cancer organoid lines, including five from SCLC. These SCLC organoids accurately reproduced the tissue structure of the original tumors and maintained key SCLC diagnostic markers such as CD56, SYP, and TTF-1. It was

also noted that the culture conditions for non-small cell lung cancer (NSCLC) PDOs and SCLC PDOs differed, with R-spondin1 and Wnt3a being crucial for the long-term culture of SCLC tumor organoids [57, 65]. In addition, Zhang et al. were able to establish 3D co-culture models to expand circulating tumor cells (CTCs) *ex vivo* from early-stage SCLC patients [66, 67].

Recent studies using engineered mouse lung cancer organoids (LCOs) have shed light on SCLC metastasis mechanisms, showing that KMT2C deficiency leads to extensive metastasis [68, 69]. This particular SCLC model, driven by Trp53 and Rb1 (mouse homologs of human TP53 and RB1, respectively) deficiencies and Myc overexpression, displayed multiple diagnostic markers of SCLC and developed significant distal metastases in multiple organs [68]. In SCLC research, brain organoids can be propagated on a large scale, facilitating the testing of various cell subtype combinations [70]. However, creating a PDO model is time-intensive, costly, and technically challenging, necessitating further research into PDOs [54].

Patient and circulating tumor cell xenografts (PDX/CDX)

The *in vivo* preclinical methods of SCLC research include the application of mouse xenograft models. These models are either cell line-derived xenografts, created from SCLC cell lines, or PDX models, which involve directly implanting tumor material into immunocompromised mice like NOD/SCID or NSG [45, 71] (Figure 2B). The advantage of these models can be seen in the example of BH3 mimetics (BCL2/BCLxL inhibitors), where significant effectiveness in SCLC cell line models has been observed [45, 72, 73], with limited sensitivity in SCLC PDX models [74]. The discrepancy between PDX models and cell-line models in drug sensitivity underscores the potential impact of *in vitro* selection artifacts on clinical outcomes, suggesting that PDX models may better reflect the expression profiles and drug sensitivities of SCLC patient tumors [75, 76].

Obtaining SCLC samples is unfortunately very challenging as it is rarely surgically removed, and invasive tumor sampling is typically unnecessary after diagnosis [45]. To bridge this gap CTCs from the blood of cancer patients can be sampled noninvasively and are highly abundant in SCLC patients [77, 78]. In the CDX technique, changes in CTC numbers are closely aligned with chemotherapy responses, indicating that CTCs may reflect the biology of SCLC tumors. Both PDX and CDX techniques maintain the original human tumor's histopathological and genetic characteristics, preserving its heterogeneity and complexity [45, 79]. This significantly improves the ability to identify and test biomarkers for treatment and prognosis [64].

PDX models are thought to preserve the tumor microenvironment and epigenetic features, which are crucial for tumorigenesis, invasion, metastasis, and the effectiveness of anticancer therapies [60]. However, PDX has several limitations, including chances of tumor tissue engraftment failure, a long tumor development timeline, dissimilarity of the tumor microenvironment between human and murine models, and low throughput for drug screening [80]. Furthermore, the requirement for immunocompromised hosts limits their use in studying cancer-immunity interactions. Advances in humanized mice and mice with reconstituted human immune systems offer potential solutions [54]. Sequencing studies using next-generation sequencing on SCLC PDX models have proven valuable for unraveling the molecular landscape of this disease [78], and also reported the presence of a concordant somatic TP53 mutation in all CTCs [77].

Genetically engineered mouse models (GEMM)

Research in lung cancer has progressed significantly due to studies using genetically engineered mouse models (GEMMs), which facilitate the examination of tumor biology and environmental interactions *in vivo* conditions [78]. The first SCLC GEMM model was developed in the laboratory of Anton Bern in 2003 [10]. It incorporated loss-of-function mutations in the Rb1 and Trp53 tumor suppressor genes (double knockout), mirroring mutations found in over 90% of SCLC patients [81] (Figure 2A). These mutations were believed to exhibit significant histological and molecular biological similarities to the human disease, though the rate of tumor development in the model was slower than typically observed in human cases [82].

GEMMs laid the groundwork for numerous subsequent studies, which explored the roles of various potential tumor suppressors and oncogenes in SCLC [83]. A notable study within this framework examined P130, also known as Rbl2 (RB Transcriptional Corepressor Like 2), a member of the retinoblastoma (Rb) family (Figure 2B). The deletion of the P130 gene in mice already having Rb1 and Trp53 gene knockouts led to faster tumor growth, thereby affirming P130's tumor suppressor function in SCLC (triple knockout) [81, 84]. Similar methods were used to demonstrate the importance of PTEN and NOTCH tumor suppressors [7, 85].

Conditional Trp53/Rb1 (double knockout) and Trp53/Rb1/Rbl2 (triple knockout) knockout mouse models displayed traits typical of the ASCL1-high/NEUROD1-low subtype of human SCLC [35]. The double knockout model was further developed by introducing a CRE-activated Myc-T58A mutation. The stabilization of MYC in this model hastened tumor development and growth processes, and the resulting invasive tumors were representative of the high NEUROD1 subtype [35, 82].

Since the 2010s, CRISPR/Cas9 GEMMs have been applied in cancer research [86, 87], and significant breakthroughs occurred in 2023. These innovations include the development of mice

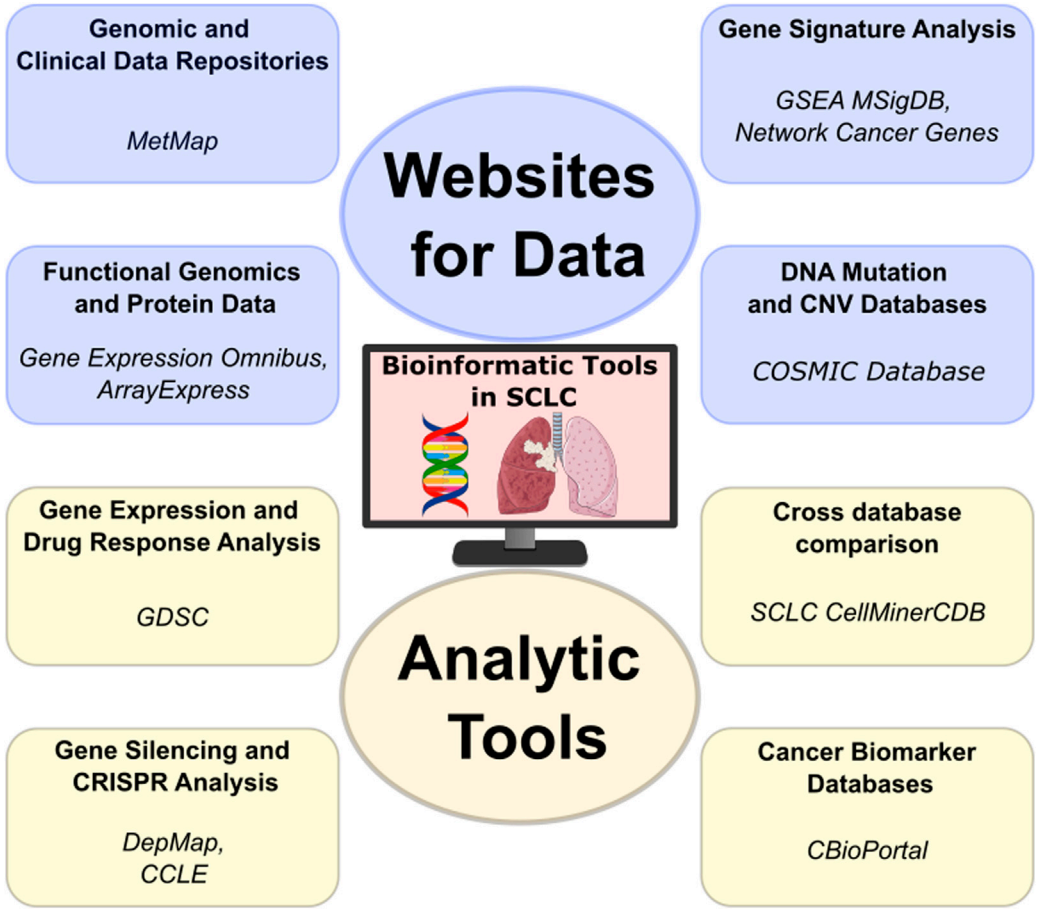

FIGURE 3
The analytic tools used in SCLC research are pivotal not only for their analytical capabilities but also for the downloadable databases they offer. This feature allows for versatile data manipulation, enabling researchers to conduct customized analyses that can lead to novel insights into SCLC's molecular intricacies and potential treatments. (Lung—lung-cancer icon by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/, DNA—dna-nucleotides-ribbon icon by Servier https://smart.servier.com/ is licensed under CC-BY 3.0 Unported https://creativecommons.org/licenses/by/3.0/.

capable of CRISPR/Cas9 base editing [88] and prime editing [89]. Prime editing, which employs Cas9 with a reverse transcriptase, allows for precise and efficient mutation engineering, as demonstrated in a proof-of-principle study introducing hotspot mutations for Kras and Trp53 in the lung and pancreas [90].

Databases and tools to mine SCLC

Research on SCLC is guided by a wealth of data from specialized websites, analyzed using integrative approaches. This combination of resources is not only changing the paradigm of the disease but also improving its treatment. The scientific progress is centered around data repositories that provide raw genomic, proteomic, and clinical data, as well as analytical tools that interpret this data to derive meaningful disease insights.

Websites for data in SCLC research

Data repositories have become indispensable in SCLC research (Figure 3). They serve not only as collections of genomic and clinical data but also as platforms that enable intricate comparative studies and groundbreaking translational research. Data can be directly accessed through different sites, such as the SRA (Sequence Read Archive) [91] and ENA (European Nucleotide Archive) [92] in case of experimental models, or European Genome-phenome Archive (EGA) [93] and dbGAP [94] for protected patient data that requires access. These allow researchers to process and analyze data in any preferred way. In many cases, processed data is also made available, such as at the GEO or ArrayExpress, which allows users to directly access results without having to reprocess large data amounts. Recent studies leveraging published data have revealed novel gene signatures that are correlated with SCLC

progression [95–97]. Overall, these databases play a crucial role in discovering biomarkers, which aid in developing new diagnostic and prognostic tools. This, in turn, helps personalize patient care for SCLC.

Mutation databases such as Catalogue Of Somatic Mutations In Cancer (COSMIC) [98] offer a comprehensive record of genomic aberrations discovered in cancer, including SCLC. COSMIC documents genetic alterations, such as mutations and copy number variations (CNVs), which can be used to find recurrent alterations and hotspots through interactive plots. It is an essential resource for identifying genetic alterations in cancer, including SCLC, and for the continuous search for targeted therapies. In addition, the MetMap [99] provides a detailed profiling of metastatic potential of cell lines including SCLC, aiding in the identification of potential therapeutic targets.

Gene signature analysis, facilitated by platforms such as GSEA's [100] MSigDB [101], and Network Cancer Genes [102], enables the identification of predictive gene patterns [103]. This approach is essential for developing targeted and personalized therapies for SCLC, tailoring treatments to individual genetic profiles [104].

Analytic tools facilitating SCLC research

The analytical tools utilized in research on SCLC are crucial not only for their capabilities but also for the provided easy data access (Figure 3). This feature allows for versatile data manipulation, enabling researchers to conduct customized analyses that can lead to novel insights into SCLC's molecular intricacies and potential treatments.

The Genomics of Drug Sensitivity in Cancer (GDSC) [49] offers a vast repository of data specifically focused on drug response in cancer, providing insights into how various cancer cells react to different treatments. Using the GDSC portal, users can compare drug sensitivity across cell lines and tissue types, compare drug response based on mutational status and correlate compound response.

The Cancer Cell Line Encyclopedia (CCLE) [48] provides another critical piece in cancer research, offering detailed genetic and molecular information on a wide range of cancer cell lines [49, 105, 106]. Building upon this, the DepMap portal [107] presents as a precious tool for functional genomics. DepMap utilizes the data from CCLE to identify essential genes for cancer cell survival, employing cutting-edge CRISPR technology. This integration allows researchers to perform in-depth analysis of CCLE data within the DepMap framework, enhancing our understanding of cancer dependencies and paving the way for new therapeutic approaches targeting these vulnerabilities in cancer cells.

The SCLC-CellMinerCDB tool at the National Cancer Institute (NCI) stands out for its integration of diverse databases, including GDSC, CCLE, UT Southwestern (UTSW) Medical Center [108] and NCI-SCLC [17, 109]. This integration not only consolidates a wealth of data but also facilitates advanced analysis capabilities. Researchers can seamlessly explore and compare data from multiple sources, encompassing diverse omics datasets such as gene mutation/copy-number data, expression data, epigenetics data (DNA methylation and enhancer signal, [97, 110]) and gene signature enrichment, which can be compared to each other or to drug response data.

The cBioPortal [111] for Cancer Genomics is an excellent example of the power of integrative data analysis. It provides researchers with a multifaceted view of molecular data sets alongside clinical attributes. This tool is particularly adept at uncovering biomarkers for SCLC and for cancer in general. With cBioPortal, we can interrogate and visualize mutation distribution in patient cohorts, identify co-expressing or anti-expressing genes, or even compare survival between patient groups based on mutational status of selected genes. In addition, processed data and clinical information can be easily obtained, helping researcher create custom analyses from a curated set.

The integration of data repositories and analytical tools is crucial in navigating the complex molecular landscape of SCLC, representing the forefront of precision oncology. The future of SCLC research and treatment depends on the continued fusion of data acquisition with analytical sophistication, which holds the key to unlocking new realms in cancer therapy.

Developing non-invasive diagnostics

SCLC is histologically characterized as a malignant epithelial tumor composed of small cells that feature minimal cytoplasm, indistinct cell borders, finely granular nuclear chromatin, and either absent or barely noticeable nucleoli. The majority of SCLC cases, roughly 90%, fall into the category of typical SCLC, which exclusively comprises these small cells. The rest are identified as combined SCLC, where the tumor also includes elements of large cell carcinoma [112]. SCLC can be classified into two stages: limited disease (LD-SCLC), when it is limited to the hemithorax, where radiochemotherapy is effective; or extensive disease (ED-SCLC), where metastatic disease can be found outside of the hemithorax at diagnosis [113].

The diagnostic process for SCLC typically includes a physical examination, an assessment of the patient's performance status, laboratory tests, and various imaging techniques. These imaging techniques often comprise contrast-enhanced CT scans of the chest and abdomen, brain imaging through MRI or CT, and potentially FDG PET/CT for cases of limited-stage disease [114]. Prior to initiating treatment, a definitive tissue diagnosis of SCLC is necessary. The choice of sampling method for diagnosis largely depends on the anatomical location of the tumor [115].

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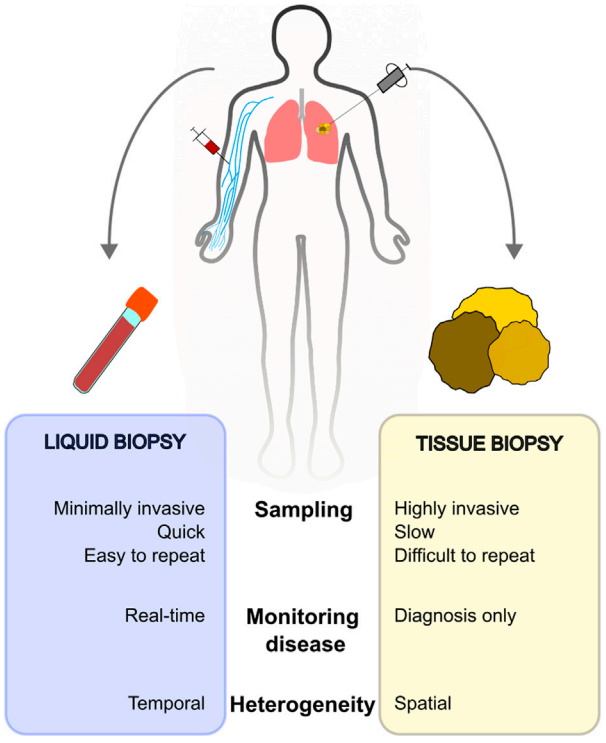

FIGURE 4
Comparison of liquid biopsy and tissue biopsy sampling.

Depending on the tumor's position in the chest, biopsies can be performed using bronchoscopy, mediastinoscopy, endobronchial ultrasound (EBUS), transthoracic needle aspiration, or thoracoscopy, if required. Obtaining biopsy samples of distant metastases is often recommended as it not only aids in diagnosing the tumor but also confirms the advanced stage of the disease [112].

Nonetheless, obtaining a tissue biopsy involves invasive methods and is not always feasible or repeatable. Moreover, the quality and quantity of the samples are frequently inadequate [116]. This underscores the necessity for investigating new diagnostic techniques.

Presently, new methods are emerging that address the limitations of traditional biopsies, such as liquid biopsy. Liquid biopsy involves analyzing biomarkers present in non-solid biological tissues, mainly blood. This technique offers significant benefits compared to conventional methods (Figure 4). The most extensively researched non-invasive cancer biomarkers include CTCs [117, 118], circulating tumor DNA (ctDNA) [119, 120], and circulating cell-free DNA (cfDNA) [96, 121, 122]. These circulating biomarkers are crucial for early cancer detection and can help determine the tissue of origin and prognosis. Additionally, they are useful in monitoring treatment responses, assessing potential resistance to therapies, and detecting minimal residual disease.

Although CTCs and ctDNAs often provide a more precise indication of tumor burden, the concentration of cfDNA still holds relevance in cancer management. Measuring total cfDNA concentration is more cost-effective than analyzing ctDNA or CTCs, which necessitate the use of expensive assays [123, 124]. While cfDNA can be increased in healthy patients for various reasons, ctDNA detection is more specific to tumors. Mutations identified in ctDNA samples are highly similar to those identified in the matched tumor tissues [125].

Due to the rapid growth and highly metastatic capacity of SCLC tumors, ctDNA levels can be valuable markers. Among others, TP53 and RB1 alterations play an important role in SCLC tumorigenesis, and can be used for monitoring of relapse through ctDNA sequencing [121, 125–127]. Fernandez-Cuesta et al. studied the possibility of detecting TP53 mutations from ctDNA. They were able to detect TP53 mutations in 35.7% of early-stage SCLC patients and 54.1% of late-stage SCLC patients [128]. Herbreteau et al. extracted circulating DNA from plasma and detected mutations in the TP53, RB1, NOTCH1, NOTCH2 and NOTCH3 genes using targeted next-generation sequencing [126]. Circulating tumor DNA was detectable if at least one somatic mutation was identified. Overall, mutations in TP53, RB1, and NOTCH1–3 genes were identified in 49 of 68 patients (70.6%), where the most frequently identified mutations affected TP53 (32/49; 65.3%) and RB1 (25/49; 51.0%) genes. Interestingly, almost a quarter of the patients harbored at least one mutation in one of the NOTCH genes (12/49; 24.5%), consistent with results seen in tumor samples [7].

In order to understand the subclonal architecture of SCLC, Nong et al. analyzed the cfDNA samples of 22 SCLC patients before and at different points in therapy using a panel of 430 genes [125]. All patients had a somatic mutation at baseline, the most common being the TP53 mutation, which was observed in 91% (20/22) of patients, and the RB1 mutation, which was observed in 64% (14/22) of patients. Overall, over 90% of patients had mutations in TP53, RB1, or both genes, and 27.3% had NOTCH1–3 mutations. In addition, plasma and tissue samples from eight patients were analyzed, showing a 94% concordance for mutations, indicating that cfDNA sequencing is a sensitive tool for detecting somatic mutations in SCLC patients. Despite the high concordance in the patient cohort, in one case none of the 26 mutations detected in tumor tissue were found the matched cfDNA sample. Also, two of the discordant cases became positive after increasing the sequencing depth. Importantly, in some patients a subset of mutations was detected exclusively in cfDNA, which may be a cause of tumor heterogeneity. Overall, a similar subclonal architecture was revealed between tissue and cfDNA, supporting the use of cfDNA to detect somatic mutations and study molecular heterogeneity in SCLC.

Serial plasma samples from 27 SCLC patients were analyzed by Almodovar et al., where disease-related mutations were detected in 85% of patients. TP53 and RB1 were the most frequently altered genes, and 10 additional genes (PTEN, NOTCH1–4, MYC, MYCL1, PIK3CA, KIT and BRAF) were detected in 52% of patients. In nine patients, cfDNA changes preceded radiological evidence of relapse [127]. Consistent with other studies, ctDNA monitoring has also been shown to identify disease recurrence prior to disease progression seen on imaging or in cases where imaging is equivocal [121, 122, 127]. Similar results were found in other studies, where cfDNA levels were found to be associated with disease outcome, as patients with high levels had a worse prognosis [129].

Conclusion

The fight against SCLC has been a path filled with both obstacles and progress. The disease's rapid spread, limited treatment choices, and the typically brief survival periods of patients have highlighted the urgent need for ongoing improvement and innovation in treatment methods and diagnostics. Our growing knowledge of SCLC is being fueled by the use of cell lines, patient-derived organoids, and mouse models, coupled with the rise of multi-omics studies and cutting-edge computational techniques. These help us better understand genetic and epigenetic changes that regulate SCLC, which may be exploited as potential therapeutic vulnerabilities. In addition, the field of diagnostics has undergone significant transformation. The limitations of traditional, more invasive biopsy methods and the scarcity of surgical specimens have given rise to advanced techniques such as liquid biopsies. These modern approaches, which analyze biomarkers like circulating tumor cells, circulating tumor DNA, and circulating cell free DNA, provide a less invasive and more dynamic perspective on the genetic makeup of the tumor and its response to treatments. As this journey progresses, each new breakthrough offers renewed hope and enhances our understanding of this complex and formidable disease.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. All authors contributed in writing the manuscript and preparing the figures.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The role of chemoradiotherapy and immunotherapy in stage III NSCLC

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Locally advanced non-small lung cancer encompasses a diverse range of tumors. In the last few years, the treatment of stage III unresectable non-small lung cancer has evolved significantly. The PACIFIC trial opened a new therapeutic era in the treatment of locally advanced NSCLC, establishing durvalumab consolidation therapy as the new standard of care worldwide. A careful evaluation of this type of lung cancer and a discussion of the management of these patients within a multidisciplinary team represents a crucial step in defining the best treatment strategy for each patient. For unresectable stage III NSCLC, definitive concurrent chemoradiotherapy (CCRT) was historically recommended as a treatment with a 5-year survival rate ranging from 20% to 30%. The PACIFIC study conducted in 2017 compared the use of chemoradiotherapy and maintenance therapy with the anti-PD-L1 monoclonal antibody durvalumab to a placebo in patients with locally advanced NSCLC who had not experienced disease progression. The study was prospective, randomized, and phase III. The administration of this medication in patients with locally advanced non-small cell lung cancer (NSCLC) has demonstrated a notable improvement in overall survival. Multiple clinical trials are currently exploring various immune checkpoint inhibition regimens to enhance the treatment efficacy in patients with stage III cancer. Our goal is to offer an up-to-date summary of the planned clinical trials for treatment options, focusing on the significant obstacles and prospects in the post-PACIFIC era.

KEYWORDS

locally advanced, NSCLC, chemoradiotherapy, immunotherapy, durvalumab

Abbreviations: ICIs, Immune checkpoint inhibitors; RT, Radiotherapy; LA-NSCLC, locally advanced non-small cell lung cancer; NSCLC, Non-small cell lung cancer; LA, Locally Advanced; SoC, Standard of care; OS, Overall survival; ORR, Objective Response Rate; TME, tumor microenvironment; PD-1, Programmed cell death 1; PD-L1, Programmed cell death-ligand 1; CTLA-4, Cytotoxic T-lymphocyte associated antigen 4; PFS, Progression-free survival; CRT, Chemoradiotherapy; AEs, Adverse events; PR, Partial response.

Introduction

Many diseases became more common as a result of the altered smoking habits that emerged in the first half of the 20th century and the air pollution that was seen to coincide with industrial development. Lung cancer is currently the most common cause of cancer-related mortality in developed nations [1]. The 5-year survival rates are 15%–40% in stage IIIA and 5%–10% in stage IIIB lung cancer, respectively [2]. Three subgroups exist for locally advanced stage III non-small-cell lung cancer (NSCLC) according to the 8th edition of the TNM classification: stage IIIA, IIIB, and IIIC tumors [2, 3]. However, in clinical practice, we can also identify stage lung cancer that is resectable, potentially resectable, and unresectable [4]. Lung cancer requires meticulous examination and staging [3]. The imaging and diagnostic methods include bronchoscopy, endobronchial ultrasound (EBUS), cryobiopsy, liquid biopsy, computed tomography (CT), FDG positron emission tomography (PET), magnetic resonance imaging (MRI), and in some cases it is necessary to perform video-assisted thoracoscopic surgery (VATS) [5]. After cytological or histological confirmation of non-small cell lung cancer, especially of adenocarcinomas, it is essential to determine the status of EGFR, ALK, and PDL1 is essential [5]. Therapy of stage III non-small cell lung cancer (NSCLC) needs a multimodal approach that involves chemotherapy, radiotherapy, surgical treatment, and in special cases targeted therapy and immunotherapy [2, 3].

Tumor location, volume, histology, immunohistochemical and molecular pathological features, patient age, performance level, and comorbidities are some of the variables that affect the treatment plan [1–3].

Before 2018, the standard of care therapy of locally advanced non-small cell lung cancer was definitive chemoradiotherapy, that contains platinum-based doublet chemotherapy regimen combined with either concurrent or sequential radiation therapy (RT) [6, 7]. The progression-free survival (PFS) was 8 months, and the 5-year survival was less than 20% in these patient population [8, 9].

A phase III clinical trial conducted in 2002 by Albain et al. showed that concomitant cisplatin, etoposide chemotherapy, and thoracic radiation improved the median overall survival (OS) to 15 months, with 3- and 5-year survival rates of 17% and 15%, respectively [10].

Subsequently, Govindan et al. reported that combination chemoradiotherapy using pemetrexed and carboplatin demonstrated fewer adverse effects while still demonstrating the same efficacy as cisplatin and etoposide [11].

Chemoradiotherapy and immunotherapy for stage III unresectable NSCLC

The introduction of immune checkpoint inhibitors (ICI) opened a new era in the treatment of lung cancer 8 years ago [8, 12]. Previous studies showed the efficacy of immunotherapy after definitive chemoradiotherapy for stage III unresectable NSCLC. CRT is administered to reduce local recurrence and prevent the onset of distant metastases and is administered during or after the completion of chemoradiotherapy [12, 13].

The efficacy of durvalumab as maintenance therapy and the completion of concurrent chemoradiotherapy in locally advanced non-small cell lung cancer was assessed in 2017 by the phase III PACIFIC trial [12]. In the PACIFIC study, durvalumab (a human IgG1 monoclonal antibody against PD-L1) was added to concurrent chemoradiotherapy for stage III non-small cell lung cancer after a year of maintenance. 713 patients with stage III (TNM 7th edition) who had not received prior anticancer treatment were enrolled in the PACIFIC trial and were randomized to receive durvalumab or placebo beginning 1–42 days after combination chemoradiotherapy [5]. Concurrent radiotherapy (54–66 Gy) and platinum doublet chemotherapy (cisplatin or carboplatin plus paclitaxel, docetaxel, vinblastine, vinorelbine, etoposide, or pemetrexed) were administered to the patients [12]. Patients who did not progress after chemoradiotherapy were randomly assigned to receive durvalumab or placebo [12, 14]. Durvalumab was administered intravenously every 2 weeks at a dose of 10 mg/kg [12]. Durvalumab maintenance therapy for a year resulted in increases in overall survival (47.5 vs. 42.9 months) and progression-free survival (PFS; 11.2 vs. 10.9 months), with about one-third of patients continuing to live without distant metastases or local recurrence. Better 5-year OS (42.9% vs. 33.4%) and median OS (47.5 months vs. 29.1 months) were reported in the updated analysis for 2022 [13, 15].

In addition, patients treated with consolidation durvalumab had a longer median time to death or distant metastases (28.3 months against 16.2 months) and a lower incidence of brain metastases (6.3% vs. 11.8%) compared to placebo [13, 14, 16]. The efficacy of durvalumab in combination with concurrent or sequential radiation therapy in patients engaged in an early access program is being investigated in real-world data by an international retrospective trial known as PACIFIC-R [17]. Compared to PD-L1 negative patients, PD-L1 positive patients had a longer PFS (22.4 vs. 15.6 months) [13]. concurrent chemoradiotherapy with durvalumab is the current standard of care [5, 12, 18].

The optimal sequence of chemoradiotherapy and immunotherapy

Sequential radiochemotherapy is less effective than concurrent platinum-based chemoradiotherapy (CRT) [6, 10, 19, 20]. However, the patient's age, overall health, comorbidities, financial situation, and logistical challenges all affect the availability of competing radiation therapies [20].

The timing of immunotherapy is under investigation. Sequential immunotherapy is preferable because it has fewer side effects, can enhance treatment outcomes, and prevents resistance to immunotherapy through the immune system's interaction with radiation [12]. According to the PACIFIC study's subgroup analysis, patients who started their ICI earlier, in 30 days had a greater OS rate at 30 months compared to patients who received durvalumab after 1 months following chemoradiotherapy (90% vs. 44%) [7, 10].

In the LUN 14-179 [21], GEMSTONE-301 [22, 23], PACIFIC-6 [24], DETERRED [25, 26], and KEYNOTE 799 [26, 27] trials, immunotherapy was administered as a maintenance therapy after concurrent chemoradiotherapy.

In phase II DETERRED trial, chemoradiotherapy with concurrent and consolidative atezolizumab led to an efficacy similar to consolidative durvalumab in the PACIFIC trial [25, 26]. The patients received in part 1 chemoradiotherapy and consolidation of atezolizumab treatment, in part 2 they took concurrent maintenance atezolizumab. An updated analysis of this trial showed that the median progression-free survival for concurrent vs sequential atezolizumab was 15.1 vs. 18.9 months [25, 28].

The phase III KEYNOTE 799 study is similar to the PACIFIC trial. The KEYNOTE 799 trial evaluated the safety and efficacy of pembrolizumab and concurrent chemoradiotherapy in stage III non-small cell lung cancer. KEYNOTE 799 demonstrated an increase in ORR and an accelerated effect in establishing the antitumor immune response [27].

Phase III GEMSTONE 301 compared the efficacy of sugemalimab after chemoradiotherapy in patients with stage III unresectable driver mutation negative stage III NSCLC in China [22, 23]. PFS was significantly longer with sugemalimab than with placebo (median 10.5 vs. 6.2 months), but OS in the sugemalimab and placebo groups was inconclusive [29].

Phase II trial LUN 14–179 evaluates the efficacy and safety of pembrolizumab as consolidation therapy after concurrent chemoradiotherapy [21]. Consolidation with pembrolizumab after chemoradiotherapy prolonged PFS and OS compared to chemoradiotherapy (CRT) alone and did not increase the rates of grade 3–5 pneumonitis [21].

The single-arm phase II NICOLAS trial demonstrated the safety of nivolumab combined with radiation therapy in stage IIIA and IIIB disease. Significant overall survival differences were observed between patients with stage IIIA vs IIIB disease (2-year OS 81% vs. 56%) [30].

Novel agents

The ongoing phase III PACIFIC-8 study investigates the efficacy and safety of domvanalimab and durvalumab compared to durvalumab. The phase III PACIFIC-9 trial compares durvalumab + chemotherapy treatment with the combination of oleclumab (CD73 inhibitor) or added monalizumab (NKG2 inhibitor). The SKYSCRAPER-03 study investigates consolidation therapy with atezolizumab and the TIGIT inhibitor tiragolumab after chemoradiotherapy. CHORUS is a phase III study that compares canakinumab combined with chemoradiotherapy and durvalumab. The KEYVIBE-006 study evaluated pembrolizumab/vibostolimab (TIGIT inhibitor) in combination with concurrent chemoradiotherapy followed by pembrolizumab/vibostolimab versus cCRT followed by durvalumab. The KEYLYNK-012 study assesses pembrolizumab in combination with concurrent chemoradiotherapy followed by pembrolizumab with olaparib placebo or olaparib compared to concurrent chemoradiotherapy followed by durvalumab.

Next step: immunoradiotherapy, treatment without chemotherapy

In an attempt to avoid overtreating patients while dealing with the side effects of chemotherapy, a novel strategy known as radiation therapy with immunotherapy has been developed. The ongoing SPRINT study aims to evaluate the efficacy of a shortened 4-week radiation therapy course for high-grade PD-L1 patients with locally advanced non-small cell lung disease [31, 32]. The DUART trial, a phase II single-arm study, was finished to evaluate durvalumab's clinical efficacy in patients with non-small cell lung cancer (NSCLC) that is incurable and not amenable to treatment [31]. In the finished PARTICLE-D trial, durvalumab, the study drug, and proton beam therapy were combined [32]. The active phase I NRG-LU004 trial examines durvalumab in combination with conventionally fractionated radiation therapy or accelerated hypofractionated radiation therapy (ACRT) in patients with locally advanced non-small cell lung cancer. It also investigate the safety of combining durvalumab with conventional radiation therapy in addition to monalizumab or oleclumab [33]. CHECKMATE 73L is a trial that is currently in progress. The main objective of the study is to examine the effectiveness of nivolumab plus concurrent chemoradiotherapy (CCRT) followed by nivolumab plus ipilimumab against CCRT followed by durvalumab in patients with untreated stage III unresectable non-small cell lung cancer [34].

Driver mutations and advanced non-small lung cancer treatment

The therapy of non-small cell lung cancer with driver mutations in stage III disease raises many questions [35]. Targeted therapies especially tyrosine kinase inhibitors are

evaluated in unresectable stage III NSCLC with actionable genomic alterations.

Unfortunately, there is no optimal treatment strategy for patients with driver mutation in locally advanced NSCLC. The PACIFIC trial enrolled patients with the EGFR mutation. Antonia et al. presented their exploratory subgroup analysis, showing that PFS and OS with durvalumab were similar to placebo in patients with EGFR mutation [15, 36, 37]. Hellyer et al. found that patients with LA- NSCLC mutations of the human epidermal growth factor receptor 2 (HER2) or EGFR had shorter PFS than those with wild-type genes (7.5 months vs. not reached) [35, 38]. We must use these data with caution due to the small number and characteristics of the patients (male, smokers, squamous cell carcinoma). The safety profile was the same in the durvalumab arm as in the overall population [15, 30]. The DETERRED trial examined individuals with specific oncogene mutations that can be targeted, which resulted in a poorer progression-free survival (PFS) [25]. The LAURA (phase III) trial enrolled patients with locally advanced, unresectable epidermal growth factor receptor mutation-positive stage III NSCLC. Patients received at least two cycles of concurrent/sequential platinum-based cCRT and were randomly assigned 2:1–80 mg or placebo once a day [39]. Currently, the effectiveness of osimertinib treatment is under investigation. The latest consensus of ESMO guideline does not recommend using ICI consolidation therapy after curative intention chemoradiotherapy in EGFR-positive NSCLC [3, 5, 40]. Patients with a KRAS mutation profit from durvalumab maintenance therapy [38, 41, 42].

Toxicity and safety

Immunotherapy can affect multiple organ systems, 50% of patients treated with ICI are estimated to experience some form of irAE. AE of any grade of those who receive ICI treatment is fatigue, gastrointestinal (colitis, diarrhoea, abdominal pain, hepatitis), endocrine (alteration of thyroid function, hypocalcemia), myocarditis, renal, peripheral neuropathy, and dermatological side effects (i.e., rash). Respiratory complications, pneumonitis, and respiratory failure are the most common cause of immune-related deaths [12–14, 43–47].

Treatment-related pneumonitis with immune checkpoint inhibitors is a challenging side effect. The spectrum of symptoms moves on a wide range from the asymptomatic case to acute respiratory failure [48]. The diagnosis of ICI pneumonitis confirms the symptoms (developing dry cough, dyspnea, and other respiratory symptoms), laboratory test (inflammatory markers, i.e., neutrophil lymphocyte ratio), lung function test, microbial culture, the presence of new infiltrates on chest imaging without new infections [14, 43, 49]. Chest CT should be combined with other diagnostic tools. However, differential diagnosis is complicated and it is difficult to distinguish between radiotherapy-related pneumonitis, new-onset interstitial lung disease, tumour progression, or pulmonary infection (i.e., Covid infection) [48, 49].

Treatment-related adverse events of grade 3–4 were 29.9% in the PACIFIC trial. The most common AE was pneumonitis, with an incidence of 4.4%, and 15.4% of these patients discontinued durvalumab because of AEs [13, 14, 50].

With respect to adverse events, pneumonitis was reported to be more severe in patients who received durvalumab. However, grade 3 or 4 pneumonitis was similar in both groups: 1.9% in the durvalumab arm and 1.7% in the control group. Furthermore, radiation-related pneumonitis contributed to the discontinuation of durvalumab in 1.3% of patients, as in the placebo arm [12–15].

The novel combinations of immunochemoradiotherapy show similar data compared to those of the PACIFIC trial. In the GEMSTONE-301 trial, grade 3 or 4 pneumonitis occurred in 3% of patients in the sugemalimab group compared to 6% in the placebo group [22]. The grade 2 or higher pneumonitis rate was around 10% in the DETERRED trial [25].

Treatment of immune-related pneumonitis depends on the severity of the disease [51]. Grade 1 pneumonitis does not need special treatment. Grade 2 pneumonitis requires ICI withdrawal therapy and administration of corticosteroid treatment administration. Grade 3 and 4 pneumonitis requires discontinuation of immunotherapy, corticosteroid treatment, hospitalization, empiric broad-spectrum antibiotic therapy, oxygen administration, and mechanical ventilation in severe cases. Guidelines recommend other immunosuppressive agents: TNFα inhibitor - infliximab, intravenous immunoglobulins, and mycophenolat mofetil [51].

Progression during and after ICI consolidation therapy

Regular chest CT or PET CT controls the efficacy of disease treatment and follow-up. PET-CT scan is recommended to evaluate tumor metabolic activity if it suggests progression of the disease [12]. Changes in F-18 fluorodeoxyglucose uptake occur commonly between 8 and 12 weeks after radiation therapy on PET CT [52]. Furthermore, several conditions (such as atelectasis, consolidation, infection, granulomatous pulmonary disease, and radiation fibrosis) are challenging to differentiate from neoplasm because areas previously treated with radiation therapy can remain avid 18F-FDG for up to 2 years.

More than half patients with stage III disease will progress within 2 years of the start of treatment. Updated data from the PACIFIC trial showed that 49.0% of patients completed 12 months of durvalumab therapy, and 31.3% discontinued due to disease progression [13]. In the PACIFIC trial, 7.1% of the patients in the ICI arm received durvalumab retreatment and

completed the first year of consolidation immunotherapy, and the median time to second progression was 48 months [13, 24].

We must distinguish between oligo- and systemic progression. If primary resistance is confirmed (progression observed during durvalumab treatment), therapeutic strategies depend on the type of progression. Oligometastasis is treatable (e.g., bone, brain) with surgery or stereotaxic ablation; a tight follow-up is recommended. In systemic progression, the rechallenge of immunotherapy is doubtful and participation in clinical trials, including a PDL-1 inhibitor, can be recommended. Delasos et al. in their retrospective examination investigated pembrolizumab treatment after chemoradiotherapy and maintenance immunotherapy (durvalumab) [53]. Patients with refractory or recurrent NSCLC require more attention, their survival is worse (median OS 10.6 months, median PFS 6.1 months) than patients with metastatic NSCLC diagnosed *de novo* and treated with chemotherapy (median OS 12.9 months) [53].

Conclusion

Treatments for locally advanced, incurable NSCLC have been evolving quickly in the field of lung cancer therapy in recent years. When treating unresectable stage III non-small lung cancer, chemotherapy plus immunotherapy have demonstrated a synergistic effect on both local and distant tumor control. The current therapeutic recommendations for unresectable stage III NSCLC include chemotherapy and 1 year of ICI consolidation therapy; however, there are a number of unanswered problems and potential techniques. We are currently awaiting the results to establish the best time to administer chemotherapy, radiation, and ICI as well as the function of targeted therapy. One major clinical barrier to improving the prognosis of patients with advanced lung cancer is resistance to immune checkpoint inhibitors.

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ZO and AK participated in the design and review of the manuscript. ZO wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Targeted therapeutic options in early and metastatic NSCLC-overview

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The complex therapeutic strategy of non-small cell lung cancer (NSCLC) has changed significantly in recent years. Disease-free survival increased significantly with immunotherapy and chemotherapy registered in perioperative treatments, as well as adjuvant registered immunotherapy and targeted therapy (osimertinib) in case of EGFR mutation. In oncogenic-addictive metastatic NSCLC, primarily in adenocarcinoma, the range of targeted therapies is expanding, with which the expected overall survival increases significantly, measured in years. By 2021, the FDA and EMA have approved targeted agents to inhibit EGFR activating mutations, T790 M resistance mutation, BRAF V600E mutation, ALK, ROS1, NTRK and RET fusion. In 2022, the range of authorized target therapies was expanded. With therapies that inhibit KRASG12C, EGFR exon 20, HER2 and MET. Until now, there was no registered targeted therapy for the KRAS mutations, which affect 30% of adenocarcinomas. Thus, the greatest expectation surrounded the inhibition of the KRAS G12C mutation, which occurs in ~15% of NSCLC, mainly in smokers and is characterized by a poor prognosis. Sotorasib and adagrasib are approved as second-line agents after at least one prior course of chemotherapy and/or immunotherapy. Adagrasib in first-line combination with pembrolizumab immunotherapy proved more beneficial, especially in patients with high expression of PD-L1. In EGFR exon 20 insertion mutation of lung adenocarcinoma, amivantanab was registered for progression after platinum-based chemotherapy. Lung adenocarcinoma carries an EGFR exon 20, HER2 insertion mutation in 2%, for which the first targeted therapy is trastuzumab deruxtecan, in patients already treated with platinum-based chemotherapy. Two orally administered selective c-MET inhibitors, capmatinib and tepotinib, were also approved after chemotherapy in adenocarcinoma carrying MET exon 14 skipping mutations of about 3%. Incorporating reflex testing with next-generation sequencing (NGS) expands personalized therapies by identifying guideline-recommended molecular alterations.

KEYWORDS

NSCLC, driver oncogenes, targeted therapy, central nervous system efficacy, molecular testing

Introduction

In recent years, there has been an expansion in the treatment options of non-small cell lung cancer (NSCLC) with the advent of targeted therapies and immuno-oncology therapies [1, 2]. Lung tumors are heterogeneous, with distinct oncogenic drivers and tumor microenvironments. Tumor evolution results in distinct organ site metastases representing intratumor heterogeneity and the ongoing development of resistance mutations [3]. Recent advancements in cost-effective parallel high-throughput molecular diagnostics might drive personalized therapy beyond adenocarcinoma subtypes associated with the most targetable genetic alterations [3]. Prior to precision medicine, patients were treated uniformly without considering the differences in clinicopathological characteristics and genetic backgrounds of different patients. The careful selection for upfront treatments of brain metastases (BM) might be detrimental to outcomes [1, 2]. Now, it is clear that patients with oncogenic driver alteration-positive NSCLC are associated with better outcomes treated with frontline targeted therapy compared to chemotherapy and anti-Programmed death (anti-PD) immunotherapy [1, 2]. Today, the right choice of molecular diagnostics is increasingly important, following the careful classification of pathological diagnostics. While stand-alone single gene assays remain valid, increasing requirements for synchronous testing for multiple targets make massive parallel sequencing technology the preferred option [2]. DNA sequencing is the standard for mutation detection, and RNA sequencing is an emerging option for fusion gene detection. EGFR, ALK, and ROS1 alterations are common for young female non-smokers with adenocarcinoma [4]. While ALK and ROS1 mutations do not show ethnic prevalence, one can observe a four times elevated EGFR prevalence in the Asian population compared to the Western population [4]. KRAS and MET mutations occur in older age populations with smoker status and adenocarcinoma. There is no clear gender preference, but a distinct excess of KRAS mutations in the Caucasian population is observable. It is widely accepted that KRAS mutation in lung cancer is smoking-associated, but it is only proven for G12C [5, 6]. BRAF mutations occur in smokers without age or ethical tendencies. The BRAF V600 mutations are detected with a higher incidence for females; other BRAF mutations have a higher incidence for the opposite sex. HER2 mutations have a higher likelihood for female and never-smoker patients [4]. In contrast to the known associations of genetic alteration with clinicopathological characteristics, according to the latest guidelines, molecular testing is now the standard for advanced-stage adenoma-carcinoma-containing cancers independent of sex, ethnicity, or smoking [2]. Therefore, histology assessments are key in clinical practice, with a cautious interpretation of mixed histology specimens and NSCLC not otherwise specified (NSCLC NOS) because molecular testing is recommended for non-squamous NSCLC cases. Testing is also recommended for NSCLC cases below 50 years of age and all kinds of tobacco in patients who quit smoking more than 15 years ago, or in never (<100 cigarettes overall) or former light (≤15 6 pack-years) or long-time ex-smokers (quit >15 years ago). Importantly, the presence of any adenocarcinoma component in a biopsy specimen that is otherwise squamous should trigger molecular testing. Accordingly, cautious minimization of tissue slides used for immunohistochemistry (IHC) stainings and preserving material for molecular testing is critical. Oncogenic driver tests usually follow the Programmed death-ligand 1 (PD-L1) IHC testing for non-squamous cases. The present review summarizes the available data on targeted therapy strategies in treating NSCLC (Figure 1). A particular focus is given to central nervous system (CNS) activity that is detrimental in the era of better control of oligoprogressive disease. Additionally, for optimal treatment outcomes, we highlight the role of distinct molecular analyses based on accurate guideline-based histology classifications to avoid excluding patients from therapy. Nevertheless, the emergence of early-stage targeted therapies extends molecular testing beyond advanced-stage disease.

Adjuvant osimertinib therapy in the treatment of NSCLC

As adjuvant treatment in NSCLC, osimertinib is the first targeted therapy approved based on the ADAURA trial. The ADAURA trial enrolled stage I/B -III/A patients with classical epidermal EGFR mutations (ex19del/L858R) who underwent complete tumor resection [7]. Patients were allowed to receive adjuvant chemotherapy before osimertinib, and optionally, they were allowed to start osimertinib therapy after surgery. According to the 1:1 randomization, the study group received osimertinib, and the other group received a placebo at a planned interval of 3 years. The primary endpoint was disease-free survival (DFS) in stage II/IIIA patients. DFS in the osimertinib group compared to placebo showed a significant benefit (hazard ratio (HR): 0.23, 95% CI 0.18–0.30) median DFS was 65.8 months for the osimertinib compared to 21.9 months for the placebo arm [8]. Subgroup analysis (gender, age, race, stage, mutation type) revealed significant benefits in DFS. Adjuvant osimertinib also showed efficacy without cytotoxic chemotherapy, with a significant benefit in DFS (HR: 0.23), and osimertinib given followed by adjuvant chemotherapy showed a similar significant benefit in DFS (HR: 0.16) [8].

NSCLC with actionable EGFR mutations

The HER/Erb family epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase (TK) receptor

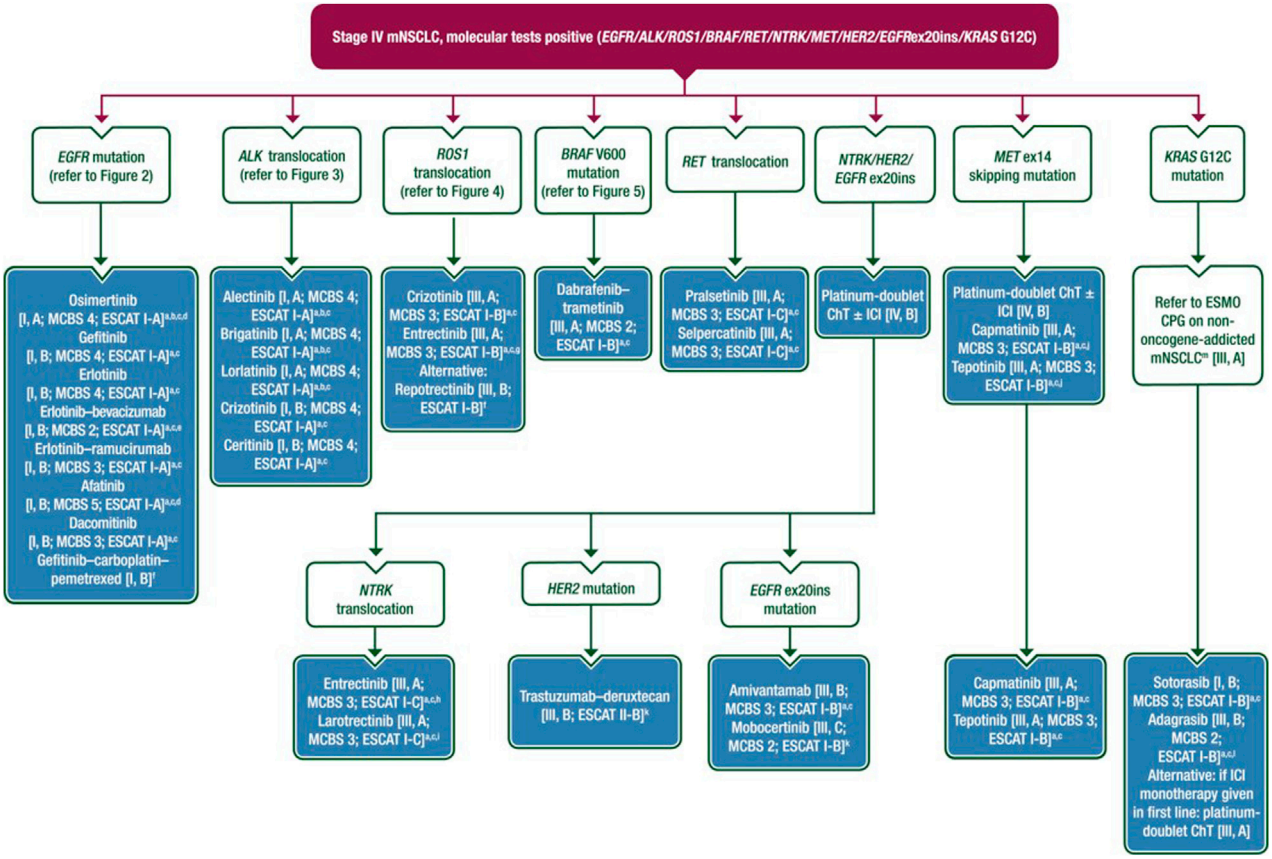

FIGURE 1
Treatment recommendation for stage IV mNSCLC based on the ESMO 2023 guideline [1].

that stimulates cell proliferation, differentiation, survival, and motility through MAP kinase and PI3K signaling pathways [9]. Overexpression and increased activity of EGFR tyrosine kinase in non-small cell lung tumors were first described in 2004, which may result from mutation, deletion, or amplification of the tyrosine kinase coding region [10].

The mutation most commonly affects exons 18–21 and occurs in 10%–20% of the Caucasian population, predominantly in young, never-smoker women. In almost 90% of cases, a so-called "classical mutation" is encountered, a deletion of exon 19 or a point mutation of exon 21 (L858R). The exon 20 insertion is the third most common EGFR mutation, accounting for 4%–12% of all EGFR mutations, it is more common in women, non-smokers, and Asians and is associated with a worse prognosis. In addition to the exon 20 insertion mutation, the most common rare mutations include exon 18 G719X, exon 20 S768I and exon 21 L861Q, which occur in 1%–3% of cases and smoking history [11].

In locally advanced or metastatic non-small cell lung cancer, EGFR tyrosine kinase inhibitor (TKI) treatment is the recommended first-line therapy with confirmed actionable EGFR mutations. EGFR tyrosine kinase inhibitors are classified into three generations based on their appearance in chronological order. The first-generation includes the reversible binding agents gefitinib and erlotinib, the second-generation includes the irreversible ErbB/HER2 inhibitor afatinib and the EGFR/pan-HER inhibitor dacomitinib, while the third-generation is osimertinib. Since then, several studies have demonstrated the benefit of EGFR tyrosine kinase inhibitors in terms of tumor response, safety, quality of life, and progression-free survival (PFS) compared with conventional chemotherapy regimens [12].

First-generation reversible EGFR inhibitors

Pioneering in the treatment of adenocarcinoma patients, gefitinib, in its pivotal phase 3 IPASS trial, showed a significant benefit in progression-free survival [median PFS (mPFS) gefitinib 9.5 months vs. chemotherapy 6.3 months; HR 0.48, 95% confidence interval (CI) 0.36–0.64 $p < 0.001$] and

tumor response [objective response rate (ORR) 71.2% v 47.3%, $p = 0.0001$], with good quality of life maintained [13].

The efficacy of erlotinib was analyzed in the OPTIMAL trial in Asia and the EURTAC trials in Europe [14, 15]. A significant difference was demonstrated in favor of erlotinib in terms of overall survival (OS) and tumor response compared to the standard platinum-based chemotherapy regimens (EURTAC mPFS erlotinib 9.7 months vs. chemotherapy 5.2 months; $p < 0.0001$; ORR 64% vs. 18%; $p < 0.0001$) (OPTIMAL mPFS erlotinib 13.1 months vs. chemotherapy 4.6 months; $p < 0.0001$; ORR 83% vs. 36%; $p < 0.0001$) (5,6). Although no benefit in overall survival was demonstrated, following these trials, both products were registered in the first-line setting for treating EGFR mutation-positive stage IIIB/IV non-small cell lung cancer.

It is now well known that the first-generation drugs are most commonly associated with skin side effects (rash, xeroderma, pruritus, and paronychia), diarrhea, fatigue, and elevation of liver function, typically AST/ALT. The most common cutaneous side effects are acneiform rash, dry skin, itching, and nail bed lesions, which are well controlled by topical or systemic antibiotic treatment (doxycycline). Diarrhea can be reduced by per os medication (appropriate dose of loperamide), with drug dose reduction if necessary. Once the side effects are resolved, the original dose is often restored. Compared with chemotherapy regimens, first-generation regimens have shown a much better side-effect profile and fewer serious (grade 3–4) adverse events [13, 15–18].

A vascular endothelial growth factor (VEGF) inhibitor, Ramucirumab plus erlotinib, showed increased PFS compared to placebo plus erlotinib arm in patients with untreated EGFR-mutated metastatic NSCLC (mNSCLC) [19]. Safety was consistent with the safety profiles of the individual compounds in advanced lung cancer.

Second-generation EGFR inhibitors

The second-generation EGFR tyrosine kinase inhibitors (afatinib and dacomitinib) are more potent EGFR and HER2 inhibitors, forming irreversible binding. The efficacy of afatinib was analyzed in LUX-lung studies. In the phase 2 LUX-lung 3 and LUX-lung 6 trials, afatinib showed significantly better tumor response and progression-free survival than platinum-based chemotherapy combinations (LUX-lung 3, mPFS for afatinib vs. chemotherapy, 13.6 months vs. 6.9 months; respectively, $p = 0.0004$; ORR, 56% vs. 23%; $p = 0.001$; respectively; LUX-lung 6, mPFS for afatinib vs. chemotherapy 11.0 months vs. 5.6 months; $p < 0.0001$; respectively, ORR 66.9% vs. 23%; $p < 0.0001$). [20, 21]. It should be noted that afatinib did not provide a benefit in OS in the overall patient group; however, in subgroup analyses, targeted therapy in patients with exon 19 deletion showed a significant benefit in overall survival (LUX-lung 3, OS for afatinib vs. chemotherapy 33.3 months 21.1 months respectively; LUX-lung 6, OS for afatinib vs. chemotherapy 31.4 months vs. 18.4 months, respectively). This benefit was not confirmed for point mutations [22]. Based on these studies, afatinib was registered as a first-line treatment for non-small cell lung tumors carrying EGFR mutations.

The LUX-lung 7 phase 2 trial comparing first- and second-generation EGFR tyrosine kinase inhibitors compared afatinib with gefitinib, where afatinib was shown to be superior in terms of progression-free survival (mPFS for afatinib vs. gefitinib, 11.0 months vs. 10.9 months; respectively, $p = 0.017$), however, the study did not show a significant benefit in terms of overall survival and more toxicity leading to dose reduction was observed when using the second-generation drugs [23]. A higher rate of G3-severe skin rash (9.4% vs. 3.1%) and G3 diarrhea (12.5% vs. 1.3%) was also observed in the afatinib group. Dacomtinib, also a second-generation irreversible EGFR and panHER inhibitor, was superior to gefitinib in first-line use in the ARCHER1050 phase 3 trial in terms of both PFS and OS [PFS for dacomitinib vs. gefitinib, 14.7 months vs. 9.2 months, respectively, $p < 0.0001$; median OS (mOS) dacomitinib vs. gefitinib, 34.1 months vs. 26.8 months, respectively], but again a less favorable side effect profile was observed with dacomitinib [24, 25].

Third-generation EGFR inhibitors and T790 resistance mutation

Acquired resistance to first- and second-generation drugs develops after 9–13 months, with a T790 resistance mutation affecting exon 20 being confirmed in 50%–60% of cases [26]. This has led to the development of third-generation therapies [26]. Osimertinib is a third-generation irreversible EGFR TKI that is also effective in the presence of T790 resistance mutations. The efficacy of the first mutation selective TKI was analyzed in AURA trials. In the AURA 3 phase 3 trial, compared with platinum-based chemotherapy, osimertinib showed a significant PFS benefit in T790 resistance mutation-positive, locally advanced or metastatic NSCLC (mPFS osimertinib vs. chemotherapy, 10.1 month vs. 4.4 months, respectively, $p < 0.001$) [27]. This was followed by the phase 3 FLAURA trial, which compared the efficacy of osimertinib with first-generation agents; osimertinib achieved a significant benefit in both progression-free and overall survival, with osimertinib providing an 8.8 months OS benefit, reducing the risk of death by 20% (mOS osimertinib vs. first-generation EGFR inhibitor, 38.6 months vs. 31.8 months; respectively, HR 0.80; 95% CI, 0.6410 .997; $p = 0.046$). The side effect profile was similar, but G3 side effects were less frequent with osimertinib [28]. The most common osimertinib-induced adverse events were acneiform rash, diarrhea, and paronychia, and a small percentage of cardiomyopathy (~1.4%–2.4%), QT prolongation (2.7%), and interstitial lung disease (ILD) (3.3%) were also described [29]. After 3 years of follow-up, 28% of patients received osimertinib, compared with only 9% in the

comparator group. Notably, first-line osimertinib reduced the risk of CNS progression by 52% (HR, 0.48; 95% CI, 0.260.86; $p = 0.014$) and fewer *de novo* CNS metastases were recorded [30]. The importance of these results is demonstrated by the fact that 10%–30% of patients with NSCLC develop CNS metastases, which are associated with a worse prognosis and worse survival. EGFR mutations are present in 40%–60% of non-small cell lung tumors affecting the central nervous system, and the risk of central nervous system metastasis is higher in the presence of EGFR mutations, making the concentration of each drug in cerebrospinal fluid a key determinant. First- and second-generation drugs have limited brain penetration, while osimertinib rapidly crosses the blood-brain barrier (BBB) and reaches higher concentrations. Osimertinib, which has the best efficacy and most favorable side-effect profile of all EGFR inhibitors, is currently the drug for first-line treatment of EGFR mutant NSCLC, particularly in patients with BM [31]. If not available in the first line, first or second-generation agents should be administered, and in case of progression, efforts should be made to confirm T790 resistance mutation from liquid biopsy or repeated histological sampling. In the presence of a T790 resistance mutation, osimertinib is preferred while platinum-based chemotherapy is recommended in case of negative resistance mutation status. With the widespread use of osimertinib, the development of new resistance mutations is expected. Most commonly, mutation of exon 20 C797X, MET amplification, and HER 2 amplification have been described in addition to aberrations of other non-EGFR mediated pathways [1].

Immunotherapy and targeted treatment options

The IMpower 150 phase 3 three-arm trial compared the combination of chemotherapeutic doublet immunotherapy and VEGF inhibitor with VEGF inhibitor-chemotherapeutic doublet and chemo-immunotherapy. EGFR mutant patients were also eligible for inclusion in the study. Subgroup analyses showed that a significant OS benefit was achieved with the combination of four regimens vs. VEGF inhibitor-chemotherapy doublet, regardless of the presence of EGFR mutations (EGFR positive subgroup mOS 26.1 months vs. 20.3 months; respectively), making the combination of four regimens an additional option after exhaustion of targeted therapies [32].

NSCLC harboring a rare EGFR mutation

Approximately 10%–20% of non-small cell lung tumors carrying EGFR mutations carry rare EGFR mutations [33]. While the presence of classical activating mutations is a strong predictor of favorable tumor response to EGFR tyrosine kinase inhibitors, rare EGFR mutations, such as the exon 20 insertion mutation, are heterogeneous, largely resistant to first- and second-generation drugs, and the efficacy of third-generation osimertinib is limited. In recent years, several new products have been developed for this patient group. Mobocertinib is an irreversible EGFR and HER inhibitor targeting exon 20 alterations. In the phase 1/2 EXCLAIM trial, mobocertinib in the multilineage setting resulted in a 32% tumor response and a median progression-free survival of 7.3 months. Based on the phase 1/2 results, mobocertinib received Food and Drug Administration (FDA) approval. Amivantamab is a bispecific monoclonal antibody that prevents tumor growth and progression by blocking EGFR and c-MET pathways and stimulates immune-mediated destruction of EGFR and cMET-expressing cells. In the CHRYSALIS phase 1 study, patients with exon20 insertion mutations received multiple lines of amivantamab treatment, with an mPFS of 8.3 months and an ORR of 40%. Based on this study, the FDA approved amivantamab for the treatment of patients progressing on platinum-based chemotherapy. Although the use of amivantamab and mobocertinib has improved the tumor response and progression-free survival of this poor prognosis patient group, the presence of EGFR exon 20 insertions is still associated with poor prognosis and unfavorable survival, and further studies on drug development are needed [33].

In addition to the exon 20 insertion mutation, the most common rare mutations include exon 18 G719X, exon 20 S768I, and exon 21 L861Q, often in associated with other mutations [2]. Most clinical trials conducted to date have recruited patients with classical EGFR exon 19 deletion and exon 21 point mutations. An exception was the LUX-lung 2,3,6 trial (afatinib vs. chemotherapy), which included patients with rare EGFR mutations. Following a detailed, retrospective analysis of the trials, the benefit of afatinib in this patient group in terms of tumor response and PFS was published, leading to the registration of afatinib for the treatment of NSCLs with rare EGFR mutations. In the FLAURA 3 study comparing osimertinib and first-generation EGFR TKIs, rare EGFR mutation was an exclusion criterion, but in phase 2 Korean study (KCSG-LU15-09), patients with rare mutations showed a better tumor response and progression-free survival with osimertinib treatment, which was confirmed in some small patient studies. First-generation formulations have shown little activity in rare mutations. Although data are scarce, real-world studies to date suggest the use of second or third-generation drugs, afatinib or osimertinib, in the presence of a major EGFR rare mutation. The choice is a clinical decision, which is best made based on the side effects encountered, the patient's general condition, and the products' availability [34].

Summary of EGFR targeted therapy

Treatment recommendation for EGFR mutant metastatic NSCLC based on the ESMO 2023 guideline is shown in Figure 2. In all patients with advanced/metastatic lung tumors, molecular profiling is recommended after histological diagnosis. If an EGFR mutation is confirmed, first-line EGFR tyrosine kinase inhibitor therapy is recommended (I,A). When exon

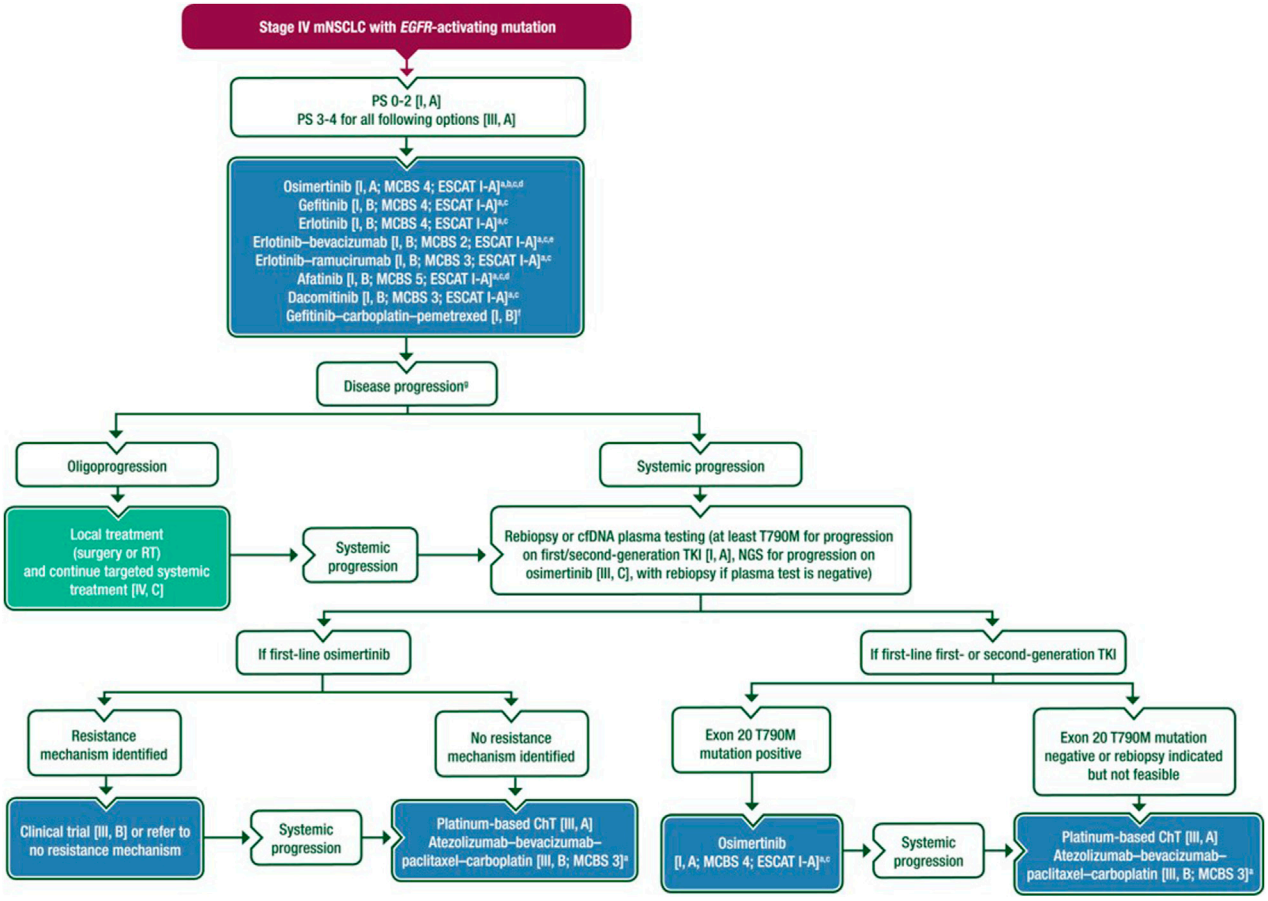

FIGURE 2
Treatment recommendation for EGFR mutant metastatic NSCLC based on the ESMO 2023 guideline [1].

19 del or exon 21 L858R EGFR mutation confirmed, osimertinib is the first choice, especially in the presence of BM (I, A). As first-line therapy, gefitinib chemotherapy and erlotinib-VEGF inhibitor therapy are also considered (I, B), but due to side effects and higher costs, EGFR TKI therapy alone is the preferred option (I, A). If osimertinib is not available, first-generation (gefitinib, erlotinib) or second-generation (afatnib, dacomitinib) agents are preferred (I, A). In case of progression, liquid biopsy or repeated tissue sampling is recommended to confirm T790 resistance mutation (I, A). In the case of a resistance mutation is present, second-line osimertinib is recommended (I, A), while platinum-based chemotherapy is the treatment of choice in case of negative results (III, A). In the case of osimertinib resistance, next-generation sequencing (NGS) is recommended to detect resistance genes (III, C). Enrolling in a clinical trial is a preferred option if available (III, B) (III, B). Otherwise, platinum-based chemotherapy is administered (III, A). After exhaustion of TKIs, chemotherapy doublet-immunotherapy-VEGF-quadruplet combination may be considered in patients with good overall Eastern Cooperative Oncology Group (ECOG PS) 0–1, if immunotherapy is not contraindicated (III, B) [1]. If oligoprogression is confirmed during EGFR tyrosine kinase inhibitor therapy, local metastasis treatment is recommended, while targeted therapy should be continued (III, A) [1]. In the presence of a rare mutation, non-exon 20 insertion, osimertinib, or afatinib is recommended (III, B). In the presence of exon20 insertion mutations, amivantamab can be given as second-line therapy in the case of progression after first-line therapy (III, B), while mobocertinib EMA approval is pending in these clinical settings (III, C) [1].

Treatment of NSCLC with anaplastic lymphoma kinase (ALK) genetical alterations

ALK fusion genes are potent, albeit uncommon, driver oncogenes of non-small cell lung cancer. Notably, the detection of ALK fusion oncogene is of great importance

because ALK-positive tumors are highly sensitive to ALK inhibitors, which significantly improves the life expectancy of patients.

Diagnosis

Molecular testing for ALK fusion can be performed as part of standard clinical care in non-small cell lung cancer, primarily adenocarcinoma, from both tumor tissue and plasma samples [35]. Methods for detecting ALK translocation include NGS, IHC, Fluorescence *in situ* hybridization (FISH), RT-PCR. The standard methodology is FISH, but immunohistochemistry with monoclonal antibodies of high sensitivity and specificity and a validated method is an equivalent method for detecting ALK fusion oncoprotein. RNA-based multigene NGS assays are also suitable instead of IHC or FISH, with the advantage of simultaneous testing for other fusion oncogenes [36, 37].

Epidemiology

The ALK fusion oncogene is present in 3%–5% of non-small cell lung cancers, with the majority of lung cancers carrying the gene being adenocarcinoma (97%). It is a disease of non-smokers or light smokers (<10 pack-years). Relative younger age at onset, with a median age of 52 years. The incidence of ALK molecular alteration in squamous cell carcinoma is limited. Cerebral metastasis is common, approximately 30% at the time of disease discovery [38, 39].

First-line treatment of ALK-positive lung cancer with ALK tyrosine kinase inhibitor (ALK-TKI)

Crizotinib is a multitarget TKI, the first ALK inhibitor to improve the life expectancy of ALK-positive patients compared to chemotherapy in both first-line and subsequent-line settings.

In the phase 3 PROFILE 1014 trial, therapy-naive patients were included, and first-generation ALK inhibitor, crizotinib, was compared with pemetrexed and platinum doublet chemotherapy, and crossover was allowed [40]. At a median follow-up of 17 months, the primary endpoint, progression-free survival, was longer with crizotinib than with chemotherapy (median 10.9 vs. 7 months; HR 0.45, 95% CI 0.35–0.60). Objective tumor response was also increased (74% vs. 45%). At 46 months follow-up, there was no significant difference in overall survival (HR 0.76, 95% CI 0.55–1.05). However, after crossover adjustment, crizotinib also improved overall survival compared with chemotherapy (HR 0.35, 95% CI 0.08–0.72). The median overall survival of more than 4 years was reported in the crizotinib arm [40].

Ceritinib, a second-generation ALK inhibitor, has also been shown to be superior to chemotherapy when administered as first-line therapy [41].

The second-and third-generation ALK inhibitors are more effective than crizotinib in metastatic disease, including BM based on phase 1 randomized controlled trials, and are considered the preferred first-line agents. Second-generation ALK-TKIs are alectinib, brigatinib, and ensartinib (not approved by the European Medicines Agency (EMA)), and third-generation agents are lorlatinib [42–45].

Alectinib indicated for therapy naïve patients in first-line or previously treated with crizotinib in locally advanced or metastatic ALK-positive non-small cell lung cancer. In the phase 3 ALEX randomized controlled trial (N = 303), the median progression-free survival (mPFS) in the first-line setting compared with alectinib plus crizotinib was 35 months for alectinib and 11 months for crizotinib (HR 0.43, 95% CI 0.32–0.58). The results are not yet mature; the median overall survival (mOS) for the alectinib arm was not yet reached, and for the crizotinib arm, it was 57 months (HR 0.67 95% CI 0.46–0.98). Immature data showed a 5-year OS rate of 63% (alectinib) and 46% (crizotinib). Time to CNS progression was longer for alectinib than crizotinib (HR 0.16, 95% CI 0.10–0.28) in the overall population. The rates of grade 3–5 toxicity were similar for alectinib and crizotinib (52% vs. 56%). In the Alectinib arm, there was a higher incidence of anemia, myalgia, se bilirubin elevation, weight gain, and photosensitivity. Nausea, vomiting, and diarrhea were more frequent on the crizotinib arm [42, 46].

Brigatinib is currently approved for use in ALK-positive non-small cell lung cancer in locally advanced or metastatic ALK-positive patients previously treated with crizotinib or not previously treated with an ALK inhibitor. Brigatinib shows efficacy in a broad spectrum of ALK mutations. The phase 3 ALTA-1L randomized controlled trial (N = 275) compared brigatinib with crizotinib in ALK-TKI naive patients. Chemotherapy administration prior to randomization was not an exclusion criterion in the trial. In ALK inhibitor naïve ALK-positive patients at 3-year follow-up, the PFS was 43% vs. 19% for crizotinib vs. crizotinib arm, respectively, according to a standardized independent evaluation. mPFS at 9–11 months follow-up was 24 months versus 11 months (HR 0.48, 95% CI 0.35–0.66). The therapeutic benefit was observed in all subgroups and was prominent in patients with BM. The brain metastasis-related tumor response was significantly higher with brigatinib compared with crizotinib (78% versus 26%). mOS has not yet been reached by either group.

There was ILD/pneumonitis occurred in 4% of patients on brigatinib and 2% on crizotinib. The incidence of grade 3–4 ILD/pneumonitis was 3% versus 0.7%. The risk decreased by gradually increasing the dose of brigatinib (90 mg once daily for 7 days, then increased to 180 mg/day if tolerated). Symptoms associated with elevated creatine kinase (myalgia, muscle pain) did not differ significantly between the two agents. Nausea, diarrhea,

constipation, peripheral edema, elevated liver function (GPT), and visual disturbances were more frequent with crizotinib. Grade ≥3 adverse events occurred in 61% with brigatinib and 55% with crizotinib [43].

Lorlatinib is recommended as monotherapy for adult patients with advanced ALK-positive non-small cell lung cancer who have not been previously treated with an ALK inhibitor. It is also for patients with advanced-stage ALK-positive NSCLC whose disease has progressed on first-line treatment with alectinib ceritinib or crizotinib.

In the phase 3 CROWN randomized controlled trial (N = 296), patients with the locally advanced or metastatic stage-naive disease were randomized to the lorlatinib or crizotinib arm. The mPFS was significantly better with lorlatinib than with crizotinib. In the first interim analysis, mPFS at 18 months of follow-up was not yet reached with lorlatinib versus 9.3 months with crizotinib (HR 0.28 95% CI 0.19–0.41). Lorlatinib showed robust CNS efficacy. Grade 3–4 adverse events occurred in 72% of patients treated with lorlatinib and 56% with crizotinib. Hypercholesterolaemia and hypertriglyceridemia occurred in >70% of patients on lorlatinib and neurocognitive side effects may affect the first-line use of lorlatinib [45, 47, 48].

Duration of treatment

Treatment with ALK inhibitors is continued until disease progression. In the case of oligoprogression, local intervention is recommended in addition to the continuation of ALK-TKI. A more potent next-generation ALK inhibitor or standard chemotherapy is indicated for extensive progressive disease.

Treatment for progression on crizotinib

For progression following crizotinib, alectinib or brigatinib is recommended, given their systemic and CNS efficacy and good tolerability.

Alectinib—In the phase 3 ALUR study (N = 107), patients with advanced ALK-positive disease pretreated with platinum-based chemotherapy and crizotinib were randomized to alectinib or mono-chemotherapy (pemetrexed or docetaxel). PFS was longer with alectinib, 7.1 months vs. 1.6 months (HR 0.32, 95% CI 0.17–0.59), and the number of grades ≥3 adverse events was lower with alectinib (27% versus 41%). CNS efficacy was also better with alectinib [49].

Brigatinib—In the phase 2 ALTA study (N = 222), patients refractory to crizotinib at 1 × 90 mg/day (arm A) or 1 × 180 mg/day (arm B) after a seven-day 1 × 90 mg/day lead-in period with brigatinib had an mPFS of 9.2 months versus 16.7 months at lower and higher doses of the agent, respectively. The median overall survival (OS) was 29.5 months versus 34.1 months. In patients with baseline BM, the independently assessed CNS objective tumor response was 50% versus 67%. Both arms had low rates of grade ≥3 toxicity [50].

Ceritinib is not preferable because it is less effective than the former in cross-trial comparisons. In the open-label ASCEND-5 study, 231 patients were randomized to ceritinib 750 mg/day or chemotherapy arm after crizotinib treatment, with ceritinib having better PFS (5.4 versus 1.6 months; HR 0.49) and ORR (39.1% versus 6.9%), both statistically significant. Nevertheless, OS analysis is still immature. Due to crossover, the OS advantage is expected to be decreased in the ceritinib arm. While initial studies used a ceritinib dose of 750 mg/day with fasting intake, a randomized open-label trial found an equivalent dose of 450 mg/day with meals was associated with lower gastrointestinal toxicity [51].

Although a phase 2 trial has shown lorlatinib to be effective in progression on crizotinib (ORR 69%, intracranial ORR 68%, mPFS not yet achieved), the EMA prescribing after crizotinib requires the prior use of a second-generation TKI [48, 52].

Treatment for progression on second-generation ALK TKI

Lorlatinib is a third generation ALK-TKI. Lorlatinib is effective against acquired resistance mutations in most ALK kinase domains, including G1202R and other ALK kinase domain mutations. Lorlatinib is the preferred agent for alectinib-induced resistance [53]. This is probably also true for other second-generation ALK inhibitors [52]. Lorlatinib is also characterized by high CNS penetration.

In a phase 2 trial, lorlatinib in patients previously treated with one or more ALK inhibitors resulted in high objective tumor response (47%), complete remission (2%) and partial tumor response (45%). Following crizotinib, treatments with lorlatinib, the ORR was 73%, and mPFS was 11.1 months. After one or more second-generation ALK inhibitors, ORR was 40% and mPFS was 6.9 months. At >30 months median follow-up, mOS was 21 months. The most common adverse events in this study were hypercholesterolemia (81%), hypertriglyceridemia (61%), edema (43%) and peripheral neuropathy (30%). Serious treatment-related adverse events developed in 7% of patients, the most common being cognitive impairment (1%). In patients who progressed with second generation ALK-TKI, the ORR for lorlatinib was higher when an ALK mutation was present in addition to the ALK fusion oncogene, suggesting that second generation ALK mutations may be associated with the development of a new oncogene. Therefore, genotyping ALK mutations after progression on second generation ALK inhibitors may identify patients more likely to benefit from lorlatinib treatment [52].

Although lorlatinib has not been studied in comparison with chemotherapy in alectinib-resistant disease, lorlatinib after

alectinib is recommended because chemotherapy can only achieve poorer survival after progression from ALK-TKI.

Alternative target therapies -ceritinib and brigatinib also show activity in progression after alectinib based on small observational studies [54, 55].

Treatment options for subsequent lines in ALK-positive NSCLC

The IMpower 150 trial suggests chemo-immunotherapy + VEGF inhibitor, the median PFS in patients with EGFR activating mutation + ALK-positive subgroup in TKI pretreated patients were 9.7 vs. 6.1 months; respectively, (HR 0.59, 95% CI 0.37–0.94). Nevertheless, there is no evidence on the efficacy of mono-immunotherapy in the presence of ALK-positivity. Chemotherapy alone is an acceptable additional option with moderate activity in this patient population. Some experts recommend a standard combination of pemetrexed, carboplatin, and pembrolizumab combinations for lung adenocarcinoma, although clinical trials demonstrating the efficacy of this combination have excluded ALK-positive patients [56, 57].

Treatment of brain metastases in ALK-positive NSCLC

In cases of BM, both for symptomatic or asymptomatic cases, second or third generation ALK-TKI is recommended, as these agents have good blood barrier penetration and CNS efficacy. The majority of patients with BM, whether TKI naïve or treated with crizotinib, are likely to respond to these agents and may be able to defer surgical intervention or radiotherapy, thereby reducing morbidity associated with local care. However, surgical treatment may be considered as initial therapy in cases of spatial disproportionation or risk of herniation due to massive BM [58].

Summary of ALK targeted therapy

Treatment recommendation for ALK translocated metastatic NSCLC based on the ESMO 2023 guideline is shown in Figure 3. NSCLC with ALK rearrangements is a subtype of lung cancer with specific clinical and pathological features. Due to the availability of effective therapies, all lung adenocarcinomas should be investigated for ALK fusion oncogenicity. In locally-advanced or metastatic stage ALK-positive NSCLC, a second or third-generation ALK inhibitor is recommended in the frontline setting. Treatment should be continued until progression or intolerable toxicity. In case of oligoprogression in mildly symptomatic or asymptomatic patients, local ablative therapy for the progressive formulation is recommended with continuation of the ALK inhibitor. For progression on a second-generation ALK inhibitor, lorlatinib is recommended over chemotherapy or other ALK inhibitors. Following the exhaustion of TKIs, platinum doublet CHT or in CHT combination with bevacizumab and/or anti-PD immunotherapy can be administered if the patient is still fit for further treatment.

Further studies are needed for TKI-treated ALK-positive cases to determine whether identifying specific tyrosine kinase domain mutation can identify appropriate next steps in therapy. Nevertheless, some preliminary data suggest that specific kinase domain mutations may impact the following line of therapy [2]. Broad genomic profiling may be the most informative approach to examining potential resistance mechanisms, which may require repeated sampling during treatments. Assay methodology selection can impact the ability to identify subclonal events in this setting.

KRAS mutant NSCLC treatment

KRAS mutations activate several additional signaling pathways, occur in about 20%–25% of lung adenocarcinomas and are usually associated with lung cancer in smokers [59]. G12G KRAS mutation subtype is associated with smoking status [6].

The presence of the KRAS mutation in early lung cancer does not seem to affect overall survival, however others have shown that it is associated with a poor prognosis [60]. The focus of targeted therapy for KRAS mutant lung cancer is on irreversible inhibitors of KRAS G12C. KRAS G12C mutations account for nearly 50% of all KRAS mutations [60].

Treatment of non-small cell lung cancer with KRAS G12C mutation

First line treatment

First-line therapy regimens are recommended similarly to non-oncogene-dependent, non-squamous NSCLC [1].

Second-line treatment

Targeted treatment for KRAS G12C mutant tumors after first-line platinum-based chemotherapy and/or anti-PD immunotherapy is considered.

Sotorasib is the first target agent to receive regulatory approval for KRAS G12C mutant locally advanced or metastatic adenocarcinoma in patients who have received at least one prior systemic therapy [61].

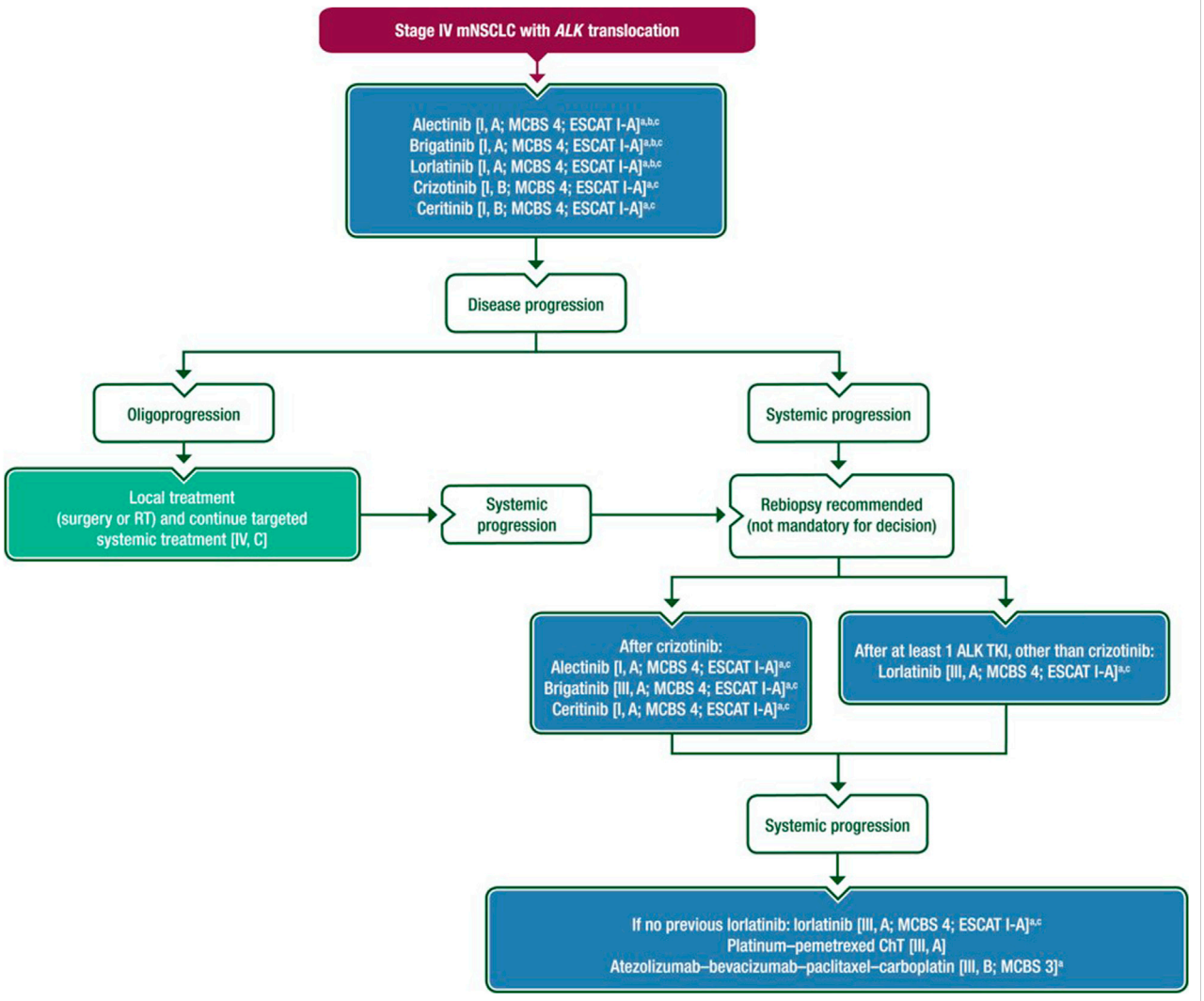

FIGURE 3
Treatment recommendation for ALK translocated metastatic NSCLC based on the ESMO 2023 guideline [1].

In the randomized, open-label, phase 3 CodeBreak 200 trial (N = 345), patients with KRAS G12C mutations were randomized to sotorasib or docetaxel after progression on platinum-based chemotherapy and anti-PD immunotherapy treatment. Better PFS was achieved with sotorasib than docetaxel based on independent unblinded assessment (5.6 versus 4.5 months HR 0.66, 95% CI 0.51–0.86), with fewer grade ≥3 toxicities (33 versus 40%) and fewer serious adverse events (11 versus 23%). Overall survival was similar in the two groups (10.6 months with sotorasib and 11.3 months with docetaxel, HR 1.0). The most common grade ≥3 treatment-related adverse events were diarrhea (12%) and elevated transaminase levels (5%–8%) [62].

In the phase 1 CodeBreak 100 trial, sotorasib achieved an objective tumor response of 41%, mPFS of 6.3 months, OS of 12.5 months, and a two-year survival rate of 33% [63].

Several drug interactions are known to occur with sotorasib. It is not recommended for co-administration with antacids such as proton pump inhibitors, H2 receptor blockers, potent cytochrome P450 3A4 (CYP3A4) inducers, and certain CYP3A4 and P-gp substrates [61].

Adagrasib has been granted conditional marketing authorization by the EMA to treat advanced non-small cell lung cancer with KRAS G12C mutation and progression on at least one prior systemic therapy [64].

In the Krystal-1 single-arm, phase 1–2 study (N = 116), KRAS G12C mutant patients received 2 × 600 mg of adjuvant adagrasib daily after prophylactic chemotherapy and PDL1 inhibitor immunotherapy. The mPFS was 6.5 months, objective tumor response was 43%, the median duration of response was 8.5 months, and OS was 12.6 months. In

33 patients with previously treated stable BM, the intracranial confirmed objective response was 33%. Grade ≥3 treatment-related adverse events occurred in 45% of patients, the most common being fatigue, nausea and elevated liver function tests. Two grade 5 events occurred: heart failure in a patient previously known to have pericardial effusion and one pulmonary hemorrhage [65].

Summary of targeted therapy in KRAS mutant NSCLC

For patients with KRAS G12C mutant NSCLC progressed after a prior line of therapy, second-line sotorasib or adagrasib may be recommended over subsequent chemo and/or immunotherapy.

Treatment of NSCLC with ROS1 genetical alterations

The ROS1 proto-oncogene encodes a tyrosine kinase of the insulin receptor family, which is structurally similar to ALK. ROS1 gene fusion was first identified in 1987 in the glioblastoma cell line U118MG [66]. Since then, ROS1 gene rearrangements have been observed in 22 adult and pediatric malignancies [67]. It is detectable in 1%–2% of NSCLC, with a higher prevalence in non-smoking, younger women. ROS1 mutations do not co-occur with other driver mutations, with rare exceptions including in *EGFR* (1/166) and *KRAS* (3/166) and no co-occurring *ROS1* and *ALK* alterations [68]. They are almost exclusively detected in adenocarcinoma, but rare cases are also found in squamous cell, pleiomorphic, and large cell lung carcinoma [69]. FISH is the gold standard method for the detection of ROS1 gene rearrangements. IHC has high sensitivity but low specificity and is not recommended as a primary determinant for treatment. In the case of a positive or inconclusive ROS1 IHC result, confirmatory FISH, NGS, and RT-qPCR should be performed [69, 70]. Treatment recommendation for ROS1 translocated metastatic NSCLC based on the ESMO 2023 guideline is shown in Figure 4.

Crizotinib was the first TKI inhibitor to be approved by both the EMA and the FDA for the treatment of ROS1 mutant non-small cell lung cancer based on the results of the phase 1 PROFILE 1001 clinical trial [71]. The study cohort included 53 patients with ROS1-positive NSCLC, most of whom (87%) had previously received first-line platinum-based chemotherapy. The ORR for crizotinib was 72%, associated with a disease control rate (DCR) of 90%. The mPFS and mOS were 19.3 and 51.4 months, respectively. The median duration of therapy with treatment was 22.4 months (15.0–35.9 months) [71]. Subsequently, several retrospective and prospective phase 2 studies have demonstrated the efficacy of crizotinib [69]. However, it is important to emphasize that the drug has a low BBB penetration and, due to the poor brain penetration with crizotinib, the primary site of progression is the central nervous system [72].

Entrectinib is a new generation TKI that inhibits tropomyosin-related kinases in addition to its anti-ROS1 activity. Based on the results of an analysis of data from three prospective phase 1 and 2 clinical trials (ALKA-372–001, STARTRK-1, STARTRK-2), the ORR with entrectinib was 67% and mPFS was 15.7 months. A significant proportion of the 161 patients included in the study (62.7%) had received prior systemic therapy and 34.8% had BM at baseline. The 24 patients who had measurable BM at diagnosis had an intracranial ORR of 79%, mPFS of 12 months and mOS of 26.3 months. The majority of adverse events associated with entrectinib were grade 1 and 2, and overall, the tolerability and safety profile of the agent was similar to other ROS1 inhibitors [73, 74]. Based on these results, entrectinib was granted a marketing authorization by the FDA in 2019 and by the EMA in 2020. Entrectinib is the first line drug for known BM based on the ESMO 2023 recommendation [1].

Ceritinib is a second-generation ALK/ROS1 TKI with significant central nervous system activity. In a phase 2 clinical trial in Korea, 32 patients with advanced ROS1-positive disease, mostly crizotinib-naive (n = 30), were treated with ceritinib. In the whole cohort, ORR was 62%, mPFS was 9.3 months, and DCR was 81%. Among patients who had not received crizotinib treatment, ORR reached 67% and mPFS 18.3 months. Of note, no treatment response was observed in the two patients previously treated with crizotinib while on ceritinib [75]. Based on these results, ceritinib may be considered for crizotinib treatment in patients with ROS1-positive NSCLC who have not previously received crizotinib; however, currently, the agent is neither FDA nor EMA-approved.

Lorlatinib is a third-generation ALK/ROS1 TKI that penetrates the brain and has been effective in a phase 1 and phase 2 single-arm clinical trial that enrolled 69 ROS1-positive patients [52, 76]. The ORR in the TKI-naïve cohort (n = 21) was 62%, mPFS 21 months, and intracranial ORR 64%, compared to a group of previously crizotinib-treated patients (n = 40), where ORR was only 35%, mPFS 8.5 months, and intracranial ORR 50%. There was also a significant difference in the median duration of response (mDOR) (25.3 months vs. 13.8 months). Along with ceritinib, lorlatinib does not have FDA or EMA approval.

Repotrectinib is a new generation ROS1/TRK/ALK tyrosine kinase inhibitor. In the phase 1/2 TRIDENT-1 clinical trial in the ROS1 TKI-naïve group (n = 71), ORR was 79% and mDOR was 34.1 months [77, 78]. In patients who had previously received ROS1 TKI therapy but did not receive chemotherapy/immunotherapy (n = 56), ORR was 38% and mDOR was 14.8 months. Based on these results, in November 2023, the FDA approved repotrectinib to treat ROS1-positive NSCLC [79].

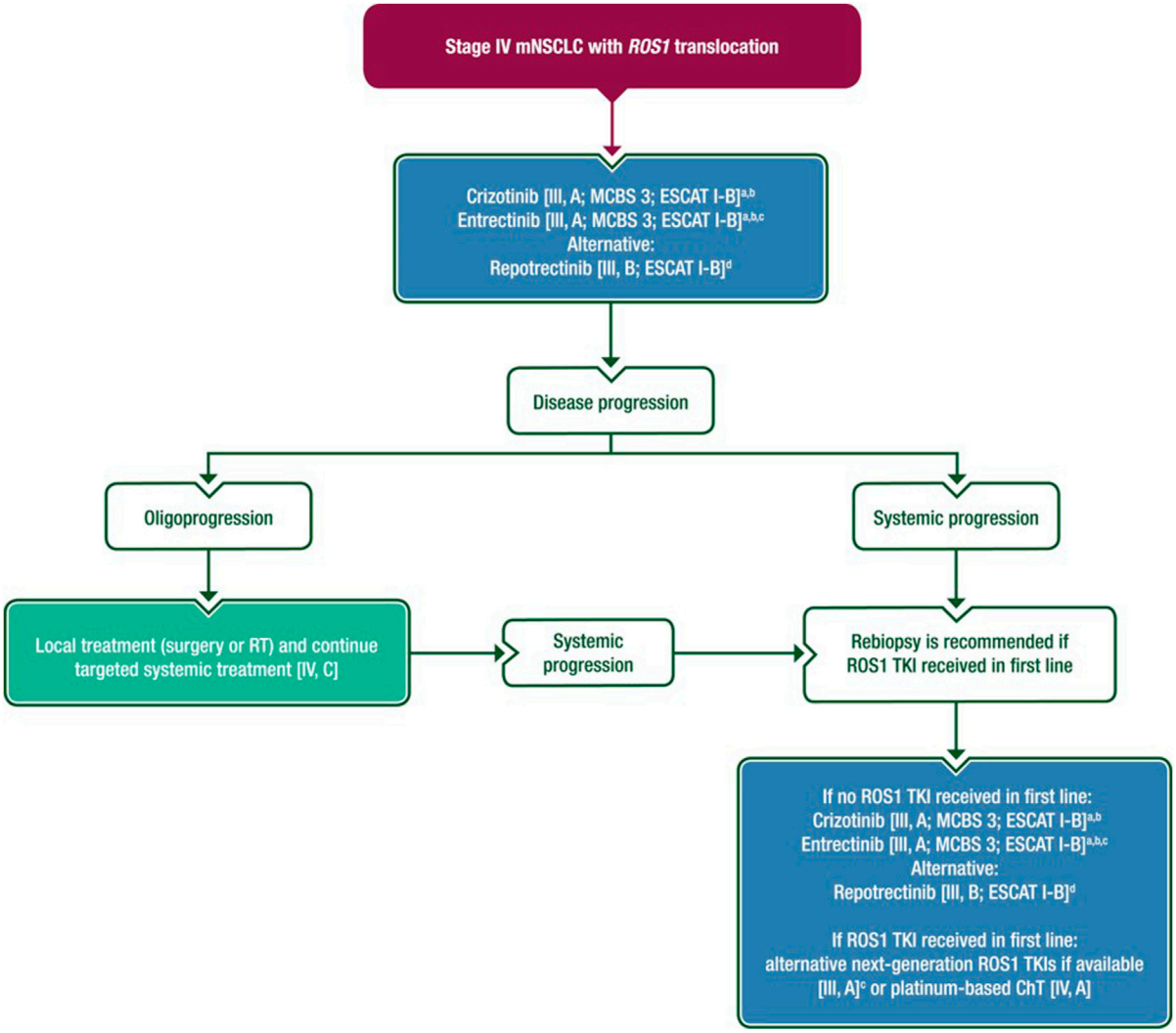

FIGURE 4
Treatment recommendation for ROS1 translocated metastatic NSCLCbased on the ESMO 2023 guideline [1].

Brigatinib, cabozantinib, thaletrectinib, and ensartinib also have ROS1 inhibitory effects based on preclinical and phase 1/2 studies [80–83].

RET-positive NSCLC treatment

The RET gene encodes a tyrosine kinase-activated membrane receptor protein, primarily involved in the differentiation of the enteric nervous system and urogenital tract [84]. Oncogenic RET alterations can be detected in several solid tumor types [85], such as thyroid cancers, NSCLCs, pancreatic, colorectal, and breast tumors, and are involved in, among others, multiple endocrine neoplasia type 2 [86], and Hirschprung's disease [87]. RET gene rearrangement is detected in 1%–2% of NSCLCs; these tumors are typically found in non-smoking, younger patients and are associated with an increased risk of BM [88]. Histologically, they are almost exclusively of the adenocarcinoma subtype; others showed 92.3% non-squamous histology [89]. Several fusion partners of RET are known, the most common being KIF5B and CCD6C [90]. IHC and RT-PCR have proven to be unreliable methods for diagnosing RET-positive NSCLCs due to their low sensitivity and variable specificity and are replaced by FISH and NGS [85, 90].

Selpercatinib is a low molecular weight drug that can penetrate the BBB and is a highly selective RET tyrosine kinase inhibitor (TKI) that has demonstrated efficacy in RET translocation-positive NSCLC in the LIBRETTO-001 phase 1/2 clinical trial [91]. The trial enrolled 105 patients previously treated with platinum-based chemotherapy and 39 therapy-naive

patients. For pretreated patients, the ORR was 64% (95% CI, 54%–73%) and the mDOR was 17.5 months. In the therapy-naïve group, the ORR was 85% (95% CI, 70%–94%). Of note, 91% of the n = 11 patients with BM observed an intracranial clinical response. The most common grade 3 or more severe adverse events were hypertension (14%), elevated alanine aminotransferase (12%) and aspartate aminotransferase (10%) levels, hyponatremia (6%) and lymphopenia (6%). Based on these results, both FDA (2020) and EMA (2021) have approved selpercatinib for the treatment of locally advanced and metastatic NSCLC [92]. The randomized phase 3 multicentre trial (LIBRETTO-431) compared the efficacy of first-line selpercatinib with or without chemotherapy (carboplatin/cisplatin + pemetrexed) with or without pembrolizumab [93]. The results of the study were presented at the ESMO Congress 2023 [94, 95]. The selpercatinib group had a significantly higher mPFS compared to the chemotherapy ± immunotherapy group (24.8 months vs. 11.2 months; HR:0.465, CI: 0.309–0.699; $p < 0.001$) [95].

Pralsetinib is the other selective RET inhibitor that will be registered by the FDA in 2020 and by the EMA in 2021, based on the results of the ARROW clinical trial. However, while the EMA approval (for both selprecatinib and pralsetinib) is only valid for patients who have not previously received RET TKI therapy, the FDA approval does not include such a restriction [96–99]. The ORR was 72% in the treatment-naïve (n = 75) and 59% in the group of patients (n = 136) who had received prior platinum-based chemotherapy. The mDOR was not reached in the therapy-naïve group and 22.3 months in the pretreated group. As with selpercatinib, pralsetinib has significant intracranial activity, with an intracranial ORR of 70% (95% CI, 35%–93%) in the group of patients with BM (n = 10, all of whom had received prior chemotherapy) in the study. The agent's tolerability and side effect profile were similar to the other TKIs [100]. In the ongoing AcceleRET phase 3 clinical trial, similar to the LIBRETTO-431 trial, first-line pralsetinib therapy is being compared with platinum-based chemotherapy ± pembrolizumab [101, 102]. Results of the trial are expected in 2024.

BRAF mutant NSCLC treatment

Mutations in BRAF (V-raf murine sarcoma viral oncogene homolog B) are mutations in the MAPK mitogen-activated protein kinase pathway, which affects downstream signaling proteins. BRAF mutations are alternative oncogenic drivers in NSCLC, which mutually exclude EGFR mutations and ALK and ROS1 rearrangements. The incidence of lung adenocarcinoma is 4.5% [103]. BRAF mutations in the serine/threonine kinase domain most commonly affect V600 [104]. Kinase inhibitors are now available for BRAF V600E mutations. These include dabrafenib, a serine/threonine kinase inhibitor, and trametinib, which has both serine/threonine and tyrosine kinase inhibitory activity [105].

The registration of the drugs was based on a prospective, multicentre, multicohort phase 2 study (BRF113928). The study enrolled 171 patients with metastatic NSCLC with BRAF-V600E mutations, of whom 78 patients received dabrafenib monotherapy (Cohort A), 57 patients received the MEK inhibitor trametinib in combination in multiple lines (Cohort B) and 36 patients received first-line combination therapy (Cohort C). The dose of dabrafenib was 2 × 150 mg/day in both monotherapy and combination therapy, while trametinib treatment was administered at 1 × 2 mg/day. With dabrafenib monotherapy, the response rate (ORR) was 33%, the mPFS was 5.5 months, and the mDOR was 9.6 months. In pretreated patients with the dabrafenib-trametinib combination, the ORR was 68%, mPFS was 10.2 months, and mDOR was 9.8 months. In previously untreated patients on dabrafenib-trametinib combination therapy, the ORR was 64%, mPFS was 10.8 months, and mDOR was 10.2 months. In patients receiving pretreated combination therapy (Cohort B), a median overall survival (OS) of 18.2 months was observed, with 4-year and 5-year survival rates of 34% and 22%, respectively, representing a significant improvement compared to both dabrafenib monotherapy and conventional chemotherapy. The combination of dabrafenib and trametinib is indicated for the treatment of patients with advanced metastatic-stage NSCLC with BRAF V600E mutations (the study on which the registry was based included only BRAF V600 mutation-positive patients, so its efficacy in wild-type BRAF mutant NSCLC has not been proven) [106].

NSCLC with distant metastases should be tested for BRAF V600 mutation status [ESMO II, A]. For NSCLC with BRAF V600E mutation in metastatic stage, first-line treatment with dabrafenib + trametinib is recommended [ESMO III, A; ESCAT: I-B]. If patients have received first-line BRAF and MEK inhibition, platinum-based chemotherapy with or without immunotherapy may be recommended as second-line treatment [ESMO IV, B] [106].

MET exon 14 skipping mutation and MET amplification in NSCLC

Oncogenic activation of the MET (mesenchymal-epithelial transition) signaling pathway can be caused by overexpression, gene amplification, gene rearrangements, and various mutations [1].

MET exon 14 skipping mutations are found in about 3%–4% of younger/smoker/gender patients with NSCLC, mostly in cases where no other driver mutation can be identified, and more often in elderly and smoker patients. In addition to adenocarcinoma, its occurrence has also been observed in sarcomatoid carcinoma. It is considered an unfavorable prognostic marker. The skipping

mutation can be detected by DNA- or RNA-based NGS (ESMO IB), while MET amplification can be detected by immunohistochemistry or *in situ* hybridization (ESMO IIB) [107–109].

Detection of MET overexpression is not associated with effective target therapy, but MET exon 14 skipping mutations or MET amplification is associated with the efficacy of MET inhibitors. MET amplification occurs in 1%–6% of NSCLC cases and may be a cause of acquired resistance to EGFR and ALK inhibitors. For MET amplification, the method of detection and the definition of high gene copy numberstill need to be standardized, while for high gene amplification the MET inhibitor capmatinib has been shown to be effective, but the FDA and EMA have not yet approved its use in this indication. In the case of MET exon 14 skipping mutations, registered targeted treatment options are available. Detection of MET exon 14 skipping mutation and MET amplification is recommended in the initial evaluation of patients diagnosed with non-squamous NSCLC (ESMO IIA).

The FDA has approved capmatinib and tepotinib for the first-line treatment of NSCLC with MET exon 14 skipping mutations, but the EMA has not approved it for first-line, only second- and multiline therapy at present. Thus, first-line platinum-based chemotherapy ± immunotherapy is recommended in these cases.

For patients with MET-amplification, platinum-based chemotherapy with/without immunotherapy is recommended as first-line treatment (ESMO IVB). Following first-line treatment, treatment with capmatinib or tepotinib monotherapy is recommended for patients with MET exon 14 skipping mutation-positive NSCLC (ESMO III A). For tumors carrying less frequent driver mutations, there is little data on the efficacy of immunotherapy, and in these cases, platinum-based chemotherapy or chemotherapy + immunotherapy is recommended if targeted therapy is not an option, while monotherapy immunotherapy is not recommended [108].

Capmatinib

Capmatinib is a potent, selective MET receptor inhibitor and has been shown to be effective in various types of MET activation *in vitro* and *in vivo* tumor models [110]. Capmatinib can cross the BBB.

The registration of the medicine was based on the results of the GEOMETRY mono-1 study [110, 111]. The phase 2, open-label, multi-arm study enrolled 364 patients with advanced (stage IIIB or IV) NSCLC who were found to have MET amplification or MET exon 14 skipping mutations. Patients who had received prior chemotherapy and subjects who had not yet received treatment were also included. Patients received capmatinib therapy in the form of 2 × 1,400 mg tablets daily.

In patients with MET exon 14 skipping mutations, the ORR was 41% in previously treated patients and 68% in previously untreated patients. The mPFS was 5.4 months in previously treated subjects and 12.4 months in patients who received first-line treatment. The effect of capmatinib treatment was typically rapid, with the vast majority of patients showing a response (82% of the previously treated patients and 68% of those who had not received treatment previously) having a tumor response at the first tumor evaluation [110]. Of the patients studied, 14 had BM, 12 of whom showed intracranial tumor control, 7 had reduced BM and 4 patients had complete remission.

Among patients, 98% reported some adverse effects, with 67% reporting grade 3–4 adverse effects. The most common symptom was peripheral edema, followed by nausea, vomiting and elevated serum creatinine. The incidence of serious treatment-related adverse events was 13%, with 11% of patients having to stop treatment. Dose reduction was required in 23% of subjects included. In one patient, pneumonitis leading directly to death may likely have been related to capmatinib treatment.

Tepotinib

Tepotinib is also a selective MET tyrosine kinase inhibitor, capable of penetrating the BBB [112]. It has demonstrated efficacy in advanced NSCLC with MET exon 14 skipping mutations in the phase 2 VISION clinical trial [113–115]. Patients with MET amplification were not included in this study. The skipping mutation was detected by histology or liquid biopsy. Patients received the investigational agent in a once-daily oral dose of 500 mg.

In the study, the ORR was 44.7% and the median PFS was 8.9 months. The median overall survival was 17.6 months. Response rates and PFS showed no difference whether the patient received first-line or multi-line treatment with tepotinib. The investigational drug also showed efficacy in elderly patients over 80 (ORR: 35.1%, PFS: 8.6 months). Intracranial tumor control was achieved in the majority of subjects with BM.

Treatment-related adverse events occurred in 86.3% of patients on tepotinib, 24.3% experienced grade 3–4 adverse events, and 12.2% experienced serious adverse events. Three cases were fatal, with death resulting from ILD-related respiratory failure or liver failure. The most common adverse events were peripheral edema, followed by nausea, diarrhea, serum creatinine elevation, and hypoalbuminemia.

HER2 mutant NSCLC treatment

HER2 is a human epidermal growth factor receptor (EGFR/ERBB2) family and is encoded by the ERBB2 gene. The

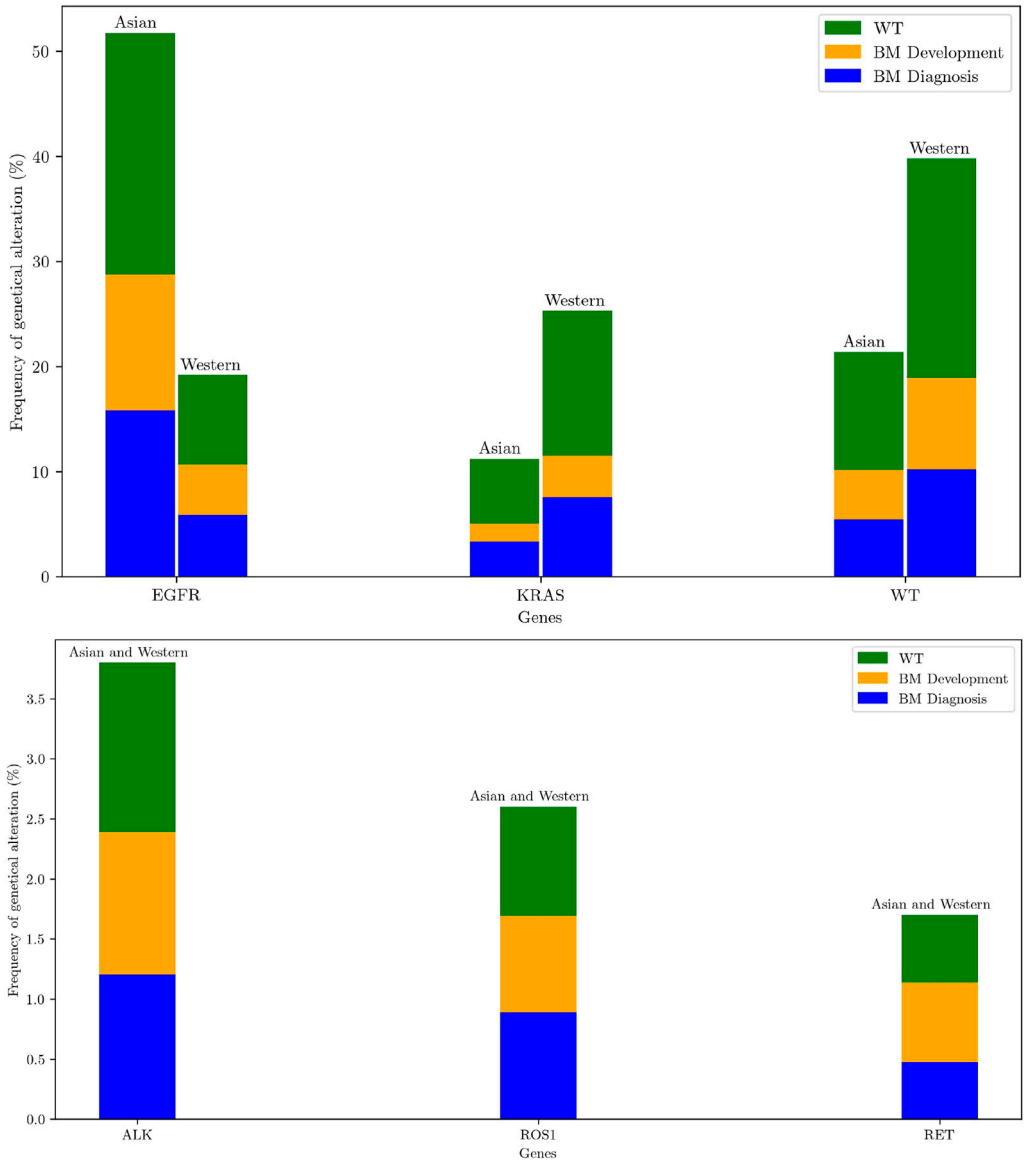

FIGURE 5
Frequency of oncogenic drivers for East-Asian and Western populations [134]. Bars are stratified according to proportions of brain metastases (BM) at diagnosis (blue) and BM development after diagnosis (orange) and pan-wild-type for all genetic alterations reported including rare ones (WT, green) [131]. For visual enhancement low frequencies of ALK, ROS1, RET genetical alterations are presented in a separate figure.

prevalence of HER2 mutations in patients with non-small cell carcinoma is between 1%–4%. HER2 lesions can develop by three mechanisms: HER2 protein overexpression, HER2 amplification and HER2 gene mutation. IHC, FISC and NGS can be used to detect these lesions. Double platinum-based chemotherapy is the first-line treatment of choice and can be complemented with immunotherapy (ESMO IV.B) [116].

In the DESTINY LUNG01 clinical trial, the HER2 antibody-drug conjugate, trastuzumab-deruxtecan treatment efficacy was investigated. In the study, 91 patients with metastatic HER2 mutant NSCLC received second-line trastuzumab-deruxtecan treatment following standard therapy. In the study, PF was 8.2 months (95% CI, 6.0–11.9), while median OS was 17.8 months (95% CI, 13.8–22.1). Treatment-related adverse events included neutropenia and drug-induced ILD, the latter resulting in 2 deaths identified in the study [117].

The DESTINY LUNG02 phase 2 randomized trial also investigated the efficacy and safety of trastuzumab-deruxtecan following platinum-based treatment in the second line, also at two different doses. At both doses (5.4 mg/kg or 6.4 mg/kg every

TABLE 1 Intracranial objective response rate in patients with BM according to the presence of drive oncogenes and targeted therapy administration [135].

Study	Year/Trial ID	Driver oncogene	Targeted therapy	Intracranial efficacy (%)
FLAURA	2014 NCT02296125	EGFR Ex19del, Ex21 L858R	osimertinib	91
ALEX	2014 NCT02075840	ALK	alectinib	81
ALTA-1L	2016 NCT02737501	ALK	brigatinib	78
CROWN	2017 NCT03052608	ALK	lorlatinib	82
STARTRK2	2015 NCT02568267	ROS1	entrectinib	79
Geometry Mono-1	2015 NCT02414139	MET exon 14 skipping mutation, MET amplification	capmatinib	54
VISION	2016 NCT02864992	MET exon 14 skipping mutation, MET amplification	tepotinib	55
LIBRETTO-001	2017 NCT03157128	RET	selpercatinib	82
ARROW	2017 NCT03037385	RET	pralsetinib	78
CodeBreaK 100	2018 NCT03600883	*KRAS* G12C	sotorasib	25

3 weeks), a significant and sustained antitumor effect with an acceptable safety profile was observed, but the lower dose had a lower rate of drug-induced ILD [118]. The study's results led to EMA approval of second-line trastuzumab-deruxtecan treatment for NSCLC with metastatic or unresectable HER2 mutations. First line trial phase 3 is recruiting [119].

Treatment of NTRK gene fusion-positive NSCLC

Neurotrophic tyrosine receptor kinase (NTRK) gene fusions initiate downstream signaling pathways, such as the AKT and MEK pathways, and are present at a very low frequency (<1) in solid tumors [1].

Entrectinib is an NTRK and ROS1 inhibitor that can penetrate the central nervous system. In a clinical study of the efficacy of entrectinib in a total of 22 NSCLC patients, the mPFS was 14.9 months (95% Cl, 6.5–30.4), median OS results are not yet available [120].

Larotrectinib is a sensitive tropomyosin receptor kinase inhibitor. In two multicentre clinical trials, a total of 20 NRTK gene fusion-positive patients were tested for the efficacy of larotrectinib. Median PFS outcome was 35.4 months (95% CI, 5.3–35.4), and the median OS was 40.7 months [121].

Discussion

Patients with guideline-recommended molecular alteration-based therapies have better outcomes with first-line targeted therapy for advanced-stage NSCLC [1]. In a retrospective study, others showed a significant increase in OS in patients with non-squamous NSCLC with molecular testing available compared to non-tested patients [122]. Importantly, comprehensive NGS vs. incomplete or no testing before initiating first-line therapy impacts the OS (22.1 vs. 11.6 months, $p = 0.017$) respectively [123]. Nevertheless, a multidisciplinary approach is essential in finding the proper diagnostic procedures and treatments to personalize NSCLC therapy. There is a broad repertoire of targeted therapies in the standard of care settings. However, there is a need for improvements; therefore, participation in clinical trials is especially encouraged [2]. Accurate imaging-based clinical staging and tissue availability influence subsequent molecular assay-based personalized therapeutic decisions in multidisciplinary teams (MDT) before first line therapy administration. The gold standard for molecular testing in NSCLC is tissue-based testing. Liquid biopsy-based ctDNA detection can guide therapy; however, it should not be used instead of tissue samples. However, the plasma-first approach is recommended if tissue is unavailable [124]. Molecular testing for stage IV NSCLC with reflex testing is associated with shorter turnaround times. There is an emerging requirement for testing in early-stage disease. Formalin-fixed paraffin-embedded (FFPE) material suits for most molecular analyses and non-acid decalcification approaches on bone biopsies. Molecular assays, such as cell blocks, direct smears, or touch preparations, are recommended in order not to miss a targetable genetic alteration. Nevertheless, adequate biopsy sampling should ensure that the sample is suitable for molecular analysis, and in small specimens, minimal IHC should be used to preserve the tissue for molecular studies [2]. Accordingly, the acceptable terms NSCLC favor adenocarcinoma or favor squamous cell carcinoma is recommended with any extent of adenocarcinoma component in a biopsy specimen that is otherwise squamous should trigger molecular testing [2]. At a minimum, EGFR and ALK testing is

recommended before initiating immunotherapy because rapid and sensitive tests are available [2, 124]. MDT is best assisted by complete-scale molecular testing for a first-line treatment decision that includes NGS and PDL1 expression [123]. DNA-based NGS oncology panels are recommended to detect EGFR, KRAS, MET, RET, HER2, and BRAF. ALK, ROS1, and NTRK1/2/3 alterations can be identified with FISH. IHC, for screening purposes with low specificity, can also be applied. Therefore, validation with NGS DNA panels with reasonable specificity may detect ALK, RET, and NTRK2 but may underdetect ROS1, NTRK1, and NTRK3 fusions. In the case of RET and METex14, skipping events, RNA-based NGS is preferable to DNA-based NGS or fusion detection [2].

Following the expansion in molecular alteration-based targeted therapy in advanced stages, recently, attention has turned to early-stage cases and resection specimens. Recent advancements in the NSCLC adjuvant treatment setting, the molecular diagnostics for EGFR and ALK in the early stage, indeed necessary to exclude targetable alterations to pave the way to proceed with immunotherapy based on PD-L1 expressors. Accordingly, molecular testing of early-stage resectable NSCLC before neoadjuvant nivolumab plus chemotherapy was performed in CheckMate 816 [125]. Additionally, molecular testing was performed to exclude driver oncogenes in the perioperative early-stage setting in the AEGEAN study on durvalumab plus neoadjuvant chemotherapy [126].

Osimertinib is the first approved targeted therapy based on the ADAURA trial that enrolled patients with classical EGFR mutant (ex19del/L858R) after complete resection, stage I/B -III/A [127, 128]. Adjuvant ALK therapy is currently in a clinical trial [129]. According to an interim analysis of the ALINA trial, adjuvant targeted treatment with alectinib was associated with significant disease-free survival (DFS) benefits compared with platinum-based chemotherapy, with favorable results for alectinib seen in both the stage II–IIIA population (n = 231; hazard ratio [HR] 0.24; 95% confidence interval [CI] 0.13–0.45; $p < 0.0001$) and the intention-to-treat (ITT) (stage IB–IIIA) population (n = 257; HR 0.24; 95% CI 0.13–0.43; $p < 0.0001$) [129, 130].

A key factor with targeted therapies includes the control rate of BM; however, there is a significant difference between targeted therapies regarding brain efficacy. New-generation targeted therapies with blood barrier penetration increased the prognosis of brain metastatic NSCLC patients [58]. 20%–30% with advanced NSCLC were found to have BM at diagnosis [131, 132]. Figure 5 shows the distribution of BM according to genetical alterations. A recent meta-analysis suggests that patients with ALK-positive and EGFR-positive NSCLC had higher rates of BM development than other genomic alterations and wild-type tumors [131]. Others showed an association with metastasis development in tumors with ROS1, MET, and RET alterations [131]. However, a meta-analysis does not support a higher rate of BM in these cases compared with wild-type cohorts [131]. BM are frequent in advanced EGFR-mutated or ALK-rearranged NSCLCs, with an estimated >45% of patients with CNS involvement by 3 years of survival with targeted therapies [133].

The intracranial tumor response to TKIs is shown in Table 1 [135]. Patients with EGFR, ALK, and ROS1 positive tumors with oligo- or asymptomatic BM should be treated by upfront systemic targeted therapy [ESMO: III, B] [136]. Of note close MRI surveillance is strongly recommended [1]. The upfront use of radiotherapy might be considered upon BM progression [137]. However, there is no available data on trials comparing the two strategies to assess the impact of delayed radiation in terms of survival or neurologic deficit [138]. ALK inhibitors with CNS activity include Brigatinib, Lorlatinib, and Alectinib [43, 45, 46]. ROS1: Entrectinib is recommended in patients with BM [ESCAT: I-B]. Compared with earlier-generation drugs, CNS activity of the EGFR TKI, osimertinib showed better intracranial response rates, including stable CNS metastatic cases, in 60% [23, 139].

Conclusion

Recent expansion in the targeted treatment options into the adjuvant setting of non-small cell lung cancer using accurate pathology diagnostics can minimize the number of excluded patients from molecular diagnostics. Accordingly, careful planning of subsequent hierarchical steps of diagnostic and therapeutic aspects can lead to improved outcomes without excluding patients from best-match targeted therapy. The selection of biopsy procedures and sites, tissue processing, and interpretation, followed by accurate molecular testing-based biomarker identification, is critical. Accordingly, the complexity of theranostics and possible resistance mechanisms can lead to better quality of life and outcomes in special populations, in patients with BM. Future trials should address drug properties such as CNS activity and other special populations, including oligometastatic disease and the emergence of resistance genes to maximize patient survival. Despite the novel standard of care therapies, clinical trials are guideline-recommended options to improve patient outcomes. Accordingly, the therapeutic options are expanding based on the innovative and positive trial results.

Author contributions

GG, ÉM, RK, RH, RB, RP, TL, EG, KY, PK, and ZL: writing, original structure and validation. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Stereotactic body radiotherapy in lung cancer: a contemporary review

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The treatment of early stage non-small cell lung cancer (NSCLC) has improved enormously in the last two decades. Although surgery is not the only choice, lobectomy is still the gold standard treatment type for operable patients. For inoperable patients stereotactic body radiotherapy (SBRT) should be offered, reaching very high local control and overall survival rates. With SBRT we can precisely irradiate small, well-defined lesions with high doses. To select the appropriate fractionation schedule it is important to determine the size, localization and extent of the lung tumor. The introduction of novel and further developed planning (contouring guidelines, diagnostic image application, planning systems) and delivery techniques (motion management, image guided radiotherapy) led to lower rates of side effects and more conformal target volume coverage. The purpose of this study is to summarize the current developments, randomised studies, guidelines about lung SBRT, with emphasis on the possibility of increasing local control and overall rates in "fit," operable patients as well, so SBRT would be eligible in place of surgery.

KEYWORDS

SBRT, NSCLC, lung cancer, review, radiotherapy

Introduction

Lung cancer is one of the most common tumor types worldwide and the leading cause of cancer-related deaths among both women and men [1]. There are two main types of lung tumors: NSCLC (non-small cell lung cancer) and SCLC (small cell lung cancer). The cases are approx. 84% NSCLC, while about 13% SCLC [1]. Histological subtypes of NSCLC are adenocarcinomas, squamous cell carcinomas, and large cell carcinomas. Given the different aggressiveness and speed of progression of the two main histological types, their treatment strategies are different [2]. The type of treatment is determined by the histology of the disease, its stage, and the patient's status. Due to developments in recent years (screening and radiation therapy techniques), a slight decrease in mortality can be seen in NSCLC patients [3]. Ganti et al. made a cross-sectional epidemiological

analysis and calculated the most recent data in terms of incidence, prevalence, and survival [4]. The incidence of all stages per 100,000 people decreased from 46.4 to 40.9 in the United States between 2010–2017. The advanced stage decreased slightly (21.7–19.6), while the incidence of stage I patients increased to 10.8–13.2. The overall prevalence rose to 198.3/100,000, possibly because more and more young patients are diagnosed these days [4] the 5-year survival data have improved compared to the previous ones; the most significant improvement is 14.7%–25.7% in stage I patients receiving only radiation treatment [4].

The stage determines the prognosis of the disease at the time of diagnosis. The stage is defined based on the AJCC 8th edition (American Joint Committee of Cancer) since 2016; the use of the different TNM systems must be considered in the results of previous studies [5] Stage I-II disease is localized only to the lung tissue; lymph node positivity appears in the ipsilateral hilus in stage IIB. Radiotherapy plays an essential role in the treatment of early and advanced stages of lung cancer for both curative and palliative purposes [6]. In the case of lymph node-negative NSCLC, traditional treatment includes surgical resection (preferably video-assisted surgery-VATS lobectomy), which has been the standard of care, providing superior overall survival compared to other techniques. At the time of diagnosis, most of the patients are either technically or medically counted as inoperable for different reasons, such as poor overall health condition, elderly age, lung function, and multiple comorbidities, such as COPD (chronic obstructive pulmonary disease), cardiac and metabolic dysfunctions. For such patients, the primary treatment modality was conventional fractionated 3D-based radiation therapy, albeit with lower effectiveness and higher toxicity than surgery [7]. Today, conventional radiation therapy used in the treatment of small NSCLC foci located at a safe distance from important mediastinal organs has been replaced by a more effective, higher fractionation dose (ultra-hypofractionation) treatment, stereotaxic radiation therapy (SBRT).

SBRT is a radiation therapy method that is now widely spread, during which a relatively small, well-defined malignant tumor is treated in a few fractions, with a high dose per fraction. It is a radiation therapy technique with image guidance that can deliver very high radiation doses (ablative doses) to the target (tumor) with steep dose gradients outside the target while sparing the nearby healthy tissues (called organs at risk—OAR) [8] During SBRT, the size of the safety margins around the tumor can be reduced, which was made possible by advanced image guidance. With this ablative radiation dose, we precisely kill cancer cells, but we must pay attention to the fact that the radiation biology of the treatment changes during extreme hypofractionation; the tumor cells and healthy tissues also behave differently than during conventional fractionation. During the standard fractionation, we take advantage of the different repair mechanisms of tumor and healthy cells [9] This non-invasive technique has shown excellent local control rates and overall survival, with lower toxicity rates compared to traditional radiation therapy [8]. Another advantage is that the treatment lasts for a short time (1-2 weeks) and is suitable for outpatient treatment (doesn't need hospitalization) [9] Given the high fractional dose, careful and accurate delivery is the most important part of the treatment. Modern technological solutions must be used both in the planning and delivery.

Regarding the high age and comorbidity of lung tumor patients, as well as the raising number of stage I patients due to the development of screening tests, the introduction and use of effective treatment methods other than surgery are increasingly important. Surgery remains the gold standard of care for operable patients; however, SBRT is the treatment of choice for patients with early-stage medically inoperable NSCLC [10].

SBRT vs. conventional radiotherapy for early-stage NSCLC

Before the implementation of SBRT, conventional radiotherapy was the mainstay treatment for medically inoperable early-stage NSCLC patients. With conventional fractionation, 60–70 Gy was delivered with a fractional dose of 1.8–2 Gy. However, conventional radiotherapy showed reasonable local control and survival rates, and it was associated with significant toxicities [11]. In the first randomized phase 2 trial (SPACE), a high-fraction dose 3 × 22 Gy SBRT regimen was compared with 70/2 Gy conventional radiotherapy. The results of a total of 102 patients after a median follow-up of 37 months showed no significant difference between the two groups either in terms of 3-year PFS (progression free survival) (SBRT: 42%, Conventional RT: 42%) or OS (overall survival) at 3 years (SBRT: 54%, Conventional RT: 59%). The authors separately note that they were surprised by the exceptionally good results of the conventional therapy. When analysing the study, it should be considered that 36%–37% of the patients (in either group) did not undergo histological verification, during the examination, only about 60% of the patients underwent PET-CT, and there was an imbalance between the two groups in terms of tumor size, furthermore the number of T2 tumors was twice as high in the SBRT group. In terms of side effects, there were significantly fewer and lower-grade side effects in the SBRT group; the most significant difference was in esophagitis and pneumonitis, but mild side effects were encountered in both groups. Given the time of patient selection (2007–2011), SBRT treatment was still in its infancy, and 4D CT was only used in a few patients [12] The results of the RTOG 09.02 CHISEL multicentre randomized prospective phase 3 study were published in 2019. Peripheral, medically inoperable Stage I patients were studied. Comparing standard-dose RT (66 Gy/2 Gy or 50 Gy/2.5 Gy) with patients receiving SBRT (3 × 18 Gy or 4 × 12 Gy), superior local control could be achieved with SBRT without developing serious side effects [13]. Based on a retrospective study of a large number of

patients (497 patients, 525 lesions), the 3-year local failure rate was 34.1% with standard radiotherapy and 13.6% with SBRT. PS matching showed a significant improvement in OS for SBRT (38.9% vs. 53.1%) [14]. In the Ontario Clinical Oncology Group's ongoing phase 3 randomized study (LUSTRE trial), the results of medically inoperable patients receiving SBRT (4 × 12 Gy or 8 × 7.5 Gy depending on localization) are compared with modest hypofractionated radiotherapy (60/4 Gy).

Medically inoperable early NSCLC patients

The standard care for early-stage non-small cell lung cancer patients (NSCLC) was lobectomy, as this provided the best chances of cure. However, surgery is not suitable for many NSCLC patients in the early stage for various reasons, such as old age, general condition, impaired lung function, or multiple comorbidities. As such, these patients are generally categorized as "medically inoperable" [7]. Based on the patient's suitability for surgery, the American College of Chest Physicians practice guidelines categorized lung tumor patients into standard risk, high risk, or inoperable categories [15]. 25% of lung tumor patients diagnosed at an early stage are medically inoperable [16]. The exact definition is variable by studies. For patients who are not suitable for lobectomy, sublobar resection is often recommended. However, in the case of these patients, the high risk of complications and the uncertain oncological outcome must be considered. 3 prospective trials were compared in 2013: RTOG 0236 with patients receiving SBRT, patients undergoing sublobar resection (ACOSOG Z4032), and a trial examining radiofrequency treatment (ACOSOG Z40033). The overall 90-day mortality was 0% for RTOG 0236, 2.4% for surgery, and 2% for radiofrequency ablation [17]. In a prospective phase 2 study, after 7 years of follow-up, the results of SBRT in medically inoperable patients were published in 2018. 65 patients received 4 × 12.5 Gy; PET CT was performed in all cases as part of the examination. 5- and 7-year PFS were 49% and 38.2%, and OS was 55% and 47%, respectively. In terms of local recurrence, there is an increase at 7 years. Therefore, it is important to follow up on the occurrence of local recurrence even after 5 years. In addition, second primary lung cancer (SPLC) developed in 18.5% of cases; due to the high incidence, it is important to confirm the newly appeared lesions in the lungs with histology. Grade 3 side effects occurred in 4.6%, and Grade 4-5 were absent. The average age of the patients was 72.1 years [18] The Japan Clinical Oncology Group's prospective study (JCOG0403) included patients with operable and inoperable histologically confirmed NSCLC tumors smaller than 3 cm who received 4 × 12 Gy SBRT treatment. The definition of medically operable is if the expected FEV1 > 800 mL (forced exspiratory volume), PaO2 > 65 torr, and did not have severe cardiac disease or severe diabetes mellitus; if either is impaired, it is considered inoperable. In the case of 100 inoperable patients, the 3-year OS was 59.9%; in the case of operable patients, the 3-year OS was 76.5%. When evaluating the results, the high average age of the patients (median age 78-79) must be considered [19] The 5-year results of the RTOG 0236 study were published in 2018, 55 medically inoperable patients were selected and received 3 × 18 Gy SBRT treatment. The long-term results of the multicenter phase two study show that the 5-year disease-free survival is 25.5%, and cancer recurrence occurs most often in the untreated lobe. During follow-up (median follow-up of 48 months), locoregional and/or distant metastasis developed in 38% of patients. The development of dissemination depends on the T stage. In the case of T1, the 5-year disseminated recurrence is 18.2%, and in the case of T2, it is 45.5% [20].

Medically operable NSCLC- lobectomy vs. SBRT

The impact of using SBRT in the treatment of early-stage NSCLC is notable. In addition to irradiating inoperable patients, SBRT has recently been proposed as an alternative treatment to surgery, even for medically and technically operable NSCLC patients who refuse surgery. Recent clinical studies showed that with the use of SBRT, similar survival rates can be achieved to surgery without invasiveness and fewer treatment-related complications [8, 21]. Early-stage NSCLC lung cancer is a curable disease, and for medically fit patients, surgery (lobectomy with lymphadenectomy) is the gold standard treatment [22]. In the past few years, in addition to the development of radiation therapy, the surgical technique has also been modernized, open surgery was replaced by VATS in patients with early lung tumors, after which the number of hospitalization days and complications decreased, and the oncological results remained similar [23, 24]. In the case of SBRT, lymph node sampling is not performed; in several studies, this "deficiency" is considered the cause of lower locoregional control [18]. Based on the national lung cancer audit, only 60.6% of early-stage patients in the UK have undergone surgery. It can be seen that a significant portion of patients are at higher risk of surgical complications [25] Several randomized trials have attempted to compare surgery with SBRT but failed to accrue (RTOG 1021, SABRTooth) [26]. Two randomized phase 3 studies (STARS, ROSEL) aimed to compare the results of surgery and SBRT in operable patients. However, these were closed early due to slow accrual. Chang et al. analysed the two trials and processed the data of a total of 58 patients. Both in terms of 3-year estimated OS (SBRT: 95%, lobectomy: 79%) and recurrence-free survival (SBRT: 86%, lobectomy: 80%), patients who received SBRT had better results. However, we must consider the small number of patients, the short follow-up time, and the lack of modern surgical technologies (e.g., video-assisted thoracoscopic surgery) [8]. In 2021, the long-term results of the STARS study were published, as the SBRT arm was re-accrued with a larger number of participants (80 patients). The results of the SBRT-receiving patients were compared with the cohort of patients who

underwent VATS lobectomy and lymphadenectomy (80 patients). All patients underwent PET-CT during the examination. There were no Grade 4-5 side effects with SBRT; Grade 3 side effects occurred in 1 patient. SBRT 3-year OS was 91%, 5-year OS was 87%, in the case of VATS lobectomy, 3-year OS was 91%, and 5-year OS was 84%. Overall, in terms of OS, SBRT is non-inferior to surgery in operable patients. There was no significant difference between the two patient groups regarding 3- and 5-year PFS either (SBRT 80% and 77%, surgery 88% and 80% respectively). After lymphadenectomy, the incidence of occult pathological lymph nodes was 10%; these patients received adjuvant chemotherapy [26]. It is still necessary to carry out comparative studies using modern techniques, and long-term results are needed. However, due to the fundamentally different modalities, it is not possible to blind either patients or clinicians at treatment allocation [22]. A propensity-matched analysis was also performed, the results of which were published in 2012. Retrospectively, the data of 64 VATS cases and 64 patients receiving SABR were compared, and locoregional failure (LRF) was investigated. Recurrence was considered if it was within the operating bed/prior PTV or ipsilateral hilo-mediastinal lymph node metastasis appeared. The 3-year LRF was 93% in the SBRT group and 82% in the surgical group. There was no difference in OS. Notably, in nearly 50% of the patients (in both groups), no histological sampling of the lung foci was performed [21]. In a single-arm phase two study (RTOG 0618), 3 × 18 Gy were administered to stage I peripheral foci in NSCLC patients in good condition (medially operable). The median follow-up time was 48.1 months. The 4-year local control was 96%, and the 4-year OS was 56%. Grade 3 side effects occurred in 14%, and Grade 4 side effects did not occur [27]. In the US VALOR trial, which is a randomized phase 3 ongoing study, veterans are enrolled with operable early-stage NSCLC from 2017, and by 2020, the number of enrolled patients exceeded the total number of phase 3 trial patients so far. The results of this trial may help us in the future [28]. Another prospective trial that is still in progress and is scheduled to be completed in 2026 is the POSTILV phase 2 study, where radical resection is compared with SBRT for stage I patients, and one of the aims of the study is to assess whether SBRT, with the correct dose and technique, more effective than surgery [16]. The STABLE-MATES phase 3 trial will be completed in 2024, comparing sublobar resection with SBRT in high-risk operable patients [16].

Importance of localization

For an SBRT treatment, establishing an indication and choosing the correct fractionation scheme, the localization of the lesions within the thoracic cavity is the most important. In terms of localization, we distinguish between peripheral, central, and "extremely central," i.e., ultracentral lesions [29]. The difference between the localizations is determined by the distance from the centrally located critical organs (trachea, heart, main bronchi, great vessels, esophagus) [30].

Peripheral lesions

Lung lesions located at a safe (>2 cm) distance from the central OARs. The previously described studies were conducted with medically inoperable and operable peripheral lung foci. A peripheral lesion can be close to (<1 cm) or touch the chest wall, which requires special attention. Late side effects affecting the chest wall can be, for example, rib fracture and chest wall pain. Rib fractures usually develop more than 6 months after radiation therapy. Previous studies have shown that chest wall side effects are more likely to occur with higher fractional doses (10 Gy vs. 20 Gy). In a retrospective study, the data of 134 patients were examined; 7.5% of them developed Grade 1 or Grade 2 chest wall side effects, and a significant correlation was found for V30 and V60. If V30 reached 80 cm^3, side effects developed in 55%; in case it reached 100 cm^3, the ratio was 74%; if V60 was 15 cm^3, side effects occurred in 69% of patients, and if V60 reached 20 cm^3, the percentage was 88%. The size of the GTV (Gross Tumor Volume) and the distance of the tumor from the chest wall showed no correlation with chest wall side effects [31].

Central lesions

Tumors in which PTV (Planning Target Volume) overlaps with a virtually drawn isotropic 2 cm extension around the vital mediastinal organs (proximal bronchial tree, heart, esophagus, large vessels) [29]. Considering the proximity of essential OARs, SBRT treatment of centrally localized lung lesions requires more attention. Various Grade 3 side effects are likely to occur, e.g., bronchial stenosis, bronchial hemorrhage, carditis, esophagitis, etc., [32]. In the phase II study published in 2006, Timmerman and his team reported "excessive," high-grade toxicity with SBRT treatment of central tumors in medically inoperable patients. T1 patients received 3 × 22 Gy, while T2 patients received 3 × 20 Gy. With a high two-year local control rate (95%), a high percentage of Grade 3-4 side effects (11%) occurred, especially in the case of hilar/pericentral tumors. SBRT-related death occurred in 6 patients after 0.6–19.5 months after treatment [33]. The 4-year results of the study were published by Fakiris in 2009; the median survival was 32.4 months, where the distribution of lesions by localization was re-evaluated according to RTOG 0236. High-grade toxicity, Grade 3–5, occurred in 10.4% of peripheral tumors and 27.3% of central tumors. A total of 5 of the 70 patients participating in the study had Grade 5 toxicity [34]. The results of a 5-fraction SBRT treatment were reported in 2019 from the United States. In this phase I/II study, the goal was to establish the maximum tolerated fractional dose (MTD) in the case of central tumors. The MTD for 5 fractions was 12 Gy, with high local control (89.4%) [35]. A European study examined the treatment of central tumors in 8 fractions with a fractional dose of 7.5 Gy; after 35 months of follow-up, Grade 3 side effects occurred in 4 cases out of 63 patients, and there were no Grade 4-5 side effects. The 3-year local control was 92.6% [36]. In 2018, Roach et al.'s prospective phase I/II study was published, in which 5 ×

11 Gy was found to be safe for central tumors, and excellent local control could be achieved [32]. LungTech, a prospective multicenter phase II EORTC trial, is underway with an 8 × 7.5 Gy fractionation scheme with high-quality technical solutions. It will investigate the role of FDG PET-CT in monitoring tumor progression and assessing side effects [30]. Based on the evidence presented so far, the maximum 50–60 Gy seems to be optimal for the fractionation scheme of centrally located lung tumors, delivered in 5 fractions, but considering the nearby OARs, 8 × 7.5 Gy is also a suitable option [16].

Ultracentral lesions

By definition, lesions where the PTV overlaps with one of the critical central organs belong here. In the case of SBRT, the risk of developing Grade 4-5 side effects is high (e.g., fistula, hemoptysis, bronchopulmonary hemorrhage, etc.). Several retrospective institutional studies demonstrated the plausibility of SBRT treatment of ultracentral tumors with an acceptable toxicity rate. When analyzing the results, we must consider that in many cases, only tumors adjacent to the peribronchial tree were examined, and lesions near the esophagus, heart, or large intestine were excluded [37]. Chang et al. used a more fractionated scheme in case the OAR constraints could not be met; 4 × 12.5 Gy or 10 × 7 Gy were delivered with an acceptable side effect rate [38]. The SUNSET study was started in 2018 and is currently still ongoing; this is a phase 1 multi-institutional study to find the maximum tolerated dose for ultracentral lesions up to 2 years after treatment. NSCLCs smaller than 6 cm were enrolled, and the aim was to limit the occurrence of Grade 3–5 adverse events to <30%. The first dose level is 8 × 7.5 Gy (15 × 4 Gy and 5 × 15 Gy are also examined) [39]. In 2021, the results of the HILUS trial, which is a phase two Nordic multicenter study, were published. 65 patients with ultracentral tumors were examined. A high rate of toxicity was encountered when 8 × 7 Gy were delivered; 22 out of 65 patients (34%) developed Grade 3–5 side effects, of which 10 were possible Grade 5 toxicity. The most common G5 side effect was bronchopulmonary hemorrhage, which developed 2–22 months after SBRT treatments. The authors concluded that in the case of lesions located <1 cm from the main bronchus and trachea, the use of the 8 × 7 Gy fractionation scheme is dangerous and prohibited [40, 41]. However, the difference between different centers regarding treatment setup and safety margins must be considered [41]. Based on Chen's 2019 systematic review (a total of 250 patients' data), after SBRT treatment of ultracentral tumors, the probability of developing Grade 3–5 side effects is 10% on average, and the median treatment-related mortality is 5% [42]. The ISRS (International Stereotactic Radiosurgery Society) published a practical guideline in 2023, summarizing the studies published so far on this topic (27 studies, all but one retrospective). The most frequently used fractionation schemes are 5 × 10 Gy, 8 × 7.5 Gy, and 12 × 5 Gy; 96% of the studies used motion management, most often 4D-CT-ITV (Internal Target Volume). The lesions were considered ultracentral if the PTV overlapped with the proximal bronchial tree (PBT). High local control (LC) (1 year LC: 92%, 2 year LC: 89%), with low life-threatening toxicities (G5: 4%) were found. PBT maximum dose (Dmax) needs to be considered according to the data of the meta-analysis; less fatal toxicity is expected if the BED_3 (Biologically effective dose) value of PBT Dmax is < 180 Gy. The BED value of the treatments was a significant predictor for the one-year local control, and a negative trend appeared depending on the tumor size (smaller size, higher local control). In the ISRS guideline, 8 × 7.5 or 15 × 4 Gy are recommended for ultracentral tumors, and the PBT Dmax BED_3 can be <133–150 Gy. If there is endobronchial involvement, the use of the ablative dose is not recommended [43]. Using a modern MR-guided technique with daily plan adaptation can reduce the probability of high toxicities when administering a large fractional dose. In the ongoing ARO-2021-3 MAGELLAN phase I trial, the aim was to find the maximum tolerated dose (MTD) for MR-guided SBRT in ultracentral localization for primary and secondary lung tumors from the 10 × 5.5 Gy scheme ($_{BED10}$ = 85 .25 Gy) up to the 10 × 6.5 Gy scheme (BED_{10} = 107.25 Gy) [44].

Dose schemes—BED

In 2015, Guckenberger et al. summarized the most common fractionation schemes of SBRT treatments used in lung tumors. Stereotactic body radiotherapy treatments were performed with a dose of 5–34 Gy per fraction in 1–10 fractions; comparing the biologically effective dose of the treatment regimens at alpha/beta 10, more than 200%–300% differences were found [45]. Several studies proved that increased biological effective dose increases local control. During SBRT planning, we must consider the expected toxicity and the oncological outcome to choose the appropriate fractionation. Based on the literature data, it is established that if BED< 100 Gy, low local control is expected [46]. Compared to conventional radiotherapy, SBRT with BED >100 Gy reduces local failure and may also increase overall survival [45, 47]. In 2007, during the Japanese retrospective multi-institutional study, 257 patients' data and the details of the hypofractionated radiation treatments were examined, based on which a difference in 5-year overall survival was observed (BED_{10} < 100 Gy: 30.2%, BED_{10} > 100 Gy: 70.8%) [48]. The M.D. Anderson Cancer Center 2019 published the results of a retrospective study, where the data were obtained from the national cancer database, and the SBRT of maximum T2a tumors was compared. Radiation treatments were divided into two groups according to BED_{10}: LowBED: 100–129 Gy and High BED: >130 Gy. Based on the aggregated results, the 5-year OS was 26% in the Low BED group and 34% in the High BED group. The study's results suggest that a higher OS can be achieved with a higher BED above 130 Gy. It is important to emphasize that the exact localization of the lesions was not determined, which is an important factor for OS [49]. Examining small T1 and T2 tumors separately, Koshy et al. found

no OS benefit for small tumors >150 Gy BED, which may suggest that we can achieve high OS in small tumors with BED_{10} 100–150 Gy [50]. When choosing the fractionation scheme, the localization of the lesion and its proximity to the various critical organs must be taken into account. In the case of small peripheral lesions, extreme hypofractionation schemes have proven to be effective and safe. In the RTOG 0915 prospective randomized phase 2 study, two regimens (1 × 34 Gy or 4 × 12 Gy) were compared for small peripheral lesions. Out of 84 patients, 10.3% received a single fraction, and 13.3% of the other group had at least G3 side effects within 1 year. The 2-year local control data was 97% and 92.7%, respectively. There was no difference between the two arms regarding two-year OS and DFS [15]. Based on the long-term results of this study (after a median follow-up of 4 years), no difference was found in late G3-G5 side effects. The 5-year progression-free survival in the single fraction group was 19.1%, and in the 4-fraction group, 33.3%, there was no significant difference. Therefore, it was established that there was no significant difference in the toxicity, the 5-year local control, or the 4-year survival data [51]. Another prospective trial compared single-fraction lung SBRT (1 × 30 Gy) with a 3-fraction (3 × 20 Gy) variant, also examining small peripheral lesions. The results of this phase 2 study were published in 2019. After a median follow-up of 53.8 months, out of 98 patients, Grade 3 or more severe side effects were described in 16% of the patients receiving one fraction and 12% in the group receiving 3 fractions. There were no differences in the survival and local control results [52]. Comparing the different fractionations, especially the less frequently used single fraction SBRT, using advanced planning techniques and image guidance is important to ensure safety. In the case of small, peripheral, early-stage NSCLC, SBRT given in 3–5 fractions is common. However, in 2022, a literature summary was published regarding SBRTs applied in one fraction, which may have the advantage of, e.g., fewer clinic visits [53]. This advantage could also be used during the COVID-19 pandemic, related to this, the ESTRO-ASTRO consensus was published in 2020. During the COVID-19 pandemic, 1 × 30–34 Gy was strongly considered if a small (<2 cm) lesion is located >2 cm from critical mediastinal organs and is also >1 cm from the chest wall [54]. If we use only one fraction, it must be considered that the BED used to determine the effectiveness of multi-fraction regimens cannot be applied due to radiobiological differences. Therefore, an SFED (single fraction equivalent dose) concept was created based on a new linear quadratic-linear model [55]. Besides choosing the exact fractionation scheme, it is important to take into account the dose prescription. Previous studies show that the PTV maximum dose correlates with the local control [56]. In his summary, Guckenberger points out the differences for peripheral tumors between the dose prescriptions defined in the studies: 60%–90% isodose line encompassing the PTV, and for SBRT treatments using the multi-fraction regimen, the PTV max dose was between 38–57 Gy [45]. BED values for the different SBRT schemes are listed in Table 1.

TABLE 1 BED_{10} values corresponding to different SBRT fractionations.

BED_{10} (Gy)	SBRT fractionation scheme (number of fractions × fractional dose [Gy])
100	5 × 10
106	4 × 12
113	4 × 12,5
132	5 × 12
151	3 × 18
180	3 × 20

Tumor size (>5 cm)

By definition, SBRT means the treatment of small-sized tumors with a high fractional dose, but for now, the exact definition of the maximum size limit remains a question of debate. In the case of early, peripheral, medically inoperable lung tumors, early prospective studies proved to be successful treatment options where the size of the tumors was smaller than 4-5 cm. Retrospective studies have also found differences in overall survival according to tumor size. In the case of ultracentral tumors, the 5-year survival was determined by the size of the PTV, if the PTV <53 cm^3 61.6%, if PTV >53 cm^3 37.4%, respectively [57]. There is limited information from prospective randomized trials on large lung tumors treated with SBRT. In the multi-institutional retrospective study with the most significant number of patients (92 patients), 5–7.5 cm tumors were treated with 5 × 10 Gy with 2-year LC 73.2% and 2-year OS 46.4%. The OS correlated with the SUVmax (standardized uptake value) measured on the pre-treatment PET-CT. Distant failure occurred more often after treatment than local failure [58]. In the case of peripheral lung tumors larger than 5 cm, SBRT treatment can be administered with due caution, considering other treatment options and taking into account that lower local control and lower OS can be achieved compared to small lesions.

Simulation

CT imaging needs to be prepared after staging, establishing the indication, and planning the session. The planned radiation therapy technique and motion management determine positioning during the simulation CT. Reproducibility is an important aspect when choosing the patient's treatment position and positioning systems and ensuring small safety zones ideal for stereotactic treatment of the target volume. The total volume of the OARs in the thorax and the subsequent total planning target volume must be visible on the simulation CT [59].

Motion management

The introduction of stereotactic treatments (SRT) appeared in the treatment of intracranial tumors; the SRT of extracranial lesions was delayed because new problems had to be dealt with, such as physiologic motion and the precise definition of the target volume [9]. Intrafractional movement that occurs during treatment, such as deviations resulting from breathing, can result in anatomical changes of several centimeters [47]. When performing SBRT of the lung, the "movement" of the tumor must be taken into account so that the planned dose is delivered to the right place and the surrounding tissues can be adequately protected. An essential and unmissable element of this is IGRT (image guided radiotherapy) before or during each faction. When selecting the IGRT method, we must consider the technique we will use and the treatment machine (linear accelerator, cyberknife). MV or kV imaging (3D CBCT, 4D CBCT, orthogonal x-ray) can be used as IGRT or optical verification, e.g., surface guided radiotherapy (SGRT). According to the phases of the respiratory cycle, the lesion in the lung changes its position. Motion management can be done in free breathing with motion-encompassing methods [60]. The most used is 4D CT, where we determine the breathing cycle and divide it into phases according to either the time phase or the amplitude. Several techniques can be used with 4D planning CT, such as internal target volume (ITV) determination or mid-ventilation determination. ITV is created from the union of GTVs delineated on CT slices of at least 3 breathing phases (max exhale, max inhale, and intermediate phase). Maximum intensity projection (MIP) can also be used to determine ITV. Comparing the two ITV approaches, it was found that ITV created with MIP is smaller compared to the GTV-based ones; in this case, tumor-miss may occur, although the MIP requires a shorter time [54]. Under free-breathing, real-time tracking is also a method of cyberknife [61]. Treatment in a specific part of the breathing cycle reduces the size of the target volume. Gating has been employed in some institutions to control respiratory motion [62]. Such gated methods can be ABC (active breath control) when the patient's exhalation is inhibited after determining the breathing volume with the help of a spirometer. Another method is the deep inspiration breath hold (DIBH), when the patient holds the air in a certain deep inhalation level for about 10–15 s, most often in a voluntary manner (with audio-visual feedback), during which the pre-treatment verification takes place (it can be kV imaging or SGRT) and also delivery. Forced shallow breathing can be done with abdominal compression. In the case of tumor tracking, with the help of external or internal markers, the treatment is delivered only in certain breathing phases using intrafractional IGRT [60, 61]. The proper implementation of image-guided positioning and motion-management techniques can significantly reduce planning margins necessary for planning target volumes and, hence, the dose to the surrounding normal lung tissues [62].

Target volume determination

Before radiation treatment of lung tumors, we define the target volumes using the ICRU 62 (Interational Commission on Radiation Units and measerments) and ICRU 83 definitions. Using contrast-enhanced diagnostic chest CT and PET-CT fusion, the GTV is first determined on the planning CT based on the visible extent of the tumor. The extension of GTV depends on the type of tumor according to the microscopic spread and the characteristics of the tumor (spread to surrounding organs), thus creating the CTV (clinical target volume). For SBRT treatments, 4D simulation is often performed, considering the changing tumor movement during the breathing cycle. In this case, the breathing cycle is divided into several phases, and GTVs must be defined separately in the phases. An ITV is obtained by summing the GTVs from all respiratory motion phases instead of CTVs, and the PTV is then obtained by applying a margin to the ITV to account for the setup and positioning uncertainties [6, 62].

Dose constraints

When assessing radiation therapy plans, in addition to the coverage of the target volume and the use of adequate doses, we must pay close attention to the doses to the surrounding critical organs to reduce the development of early and late side effects. This way, we can ensure that SBRT is safe. In contrast to conventional radiotherapy, during SBRT, a high fractional dose is delivered in a few fractions, so the fractional dose received by the OARs is higher as well; therefore, we cannot use the restrictions defined for conventional radiotherapy [63]. Based on trials and published reports related to SBRT, the relevant dose constraints are determined, and due to the increasing number of treatments, these are frequently updated. When treating lung lesions, the OARs of the thorax must be considered (lung, heart, pericardium, esophagus, trachea, main bronchi, chest wall, brachial plexus, and liver, depending on localization). Most of the dose constraints for SBRT treatment are defined for 3, 5, and 8 fractions, but due to the use of the single fraction, which is becoming increasingly popular, the latest guidelines also help us with 1 fraction [64]. Depending on whether an organ is parallel (e.g., lungs) or serial (e.g., spinal cord), different parameters must be used in the treatment planning [59]. According to the latest data, the maximum dose is relevant to 0.1 cc for serial organs. In the case of parallel organs, it is crucial to determine, in addition to the maximum critical volume, the minimal volume that receives above the threshold dose ("minimum critical volume-cold constraints") [64]. One of the first extensive summaries that defined the dose constraints used in SBRT is the AAPM task group was 101st in 2010. Here, the max dose applied to <0.35 cc, and there were no restrictions on the SBRT delivered in 8 fractions [59]. The constraints defined in the

RTOG0813 trial can be used for 5 fraction lung SBRTs [35]. Based on a review published in 2021, the purpose is to systemically pool several published peer-reviewed clinical datasets and extract them in a clinically valid format [65]. The UK consensus guideline issued new guidelines 2022 based on updated data [64].

Treatment delivery

Given that a high dose is delivered during SBRT, maximizing the protection of the organs at risk, in addition to the exact target volume coverage, with highly conformal dose distribution (even in the case of complicated, inhomogeneous anatomy) is crucial. This is one of the reasons for the advancement of modern delivery techniques in SBRT planning. Such techniques include intensity-modulated radiotherapy (IMRT) and volumetric-modulated arc therapy (VMAT) [66, 67]. Lung SBRT VMAT plans of 218 patients were compared to the previously more common 3D conformal technique. In terms of dose conformity/target coverage, there was no difference (V95% > 95% with both techniques); however, from the point of OARs, the dose constraints of the ipsilateral lung (V5, V10, V20, and MLD) were much easier to comply with in the case of VMAT technique. By using the FFF (flattening filter free) mode, the radiation treatment time can be significantly reduced; the delivery of 12 Gy in the case of FFF is approximately 1.5 min, and with FF, it is 8.3 min. The length of the radiation treatment is a very important aspect of radiation treatments using a high dose per fraction, as the patient must lie motionless throughout due to precise targeting [67].

The importance of a PET-CT during examination and planning

In addition to biopsy, PET-CT also provides metabolic data and is essential in examining NSCLC patients. It helps establish a diagnosis and is also used in planning radiation therapy, including high-dose SBRT treatment [68–70]. The PET-CT performed for planning often verifies a stage change compared to the previous imaging, which is also vital before lung SBRT treatments to exclude unknown novum distant metastases and locoregional pathological lymph nodes. In 2014, 47 NSCLC patients were examined, and the results of PET-CT performed as part of staging were compared with those of planning PET-CT. A new locoregional or distant metastasis was found in 51% of the patients. In the study, it was determined that if 6 weeks pass between the two PETs, the treatment of the disease changes in 26% of patients due to upstaging [71]. In finding pathological mediastinal lymph nodes, PET-CT has higher accuracy rates than diagnostic chest CT. The fact that different radiation therapists can delineate a target volume of much more similar size and shape to each other due to the metabolic data is of great help in radiation therapy planning, especially in determining the GTV. A good correlation was found when comparing the tumor sizes determined based on PET-CT with the pathological sizes after the subsequent surgery [68, 70] The extent of FDG (fluorodeoxyglucose) accumulation within the tumor is most often determined by the SUVmax (standardized uptake value). Several studies have proved the predictive value of the SUVmax before treatments on local control; with an SUVmax of >3, local or distant failure is more likely to occur [72]. Chang examined 130 patients after 4 × 12.5 Gy was given to peripheral small lesions (<T1) and found that if the SUVmax on the staging PET-CT was below 6.2, a significantly higher OS was expected. The staging PET-CT was the only independent significant predictor for OS [73]. After SBRT treatment, PET-CT can also help with the question of recurrence-fibrosis arising on chest CT. If the SUVmax is > 5 on the 12-week PET-CT after treatments, it is more likely to indicate recurrence [72] In ongoing trials, they also investigate the effect on local control if the dose is escalated to areas with a high SUVmax within the tumor [68].

Guidelines

The European guidelines for treating early-stage NSCLC patients have not been updated since 2014–2017. The results of the currently ongoing, critical phase 2 and 3 studies are still in progress, which can determine the place of SBRT compared to lobectomy and will provide additional help in choosing the appropriate fractionation.

1. ESMO (European Society of Medical Oncology—2014): From the point of view of the feasibility of the surgery (to estimate the morbidity and mortality that may occur after the operation), the patients must be grouped in terms of risk; a cardiac and pulmonary functional assessment is required for this evaluation. The recalibrated thoracic revised cardiac risk index (RCRI) determines cardiac high risk. The case is of low risk regarding respiratory function if both FEV1 and DLCO are >80%. In the case of invasive NSCLC, the gold standard treatment is still lobectomy; lymph node dissection is not mandatory in all cases (it can be omitted if it is cN0 on PET-CT). If lobectomy cannot be performed, SBRT treatment should be chosen. In terms of fractionation, delivery, and motion management it is not covered by the ESMO guidelines. Even in multifocal cases, surgery is the primary choice; SBRT is only chosen if surgery cannot be performed [74].
2. ASTRO (American Society for Radiation Oncology—2017): SBRT can be offered as an alternative to lobectomy for “high risk” peripheral early-stage NSCLC patients, but in the case of “standard risk,” operable patients, SBRT can only be

recommended in a clinical trial as an alternative to surgery, considering that long-term side effect and survival data >3 years are still missing. SBRT is recommended in medically inoperable cases. In central localization, at least 4 fractions are recommended, but in the case of a higher risk of side effects, 6–15 fractions are also possible. SBRT can also be given in histologically unverified cases. After pneumonectomy, SBRT is recommended for novum lung cancer [75].

3. NCCN (National Comprehensive Cancer Network—2023.3): The use of advanced technologies is important for precise, high-dose curative RT (planning 4D CT, PET-CT fusion, IMRT/VMAT screening, motion management, appropriate high-level IGRT). For medically operable patients, lobectomy plus mediastinal lymph node dissection is recommended. For high-risk patients (from a surgical point of view), SBRT is a suitable alternative to lobectomy. For medically inoperable early-stage NSCLC patients, SBRT is recommended for tumors smaller than 5 cm if the constraints of the surrounding organs permit. Based on tumor localization, the recommended fractionation: small peripheral: 1 × 25–34 Gy, peripheral tumors: 3 × 18–20 Gy, central and peripheral tumors smaller than 5 cm: 4 × 12 Gy, 4 × 12.5 Gy, 5 × 10–10.5 Gy, for central lesions 8 × 7.5 Gy. According to the NCCN guideline, SBRT can be performed with a higher number of fractions (max. 10 fractions) in the case of ultracentral location. Treatment in 3 or fewer fractions is prohibited for central and ultracentral tumors. Dose constraints for healthy organs were determined based on RTOG 0618, 0813, and 0915 [6].

SBRT treatment and immunotherapy

During SBRT treatment, a high dose is delivered per fraction, which helps to achieve high local control in patients with early-stage lung tumors. The probability of the appearance of distant metastases is 10%–20%. The increased effect of the immune system may cause out-of-field tumor regression during radiation therapy [29]. The reason for this is that the release of tumor antigens increases due to the ionizing radiation, and thus, the adaptive immune system recognizes them more easily; this is called the abscopal effect [76]. During SBRT, we detect tumor shrinkage, but tumor cell fragments are also present and can behave immunogenically. It is of therapeutic importance that, in the case of lung tumors, an exceptional antitumor immune response is developed to target tumor antigens. The immune response can be inhibited by regulating different checkpoint pathways. For example, antibodies against PD1 and CTLA-4 can increase the antitumor effect [29]. Combined, SBRT and immunotherapy can be synergistic, effective, and safe treatments [77]. In advanced NSCLC patients, it was investigated that progression-free survival and overall survival were higher if the patients also received radiotherapy before immunotherapy [78]. Immunotherapy with SBRT treatment resulted in a good clinical response in melanoma and advanced lung tumor patients [79, 80]. PD-1/PD-L1 inhibitors such as pembrolizumab and atezolizumab are recommended as first-line treatment in advanced NSCLC patients with high PD-L1 expression [77]. A higher immunological antitumor effect can be achieved with a higher fractional dose than conventional radiotherapy. The SBRT treatment creates a supportive immune microenvironment for subsequent immune checkpoint inhibitors; in return, the immune checkpoint inhibitors reduce radiation resistance and boost the abscopal effect [77]. Administering immunotherapy (immune checkpoint inhibitors—ICI), enhancing the immune response, together with SBRT treatment decreases the probability of developing regional and distant metastases, so, e.g., in the case of central-ultracentral lung tumors, safer, reduced-dose SBRT treatment should be considered (BED<100 Gy) [29]. Currently, several studies are ongoing that investigate the administration of SBRT and ICIs in early-stage NSCLC; in terms of dose, there is a lack of consensus, but the most frequently studied ICIs are pembrolizumab, atezolizumab, and durvalumab [77, 81].

Radiological and pulmonary function changes after SBRT

CT and PET-CT are used for control purposes after SBRT treatments. Given that more and more "fit" patients are receiving this form of treatment, it is crucial to define the follow-up protocol precisely due to the more prolonged survival. Detecting local recurrence is often difficult because its radiological picture can be confused with early and late lung injury after SBRT. Side effects are considered early if they develop sooner than 6 months after radiation treatment (pneumonitis-consolidation, GGO) and late (fibrosis). In 2012, a systemic literature review summarized possible radiological changes after SBRT. Considering the conformal dose distribution at high doses, the resulting CT abnormalities are different from the sharp-bordered lesions corresponding to the fields seen after conventional radiation treatments. During SBRT, the lung volume receiving smaller doses will be larger [82]. The phase 3 CHISEL study compared conventional (mean BED_{10}: 65.49 Gy) and SBRT (mean BED_{10}: 125.92 Gy) treatment of medically inoperable patients with small lung cancer patients. The analysis of pulmonary function changes was published in 2022. Despite the significant BED difference, no significant difference was found after a 3- to 12-day follow-up between the patients' PFT (pulmonary function test), nor in FVC, DLCO, and FEV1 [83]. Overall, acute side effects developed in 62% of cases; consolidation is visible in almost half of the cases. Among the radiological changes on thorax CT, the most common indication of recurrence is enlarging opacity after 1 year. The blurred border, the examined area's inhomogeneity, and the air

bronchogram's disappearance can be suspicious [82]. Chang et al. examined the data of 130 patients who received 4 × 12.5 Gy for stage I peripheral NSCLC lung lesions. 9.3% of patients experienced Grade 2 pneumonitis and 2.3% Grade 3 pneumonitis. After multivariate analysis, it was determined that the probability of developing pneumonitis is significantly higher if the mean ipsilateral lung dose (MLD) > 9.14 Gy [73]. In 2014, new statistical and geometric analysis methods were used to examine the dosimetric parameters that can affect the development of lung injuries. The planned CT data of 24 patients were compared (with deformable registration) with the diagnostic thorax CT images taken at 3, 6, and 12 months (after SBRT treatment). The patients received 3 × 12–18 Gy or 4 × 12.5 Gy. There was no Grade 3-4 pneumonitis, Grade 2 in 15% of the patients. The critical dose (low-dose peak location) of lung radiographic injury was approximately 35 Gy (with a standard deviation of 10 Gy), or 70% of the prescribed dose. The larger the PTV, the smaller the critical dose. Therefore, in the case of a larger PTV, the probability of developing pneumonitis/fibrosis is higher [84]. Radiological changes do not always correlate with decreased pulmonary function. Pulmonary function deterioration occurs very rarely after SBRT treatment of peripheral lesions. Analysing the phase 2 RTOG 0236 study, the data of 55 medically inoperable patients who received 3 × 18 Gy treatment were examined. They found that poor respiratory function before treatment did not increase the likelihood of developing pulmonary toxicity, and patients who became inoperable for cardiac reasons had a lower 2-year OS. After SBRT treatment, Grade 0 and 1 PFT (pulmonary function test) changes were observed in most patients based on the RTOG SBRT pulmonary scale; > 70% did not change PFT [85].

MRI-guided lung SBRT

The use of MR in stereotactic treatments is novel. Currently, the most used image-guided strategies are techniques utilizing X-rays. The introduction of MRI-guided techniques can facilitate the isolation of different soft tissues. Compared to X-ray imaging, radiation exposure is also an important aspect. MRI can help us in many ways when performing lung SBRT, for example, by defining target volumes, planning, and using motion management. Intra- and inter-fractional deviations can be easily recognized and eliminated [47]. A clear advantage can be seen during MRI-guided adaptive radiation treatment of central lesions (SMART). After performing a breath hold 3D MRI simulation and planning CT, the plan is prepared using an inverse technique (IMRT, VMAT). Before each fraction, a 3D MRI image of the position to be treated per the anatomy of the day is taken. After the rigid registration with the gross tumor volume on the planning MRI scan, a couch shift is performed, if necessary. The OAR contours are propagated to the chosen MRI scan of the day using deformable image co-registration. The clinician then makes the necessary corrections on the GTV, and the PTV is generated with an isotropic margin of 5 mm. The online plan is then reoptimized with the same optimization objectives and field parameters. Based on real-time 2D MR images, the degree of breath-hold (gating window) can be monitored during fraction. Due to the central localization, the proximity of the OARs complicates the implementation of SBRT. Using on-table plan adaptation reduced the OAR planning constraint violations ($p < .05$), but the OAR maximum doses mainly remained stable. With the MRI-guided technique, we can reduce the size of the PTV during breath-hold gated treatments with the help of continuous lesion visualization. By increasing the coverage, the target of 95% PTV coverage was achieved in 95% of the plans, compared to the "predicted" plans (71%). As well as the daily adaptation of plans, they reduce the likelihood of side effects by monitoring the anatomical and target volume changes that occur during each fraction [86]. Finazzi et al. investigated the advantages of MRI-guided adaptive radiation therapy (SMART) during SBRT treatment of 25 peripheral lung lesions. Compared to free-breathing plans using 4D CT-based ITV, the size of SMART-PTVs has decreased, and the PTV coverage has improved. BED_{10} > 100 Gy can be delivered to PTV 95% in a higher percentage of patients, but the dosimetric benefits were modest. In conclusion, it can be said that in the case of peripheral lesions, it is not always beneficial to use SMART; it should be chosen if there is a higher probability of the development of severe side effects (if the lesion moves >1 cm, re-irradiation, previous lung surgery, severe lung disease in the anamnesis) [87]. When using this technique, we must remember that it takes longer to perform compared to CT-based and non-adaptive treatments (for peripheral lesions, on average, 48 min door-to-door; for central lesions, 59 min) [86, 87]. An ongoing prospective study, with phase 1 data reported to date, focuses on ultracentral tumors. Five lesions were examined, and 5 × 10 Gy were delivered while keeping the strict OAR constraints. 70% of the 25 fractions were based on an adapted plan due to the OAR violation. No Grade 3 acute side effects were found 6 months after treatment. In the future, the application of SMART may expand the SBRTs indicated for ultracentral tumors [88]. It remains a question whether the benefits provided by MR also represent a survival advantage and whether the cost of MR LINAC is worth it [47].

Discussion

Nowadays, SBRT is an increasingly common and frequently used form of radiotherapy treatment. SBRT has revolutionized the early-stage NSCLC and oligometastatic cancer treatment paradigm, providing a highly effective and non-invasive alternative to surgery. This curative, safe treatment method can also be used for patients considered inoperable due to

some comorbidity. Elderly patients with poor respiratory function can also be treated as it does not worsen PFT after treatment [85]. The conventional radiation treatment, previously chosen for medically inoperable patients, has now been replaced by SBRT if the localization of the tumor allows it [13, 14]. High-fractionation SBRT can be given safely to peripheral lesions, but the results of modest hypofractionation treatments for local control are still ongoing (LUSTRE study). The localization determines the dose of SBRT; tumors close to the chest wall and localized centrally are treated with a lower fractional dose in more fractions [31, 35]. SBRT treatment of ultracentral lesions is not recommended based on guidelines but can be carried out under safe conditions, as long as the dose of the nearby OARs is adequate and the fractional dose is as low as possible [37, 43]. The appropriate dose is still being determined (SUNSET study) [39]. The size of the lung lesion affects the expected local control and the development of dissemination [20]. The guidelines recommend SBRT treatment for less than 5 cm lung lesions, but tumors >5 cm can also be treated with appropriate care [58]. Comparing lobectomy and SBRT in the case of operable patients, previous studies have produced similar results [26], but prospective phase 2-3 studies are still ongoing (VALOR, POSTILV, STABLE-MATES) [16].

During the follow-up after SBRT treatment, PET-CT can be an additional examination of the diagnostic CT, helping to distinguish between fibrosis and recurrence. It indicates a relapse if, in addition to increasing opacity, the SUV max value is higher than 5 [72]. Future directions involve refining patient selection (for example, not only inoperably patients benefit from it), optimizing treatment planning (VMAT planning, MR guided therapies), and integrating SBRT with novel systemic therapies like immunotherapy.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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LINAC-based SBRT in treating early-stage NSCLC patients—single institution experience and survival data analysis

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Aim: This single institute prospective study aimed to evaluate the feasibility of LINAC-based stereotactic body radiotherapy (SBRT) in treating patients with early-stage non-small cell lung cancer (NSLSC). We focused on the survival data with the local and distant control profiles and the cancer- and non-cancer-specific survival. Treatment-related side effects were also collected and analyzed.

Methods: Patients with early-stage NSCLC between January 2018 and October 2021 were included in our prospective study; a total of 77 patients receiving LINAC-based SBRT were analyzed. All patients had pretreatment multidisciplinary tumor board decisions on SBRT. The average patient age was 68.8 years (median: 70 years, range: 52–82); 70 patients were in ECOG 0 status (91%), while seven patients were in ECOG 1-2 status (9%). 52% of the patients (40) had histologically verified NSCLC, and the other 48% were verified based on PETCT results. We applied the SBRT scheme 8 x 7.5 Gy for central tumors (74%) or 4 x 12 Gy for peripheral tumors (26%).

Results: The mean follow-up time was 25.4 months (median 23, range 18–50). The Kaplan-Meier estimation for overall survival in patients receiving LINAC-based SBRT was 41.67 months. Of the 77 patients treated by SBRT, death was reported for 17 patients (9 cases cancer-specific, 8 cases non-cancer specific reason). The mean local tumor control was 34.25 months (range 8.4–41), and the mean systemic control was 24.24 months (range 7–25). During the treatments, no Grade I-II were reported; in 30 cases, Grade I non-symptomatic treatment-related lung fibrosis and two asymptomatic rib fractures were reported.

Conclusion: In the treatment of early-stage NSCLC, LINAC-based SBRT can be a feasible alternative to surgery. Although we reported worse OS data in our patient cohort compared to the literature, the higher older average age and the initial worse

Abbreviations: NSCLS, Non-small cell lung cancer; VATS, video-assisted thoracoscopic surgery; SBRT, Stereotactic body radiation therapy; LC, Local control; OS, Overall survival; CSS, cancer-specific survival; LCC, locoregional control; DFS, disease-free survival.

general condition (ECOG1-2) in our patient cohort appear to be the reason for this difference. With the comparable local control and survival data and the favorable side effect profile, SBRT might be preferable over surgery in selected cases.

KEYWORDS

VATS, NSCLC, SBRT, LINAC-based SBRT, early stage

Introduction

Lung cancer in Europe represents a leading cause of cancer cases, with more than 312,000 newly diagnosed cases per year. Hungary is the leading European country in the incidence of lung cancer and has the highest mortality rate [1, 2]. Approximately 85% of all lung cancer incidences are non-small cell lung cancer (NSCLC) [3]. The gold standard curative protocol is surgery that mainly aims to reduce the disease progression, relieve the symptoms, and increase the overall survival (OS) if possible [4]. Many patients, however, are unable to tolerate thoracotomy due to comorbidities or personal preference. [5] The video-assisted thoracoscopic surgery (VATS) method was chosen as the treatment choice since it was reported to decrease the risk of complication after treatment and a higher 5-year survival rate than the open lobectomy method [5, 6]. Nevertheless, in some cases where resection is not possible due to the tumor location, functional status of the lung, or inoperable patients [7, 8], another treatment method should be evaluated and assessed.

LINAC-based stereotactic body radiation therapy (SBRT), which is an alternative to VATS, was found to be a choice of treatment, especially for elderly patients and those patients with more than one known disease [5]. This method was comparable to the VATS in previous clinical reports [5, 9–11].

SBRT is a state-of-the-art treatment method that uses radiation therapy to deliver high-dose ablative doses to tumors [11]. This method allows for successful tumor ablation with relatively high tumor control probability while keeping the surrounding tissues intact [7, 12]. Furthermore, this technology's use and continuous improvement can improve results in potentially operable cases. Previously, limited studies have been compared between (SBRT) and (VATS) in terms of overall survival (OS), cancer-specific survival (CSS), loco-regional control (LCC), and disease-free survival (DFS). Therefore, this single institute prospective study aims to evaluate LINAC-based SBRT for NSCLC patients as 5 years of follow-up experience at Debrecen University regarding OS, CSS, LCC, and side effect profile.

Material and methods

Study population

This was a prospective mono-institutional study; the consent form was obtained from each patient. Patient data were collected and processed with the ethical permission of the Regional Research Ethics Committee. Demographic variables obtained from the electronic file database Clinic Center of the University of Debrecen (Debrecen, Hungary), called UD-MED, included age, gender, forced expiratory volume in 1 s to forced vital capacity ratio (FEV1/FVC%), and FEV1% predicted before treatment. All patients underwent pretreatment multidisciplinary tumor board before starting the SBRT at the Oncoradiology Clinic of the University of Debrecen (Debrecen, Hungary).

The average age was 68.8 years (median: 70 years, range: 52–82); 67 patients were in ECOG 0 status (87%), while ten patients were in ECOG 1-2 status (13%). 52% of the patients (40) had histologically verified NSCLC, and 48% were confirmed based on PET-CT with high FDG SUV (over the SUV value of 2.8). The mean FEV1 value was 1.06 (L), the mean FEV1 42%, the mean FVC 2.14 (L), and the mean FVC 62.69%.

Imaging

Chest CT examination is fundamental in tumor diagnostics and staging; a contrast-enhanced chest CT scan was used in all cases as a part of staging. Determining the patient's respiratory function capacity was also crucial from the point of view of the operation and the execution of the radiation treatment. Before treatment, a bronchoscopy was performed in all cases. In cases where the bronchoscopy could not give proper histological information, a CT-guided needle biopsy was conducted where a better visualization of the tumor's position was obtained when the tumor was smaller than 2 cm and when complications were more avoidable with such an examination. To decide oncological operability, enlarged lymph nodes detected on CT or PET-CT were valid only in conjunction with a positive histological examination. Suspicious patterns examined with CT can be supplemented with an FDG-PET examination, and in the case of non-small cell lung cancer, increased FDG uptake is observed. All patients receiving SBRT in our patient cohort had pretreatment FDG-PET scans within 2 weeks before the start of the treatment. A brain MRI was performed in all cases as a part of the staging.

Treatment procedures

For the 4D CT-based SBRT procedures, we used ELEKTA VERSA HD units with individual vacuum fixation systems and online 4D CBCT verification for each patient. Planning 4D CT

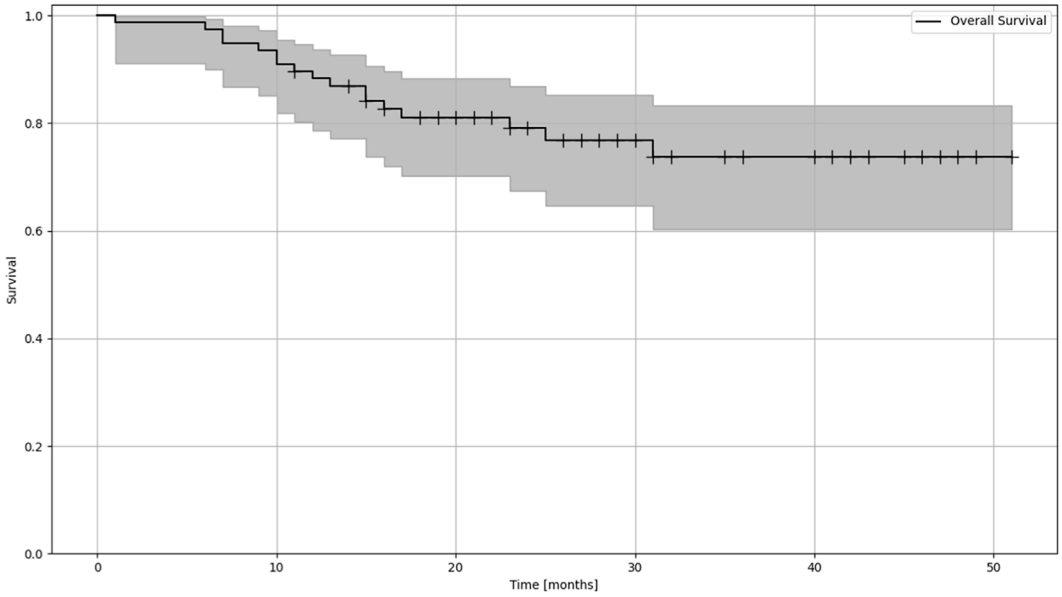

FIGURE 1
Kaplan-Meier estimation of OS (overall survival) of patients treated with lung SBRT.

was performed in the treatment position, with a slice thickness of 3 mm. No abdominal compression was applied during the process. Radiotherapy contouring and planning followed the department clinical protocol using the Pinnacle (Phillips, Netherlands) planning system (System version 16.2). To determine the exact gross tumor volume (GTV) and biological target volume (BTV), 4D planning CT-fused with FDG PET scans was used. Besides, the GTV and BTV internal target volume (ITV) was defined, using 4D CT information, to cover tumor movements. An additional 3–5 mm margin was used to generate the PTV. The mandatory OARs in planning were the lungs, heart, spinal cord, trachea, esophagus, chest wall, and great vessels per protocol. We applied the SBRT scheme of 8 × 7.5 Gy for central tumors (74%) or 4 × 12 Gy for peripheral tumors (26%). The treatments were delivered every other day (48-h shifts), with daily 4D CBCT verification and correction, if needed.

Data collection

Patients were followed up as follows: every 3 months for 2 years, every 6 months for another 3 years, and then annually. A medical history, physical examination, and chest CT were performed during the follow-up.

The primary endpoint of the study was local control (LC). We also examined systemic control, cancer-specific survival (CSS), and non-cancer-specific survival (NCSS). The analysis also focused on overall survival (OS) and treatment-related toxicity. OS was defined as the interval from the treatment date to any death or the last follow-up.

Statistical analysis

We used UD-MED, MEDSOL, and the Electronic Health Service Space (EESZT) for clinical data collection and analysis. The statistical analysis was performed with in-house-built Python scripts using the lifelines (v0.27.8) package [13].

Overall survival was estimated with the Kaplan Meier method (Figure 1). Risk estimation was performed using the Aalen-Johansen estimator to be able to assess risk in the different groups of patinets (Figure 2, Table 1).

Results

The mean follow-up time was 25.4 months (median 23, range 18–50). The Kaplan-Meier estimation for overall survival in patients receiving LINAC-based SBRT was 41.67 months. Of the 77 patients treated by SBRT, death was reported for 17 patients (9 cases cancer-specific, 8 cases non-cancer specific reason). The mean local tumor control was 34.25 months (range 8.4–41), and the mean systemic control was 24.24 months (range 7–25) During the treatments, no Grade I-II side effect were reported; in 30 cases, Grade I non-symptomatic treatment-related lung fibrosis and two asymptomatic rib fractures were reported.

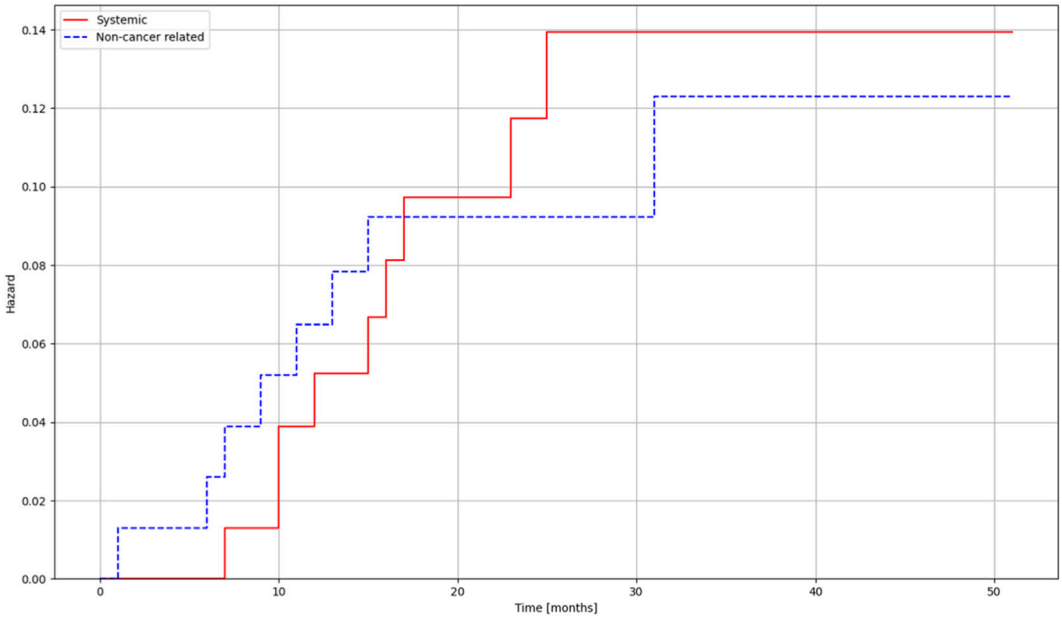

FIGURE 2
Risk estimates for deaths related to systemic progression and non-cancer-related deaths for patients treated with lung SBRT.

TABLE 1 Aalen-Johansen risk estimate for cumulative risk of patients.

	Times [months]					
	0	10	20	30	40	50
At risk	77	72	46	25	12	3
Censored	0	0	17	36	48	57
Events	0	5	14	16	17	17

Discussion

The gold standard treatment option for early-stage NSCLC patients is still surgery, considered the first treatment of choice [14–16]. The state of art surgery is usually done with VATS to reduce patient encumbrance [17–19]. Considering the Overall Survival, Loco-regional Control, and Systemic Control data of the previously reported retrospective studies, SBRT is a full-fledged alternative to surgery for early-stage NSCLC patients [5, 20–22]. Besides the comparable local control and survival data, the main advantage of the SBRT is the favorable side effect profile and excellent tolerability, even in comorbid elderly patients [23]. Using SBRT also offers lower post-treatment mortality [24]. In the literature, only a few studies focus on comparing SBRT and surgery because the comparison is made difficult by the patients' different average ages and health statuses [5, 10–12, 25]. In our patient cohort, the higher average patient age, worse general condition, and initial respiratory functions are all reflected in the general patient selection process in the clinical decisions; the younger patients in good general condition are more frequently referred to surgery.

As in the previous studies, Dong et al., in their analysis of several studies, found that the results of SBRT were comparable to those of VATS. Thus, they reported that OS was comparable between the two groups with a statistically significant difference. They also reported comparable outcomes with no significant differences in terms of loco-regional failure, with 3- and 5-year rates of loco-regional failure for radiotherapy and surgery being 93.5% and 93.5% and 94.0% and 85.9%, respectively; furthermore, they reported that distal failure was comparable for both groups with no statistical significance between the groups [5].

In our prospective study, the SBRT-related loco-regional and systemic control results are comparable to the previously reported conventional surgery results in the literature [5, 7]. In the SBRT cohort, the hazard of death due to systemic progression barely exceeds the risk of dying from non-cancer-related reasons. We recorded no deaths due to local progression. For systemic control, we also examined the lymph node and distant metastases; SBRT showed promising results in terms of non-tumor-specific survival. The scope of SBRT indications should be expanded in the future, and further studies with more cases should be considered. Currently, the gold standard therapy of choice is still surgery [14, 16, 25], the advantage of which is the possibility of histological sampling.

It is important to note that in our prospective data analysis, some data were worse for patients who underwent SBRT, as reported in the literature; this can be explained by the fact that in our SBRT group, there were medically inoperable patients with worse respiratory function and with many comorbidities. Pulmonary function values were available before and after SBRT in some patients, and we observed improvement in FEV1 and FVC. The difference between recurrence and fibrosis can be difficult during follow-up, so monitoring the patient with PET-CT is essential. Another difficulty is the separation of metastases and secondary lung tumors.

Our patient cohort also noticed no acute side effects during SBRT. No late severe side effects were also described during oncological follow-up. This study aims to help clinicians the find the proper treatment of patients with early-stage NSCLC. The findings provided valuable information in answering these and other unresolved questions regarding SBRT.

Conclusion

In the treatment of early-stage NSCLC, LINAC-based SBRT can be a feasible alternative to surgery. We report moderately worse OS data in our patient cohort compared to the literature [5]. However, the difference in average age and the initial worse general condition (ECOG1-2) of our patient cohort can be an underlying reason. With the comparable local control and survival data and the favorable side effect profile, SBRT might be preferable over surgery in selected cases.

Data availability statement

The original contributions presented in the study are included in the article materials, further inquiries can be directed to the corresponding author. All the datasets are available to access.

Ethics statement

The studies involving human participants were reviewed and approved by the Regional Ethical Commitee of University of Debrecen, registration number: 599-2021. The patients provided written informed consent to participate in this study, approved by the ethical committee.

Author contributions

KT, MB, GK, EC, DS, and JP data collection, patient follow-up and data analysis, MS statistics, manuscript review, AK program leader, manuscript preparation. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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KRASG12C mutant lung adenocarcinoma: unique biology, novel therapies and new challenges

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KRAS mutant lung cancer is the most prevalent molecular subclass of adenocarcinoma (LUAD), which is a heterogenous group depending on the mutation-type which affects not only the function of the oncogene but affects the biological behavior of the cancer as well. Furthermore, KRAS mutation affects radiation sensitivity but leads also to bevacizumab and bisphosphonate resistance as well. It was highly significant that allele specific irreversible inhibitors have been developed for the smoking associated G12C mutant KRAS (sotorasib and adagrasib). Based on trial data both sotorasib and adagrasib obtained conditional approval by FDA for the treatment of previously treated advanced LUAD. Similar to other target therapies, clinical administration of KRASG12C inhibitors (sotorasib and adagrasib) resulted in acquired resistance due to various genetic changes not only in KRAS but in other oncogenes as well. Recent clinical studies are aiming to increase the efficacy of G12C inhibitors by novel combination strategies.

KEYWORDS

KRAS, lung adenocarcinoma, G12C mutation, sotorasib, adagrasib

Introduction

The most frequent histological type of lung cancer is adenocarcinoma (LUAD) comprising half of the cases and the vast majority of the non-small cell lung cancers (NSCLC). The molecular classification of adenocarcinoma subgroup is established and is well known, where the most frequent genetic alteration among non-Asian patients is KRAS mutation (1/3) followed by EGFR (5%–15%) while in Asian patients EGFR mutation is the most frequent followed by KRAS mutation [1, 2]. Other relatively frequent mutations affecting BRAF and MET and by incidence followed by so called

Abbreviations: CI, confidence interval; CNS, central nervous system; DCR, disease control rate; GFR, growth factor receptor; HR, hazard ratio; HRD, homologous recombination repair deficiency; HRR, homologous recombination repair; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; TMB, tumor mutational burden.

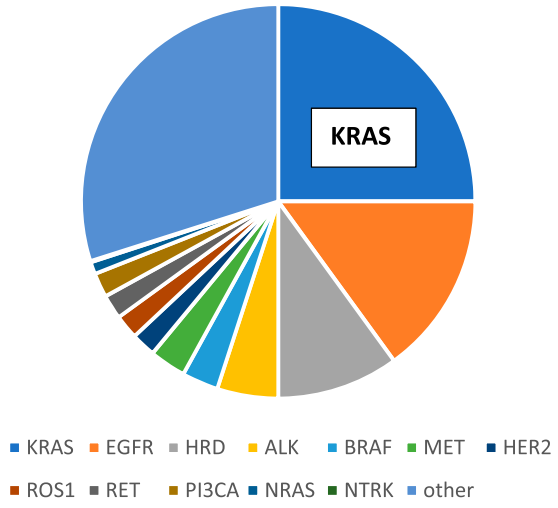

FIGURE 1
Molecular classification of lung adenocarcinoma.

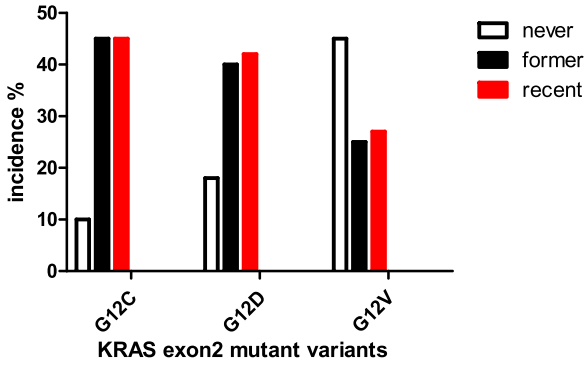

FIGURE 2
Connection between smoking history and KRAS mutant types in lung adenocarcinoma [10].

translocation cancers involving ALK/ROS1 less frequently RET or NTRK. At the same incidence levels, MET and HER2 amplifications also occur in this histological type [3]. It is of note that HRR mutations are also relatively frequent though less appreciated [4] (Figure 1). In the past decade target therapy changed the treatment of lung adenocarcinoma which left KRAS mutant lung cancer in an orphan status which changed recently significantly [5].

Molecular epidemiology of KRASG12C mutant lung cancer

KRAS mutant lung cancer has three variants: type-1 is a characterized by mucinous histology with TTF1 expression, type-2 is characterized by high TMB and PDL1 expression while type-3 group contains KEAP mutation [6]. Other studies performed subclassification based on gene expression signatures and defined a p16 mutant, a p53 mutant and a STK11 mutant forms all having different expression profiles [7].

KRAS mutation in lung cancer has three predominant forms: the most frequent is G12C (~40%) followed by ~20–20%, G12D and G12V, respectively [1, 2, 8]. It is widely accepted that KRAS mutation in lung cancer is smoking associated but it is only proven for G12C while the G12D and G12V are associated with chromosomal instability and/or mismatch repair deficiency [9]. There is a clear association between smoking and allelic variants of mutant KRAS: among recent smokers far the most frequent is G12C mutation while among non-smokers G12V is the predominant (Figure 2). The presence of G12C mutation among non-smokers (~10%) indicates the effect of passive smoking [10].

Various KRAS mutants are differ in biochemical and signaling functions: in G12C mutant the mitogenic RAS-RAF-MEK pathway is the most active, while in others the AKT signaling seems to be equally active, most probably due to changes in RAF affinity of the protein (Table 1, Ref [11]). Furthermore, individual mutants are characterized by differential alterations in GTP-ase activity or to sensitivity toward GAP proteins. Furthermore, the GDP/GTP exchange potential of the individual mutants seems also be different in various variants. There are other data supporting different lung carcinogenesis behind mutant KRAS variants: G12C mutation is associated with EGFR4 mutation, G12D mutations tend to have PDGRA mutation while G12V mutation containing tumor used to have PTEN mutation [1, 7]. Allelic imbalance of KRAS genes may also affect its function. In KRAS mutant lung adenocarcinoma heterozygous loss of the wild type allele is very frequent (~75%) leaving the mutant allele the only functioning KRAS (a kind of homozygosity), whereas the copy gains of the mutant allele is much less frequent [12]. Other analyses defined the oncogenic driver roles of various KRAS mutant forms and found that G12C is a real major driver oncogene in lung cancer, unlike G12D/V which are only "mini-drivers," cooperating with other mutant oncogens [13].

Biology and therapeutic sensitivity of KRASG12C mutant lung cancer

Analysis of a large KRAS mutant LUAD database indicated that this type of lung cancer has increased potency to metastatize to the lung but decreased one to the liver and to invade the pleural surface [14]. Furthermore, it was shown that in case of bone metastases KRAS mutant status is an independent negative prognostic factor [14]. As far as the chemotherapeutic sensitivity concerns, most of the KRAS mutant variant

TABLE 1 Biochemical characteristics of KRAS mutant proteins [11].

KRAS	Wild type (G12)	G12C	G12D	G12V
GTP affinity	High	High	High	High
GDP/GTP exchange	Fast	Medium	Slow	Slow
GTP-ase activity	High	High	Decreased	Lost
GAP sensitivity	High	Lost	Lost	Lost
(B)RAF affinity	High	High	Decreased	Decreased

GAP, GTP-ase activating protein.

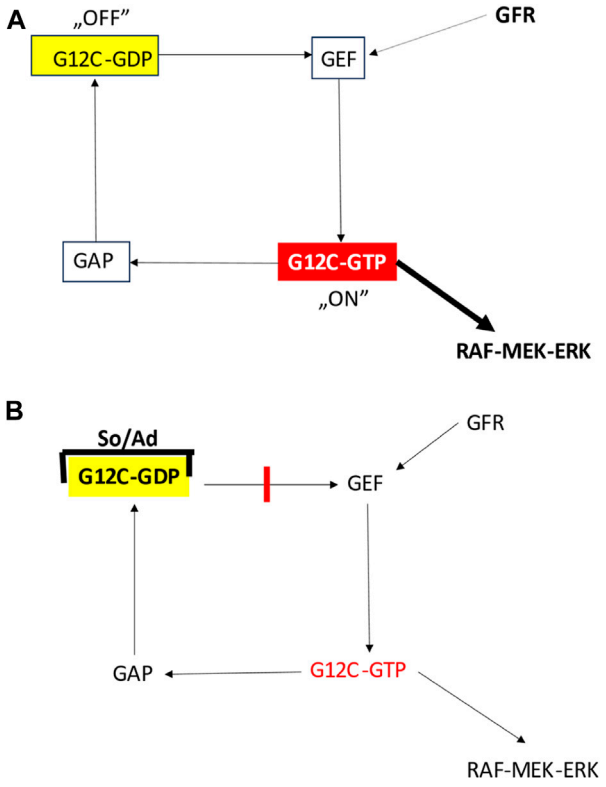

FIGURE 3
Effect of G12C inhibitors on the function of mutant KRAS protein [11]. **(A)** Function of the G12C mutated KRAS protein. **(B)** Function of the G12C mutated KRAS protein bound to inhibitors. Ad, adagrasib; GAP, GTP-ase activating protein; GEF, guanin nucleotide exchange factor; GFR, growth factor receptor; So, sotorasib.

containing tumors are equally sensitive toward platinum-based therapies, except the G12V mutant which seems to be more sensitive to this chemotherapy than others [10]. Another retrospective analysis tested the efficacy of bevacizumab in combination with chemotherapy and demonstrated that it is more efficient in KRAS wild-type tumors which was due to the resistance of the G12D mutant form [15]. Analysis of the treatment outcome of bone metastatic lung carcinoma patients indicated that the KRAS mutant tumors seems to be resistant to radiation therapy and to bisphosphonates [16] as it was predicted by the preclinical models [17]. A recent analysis of the G12D mutant lung cancers demonstrated that the density of $CD8^+$ T cells, the TMB and the tumor cell expression level of PDL1 are lower as compared to other KRAS mutants including G12C [18]. More importantly, the efficacy of immune checkpoint inhibitors turned out to be poorer in G12D mutant lung cancers.

Novel drugs to target mutant KRAS

The race for the G12C mutant KRAS inhibitor

Although it was considered undruggable, development of mutant KRAS inhibitors lastly became successful [19]. By the development of KRASG12C inhibitors. The challenge was here that—on the contrary to the various oncogenic tyrosine kinases where the increase kinase activity is the target—here in case of a GTP-ase the lost function is the target so a direct enzyme inhibitor is not an option. On the other hand, since the wild type KRAS is a critical signaling component of most of the normal cells, the inhibitor must be highly selective for the mutant isoform. As a result, a new class of inhibitors have been designed: the allele-specific (i.e., mutation specific) irreversible inhibitors. The idea was that since the KRAS is active in the GTP-bound state the novels drugs accumulate it in the off-state which is the GDP-bound KRAS (Figure 3).

The first in class of such KRASG12C inhibitor was published in 2013 [20] and a drug was approved for lung cancer in 2021 [20] which was a very rapid developmental process. The race was won by Amgen by a novel drug which is not only allele-specific (G12C) but also bind to a novel pocket (c95-99) critical in GTP-binding (ref [21], AMG-510, sotorasib). Preclinical data indicated that this novel inhibitor, not only blocks the mitogenic signaling (RAS-RAF-MEK) but is synergistic with platinum-based chemotherapy, with MEK inhibitors or with immune checkpoint inhibitors [21]. For the second place of this race arrived Mirati with a chemically distinct but functionally similar compound MRTX849/adagrasib which is characterized by very good pharmacological characteristics and which has a very good

penetrance of the blood-brain barrier, forecasting its use for brain metastases [22]. It is of note that the half -life in the circulation of AMG510 is 5 h as compared to adagrasib's 23 h. Meanwhile there are several other G12C inhibitors developed [23], some even reached clinical testing but only GDC-6036 exhibited early clinical efficacy [24].

Other mutant KRAS inhibitors

Developments in this filed continued by the G12D inhibitors which is far more frequent in other cancers but much less in lung adenocarcinoma. Unfortunately, irreversible inhibitors are nonexistent but a G12D selective inhibitor was developed: MRTX1133 which locks KRAS protein in the GTP-bound state which is in clinical development right now [25]. Furthermore, there are other novel inhibitors such as KRAS12D1-3 and RAS(ON)G12D [26].

Pan-RAS inhibitors

Other directions are the development of so-called pan-RAS inhibitors. BI-2852 induces homodimers of KRAS and turned out to be a KRASG12D selective inhibitor [27]. A real pan-KRAS inhibitor which even reached successful clinical testing is RMC-6236, a powerful RAS(ON) inhibitor which showed activity in G12V and other rare mutant forms [28].

Indirect RAS inhibitors

One of the main GTP-exchange protein of RAS is SOS1 and it serves as drug development target: there are several new molecules are on the market and some of them entered the clinic [29]. It would be interesting to see the side effect profile since these inhibitors are equally effective against all RAS isoforms and all variants, wild type or mutant. RAS proteins are phosphorylated at C32 of the exon2 by SRC and SHP2 phosphatase acting at this site. There are several SHP2 blockers in development and some of them entered clinical phase [30].

Novel treatment options for KRASG12C mutant lung adenocarcinoma

In the past nearly 20 years, treatments targeting EGFR and ALK have already become part of everyday patient care, but at the same time, the use of targeted therapy against the driver mutation present in the largest proportion, the KRAS mutation, has only become a realistic possibility in recent years. We currently have the most experience with two KRAS inhibitors; these are sotorasib and adagrasib. The phase II trial for sotorasib was the Code-BreaK100, while that for adagarasib was the Krystal-1 clinical trial [31, 32].

The Code-BreaK100 trial investigated the activity of once-daily oral sotorasib 960 mg in patients with KRASG12C mutation-positive advanced NSCLC previously treated with platinum-based chemotherapy [31]. The primary endpoint was objective response (complete or partial) based on independent central review. Key secondary endpoints included duration of response, disease control (complete response, partial response, or stable disease), progression-free survival, overall survival, and patient safety. The predictive value of some biomarkers was also analyzed. Among the 126 enrolled patients, the majority (81.0%) had previously received platinum-based chemotherapy and PD-1 or PD-L1 inhibitors. According to the central review, 124 patients had measurable disease at baseline and the therapeutic response could be evaluated. An objective response was observed in 46 patients [37.1%; 95% confidence interval (CI), 28.6–46.2], including 4 (3.2%) complete responses and 42 (33.9%) partial responses shown. The median duration of therapeutic response was 11.1 months (95% CI, 6.9-not evaluable). Disease control occurred in 100 patients (80.6%; 95% CI, 72.6–87.2). Median progression-free survival was 6.8 months (95% CI, 5.1–8.2), and median overall survival was 12.5 months (95% CI, 10.0-not evaluable). Treatment-related adverse events occurred in 88 of 126 patients (69.8%), including a grade 3 event in 25 patients (19.8%) and a grade 4 event in 1 patient (0.8%). Therapeutic responses were also analyzed in subgroups defined by PD-L1 expression, tumor mutational burden (TMB), and concurrent STK11, KEAP1, or TP53 mutations. Based on all of this, in this phase II study, sotorasib therapy showed clinical benefit in patients with previously treated KRASG12C-mutated NSCLC without new patient safety signals [31].

The Krystal-1 study evaluated adagrasib (600 mg orally twice daily) in patients with KRASG12C-mutated NSCLC who had received prior platinum-based chemotherapy and anti-PD1 or anti-PD-L1 immunotherapy [32]. The primary endpoint was objective therapeutic response (ORR), assessed by an independent central review. Secondary endpoints included duration of response, progression-free survival, overall survival, and patient safety. A total of 116 patients with KRASG12C mutation-positive NSCLC were treated until October 15, 2021 (mean follow-up: 12.9 months); 98.3% had previously received both chemotherapy and immunotherapy. Of the 112 patients with measurable disease at baseline, 48 (42.9%) had a confirmed objective response with a median duration of 8.5 months [95% confidence interval (CI), 6.2–13.8], and the median progression-free survival was 6.5 months (95% CI, 4.7–8.4). As of January 15, 2022 (median follow-up, 15.6 months), the median overall survival was 12.6 months (95% CI, 9.2–19.2). In 33 patients with previously treated stable CNS metastases, the intracranial objective response rate was 33.3% (95% CI, 18.0–51.8). Treatment-related adverse events occurred in 97.4% of patients; grade 1 or 2 in 52.6%, grade 3 or higher in 44.8% (including two grade 5 events), and it became necessary to suspend medication in 6.9% of patients. Overall, in previously treated patients with KRASG12C-

TABLE 2 Summary of the clinical efficacies of sotorasib and adagrasib.

	Sotorasib	Adagrasib
	CodeBreaK100	KRYSTAL-1
N of patients	126	116
Primary endpoint	ORR	ORR
ORR (95% CI) (%)	37.1 (28.6–46.2)	43 (33.5–52.6)
DOR (95% CI) (month)	11.1 (6.9–NE)	8.5 (6.2–13.8)
DCR (95% CI) (%)	80.6 (72.6–87.2)	80 (70.8–86.5)
PFS (95% CI) (month)	6.6 (5.1–8.2)	6.5 (4.7–8.4)
OS (95% CI) (month)	12.5 (10.0–NE)	12.6 (9.2–19.2)
Follow-up (month)	15.3	12.9
Brain metastasis, n (%)	26 (20.6)	24 (21)
Intracranial ORR, DCR (%)	33, 85	12.5, 88
PD rate (%)	16.1	5
Dose reduction/suspension (%)	22.3	Reduction: 52; suspension: 61

ORR, objective response rate; DOR, duration of response; DCR, disease control rate; PFS, progression-free survival; OS, overall survival; CI, confidence interval; NE, not evaluated; PD, progressive disease.

mutated NSCLC, adagrasib demonstrated clinical efficacy with no new patient safety alerts [32].

Below, we will review what differences can be verified between the two agents based on the results of these trials (Table 2). In phase II trials, the ORR was higher with adagrasib (43%) than with sotorasib (37%), and the rate of progressive disease (PD) was lower with adagrasib (16% for sotorasib vs. 5% for adagrasib), as shown in Table 2. However, in the absence of a head-to-head comparison, the results of such comparisons should be evaluated with caution [33]. Median PFS was similar between the two drugs (sotorasib, 6.6 months and adagrasib, 6.5 months). Drug-related adverse events were more common with adagrasib than with sotorasib, and, as a result, treatment interruption or dose reduction is more common with adagrasib (sotorasib, 22% and adagrasib, 52%). The confirmatory phase III trial for sotorasib was the Code-BreaK200 [34], while for adagrasib it was the Krystal-12 study.

In the Code-BreaK200 trial, between 4 June 2020 and 26 April 2021, 345 patients were randomized in a 1:1 ratio to the sotorasib (n = 171) or docetaxel (n = 174) arm. In the sotorasib group 169 (99%), and in the docetaxel group 151 (87%) patients received at least one course of treatment. After a median follow-up of 17.7 months, the study reached its primary endpoint, a statistically significant increase in PFS for sotorasib compared with docetaxel [median PFS 5.6 months (95% CI 4, 3–7.8) vs 4.5 months (3.0–5.7); HR: 0.66 (0.51–0.86) p = 0.0017]. Sotorasib was well tolerated, fewer grades 3 or worse [n = 56 (33%) vs n = 61 (40%)] and serious treatment-related adverse events compared with docetaxel [n = 18 (11%) vs n = 34 (23%)].

For sotorasib, the most common treatment-related adverse events of grade 3 or worse were diarrhoea [n = 20 (12%)], alanine aminotransferase increase [n = 13 (8%)], and aspartate aminotransferase increase [n = 9 (5%)]. For docetaxel, treatment-related adverse reactions of grade 3 or worse were neutropenia [n = 13 (9%)], fatigue [n = 9 (6%)], and febrile neutropenia [n = 8 (5%)]. In conclusion, sotorasib significantly increased progression-free survival and showed a more favorable safety profile compared to docetaxel in patients with advanced stage (IIIB/IV), good performance status (ECOG 0-1), KRASG12C-mutated LUAD who had already received platinum-based chemotherapy and immune checkpoint inhibitor therapy as first-line treatment and had no symptomatic brain metastases [34].

In the Krystal-12 trial, docetaxel was also the comparator agent and the inclusion criteria were the same as in the CodeBreak200 study, however, the randomization ratio was 2: 1 in favor of adagrasib. Patients received 600 mg of adagrasib twice daily, and 75 mg/body surface area of docetaxel every 3 weeks. Adagrasib produced an ORR of 42.9% and a PFS of 6.5 months. Both drugs showed the already known side effect profile, the most common toxicities were diarrhea, musculoskeletal pain, fatigue and hepatotoxicity [35].

In NSCLC approximately 30%–40% of patients develop brain metastases during the course of the disease. In 2022, brain metastasis specific activity of adagrasib has been reported by Sabari et al. [36] Retrospectively, 374 NSCLC patients with KRAS mutations (149 with G12C mutation and 225 with non-G12C mutation) were analyzed for brain metastases. Overall, 40% of patients with KRASG12C or non-G12C mutations developed

brain metastases during the follow-up period. 77% of patients had a diagnosis of synchronous brain metastases detected within 3 months of initial diagnosis. Brain metastasis occurred less frequently in NSCLC patients with KRAS mutations than in NSCLC patients with other oncogenic driver mutations [30]. In a retrospective review of 579 patients with metastatic NSCLC, the incidence of brain metastasis was highest in NSCLC patients with ROS1 (36%) and ALK (34%) mutations/fusions, followed by EGFR (28%) and KRAS (28%). In NSCLC without a driver oncogene, brain metastasis occurred in only 21% of patients [37]. The response of brain metastases to radiation therapy may vary depending on the driver oncogene. In an analysis by Arrieta et al., the response rate to radiotherapy was higher in NSCLC patients with EGFR (64.5%) or ALK (54.5%) mutations than in those without driver mutations (35%). However, in NSCLC patients with KRAS mutations, this rate is only 20%, which further emphasizes the need for effective treatments in this group [38].

Only limited data are available on the CNS activity of sotorasib in metastatic NSCLC. Although patients with active, untreated brain metastases were excluded from the CodeBreaK100 study, 2 of 16 patients with stable brain metastases had a complete response to therapy, and 12 achieved stable disease with sotorasib therapy, representing 88% of the patients with intracranial disease control [39]. In addition, several case studies have been published of patients with brain metastases in whom radiological regression was confirmed and symptoms resolved with sotorasib treatment [40, 41]. Yeh et al. reported a patient with NSCLC harboring a KRASG12C mutation with symptomatic leptomeningeal involvement and multiple brain metastases treated with sotorasib monotherapy [41]. The patient showed clinical improvement 2 weeks after the start of sotorasib treatment, and brain MRI showed clear radiological improvement in several metastatic foci and meningeal involvement. In this case, sotorasib was effective against untreated, symptomatic metastases. However, severe hepatotoxicity necessitated discontinuation of sotorasib, leading to disease progression. Therefore, although sotorasib is also effective in metastases affecting the central nervous system, further prospective studies are needed.

Negrao et al. studied the intracranial efficacy of adagrasib in KRASG12C-mutated NSCLC patients with untreated CNS metastases enrolled in the KRYSTAL-1 study [42]. 25 patients were enrolled and evaluated (mean follow-up, 13.7 months), and 19 patients had radiologically evaluable intracranial activity. Safety was consistent with previous reports for adagrasib: treatment-related grade 3 adverse events occurred in 10 patients (40%), grade 4 in 1 patient (4%), and there was no grade 5 adverse events. The most common CNS-specific adverse reactions were dysgeusia (24%) and dizziness (20%). Adagrasib showed an intracranial ORR of 42% and a DCR of 90%, as well as a PFS of 5.4 months and an OS of 11.4 months, which is promising for the treatment of patients with untreated CNS metastases.

The clinical trial results of the KRAS inhibitors sotorasib and adagrasib are promising, however, currently they are inferior to EGFR inhibitors or ALK inhibitors in terms of both therapeutic duration (PFS, OS) and side effect profile. Further extensive studies—mainly targeting predictive markers and resistance mechanisms—are necessary in order to be able to treat permanently and effectively this large group of patients with a good quality of life.

Primary and acquired resistance mechanisms

Primary resistance

There are characteristic co-occurring mutations in KRAS mutant lung cancer such as STK11 and KEAP1. STK11 mutation was shown to be associated with resistance to immunotherapy [43]. In the CodeBreak100 study the association of STK11 and KEAP1 mutations have been evaluated in relation to the efficacy of sotorasib and found that the lowest response rate was found in tumors having KEAP1 mutation/STK11 wild type genotype while the highest was seen in tumors with STK11mutant/KEAP1 wild type genotype [44]. A recent genomic analysis of a large G12C mutant lung cancer cohort treated with G12C inhibitors revealed that co-occurring mutations of KEAP1, SMARC4 and CDKN2A were independent negative predictive factors of inhibitor efficacy while mutations in the DDR genes were positive predictive ones [45].

Acquired resistance

Acquired resistance to sotorasib treatment of lung cancer patients had various pathomechanisms At the first place it was found the disappearance of G12C mutation from cancer cells or the amplification of the wild type KRAS gene. Other KRAS-related genetic alterations were the acquired novel mutation types (G13V, G12D, G12V, V8L, V141I) or the novel mutations affecting NRAS. Furthermore, mutations of the EGFR signaling pathway members such as EGFR or BRAF are also occurred [46]. Although at not high frequency, but amplifications of MET or HER2 have also been reported [47, 48].

Upon adagrasib resistance it was described histological transformation from adenocarcinoma to squamous [49] a bit similar to what was seen in case of EGFR inhibitor resistance. It can occur most probably in those cases where the original tumor is a combined adenosquamous variant since KRAS mutation is adenocarcinoma specific genetic alterations. In case of acquired resistance to adagrasib at first place also novel KRAS mutations have been identified (G12D/R/W, G13D, Q61H, R68S, H95D/Q/R, Y96C). The resistance mechanism does not involve the EGFR signaling instead the RET signaling with mutations affecting RET, BRAF and MAP2K1. Furthermore, gene amplification here also involved MET but interestingly there were several gene fusions in the resistant tumors involving, ALK, RET, FGFR3 and BRAF [49].

TABLE 3 Clinical developments of G12C inhibitor combinations [51].

G12C inhibitor	Partner	Function	NCT	Clinical phase	Cancer
sotorasib	AMG404	PD-1 inhibitor	03600883	I/II	NSCLC
	carboplatin/pemetrexed	chemotherapy	(Japan)	II	NSCLC
	palbociclib	CDK4/6 inhibitor	05178888	I/Ib	solid tumor
	afatinib	EGFR-inhibitor	04185883	Ib/II	NSCLC
	panitumumab	anti-EGFR	05198934	III	CRC
	everolimus	mTOR inhibitor	04185883	Ib/II	solid tumor
	RMC-4630	SHP2 inhibitor	04185883	Ib/II	solid tumor
	bevacizumab	anti-VEGF	05180422	I/II	NSCLC
adagrasib	pembrolizumab	anti-PD1	046113596	II	NSCLC
	cetuximab	anti-EGFR	04793958	III	CRC
	TNO-155	SHP2 inhibitor	04330664	I/II	solid tumor
	BI-17011963	SOS1 inhibitor	04975256	I/Ib	solid tumor

CRC, colorectal cancer; NSCLC, non-small cell lung cancer.

The resistance mutations of KRAS can be classified into three main categories. Mutations in the codon12 or codon61 decrease the potential of the KRAS protein to hydrolyze GTP. Mutations at codon 13 increase the GDP-GTP exchange, while mutations at R68, H95, Y96 and Q99 decreases the affinity of the inhibitors.

It is interesting that various mutational profiles of the KRAS mutant lung cancers affect the development of resistance to sotorasib or adagrasib [49] The H95 mutations may confer resistance to adagrasib but does not affect the activity of sotorasib. On the other hand, G13D, R68M, A59S/T mutations confer sotorasib resistance but retain adagrasib sensitivity [48]. Finally, m72 or Q99 mutations cause adagrasib resistance but do not affect sotorasib sensitivity [50]. Based on these data it can be hypothesized that the development of acquired resistance could be treated by sequential use of the other G12C inhibitor.

Developing combinational approaches

The observed clinical efficacy and the developing resistances both stimulated novel clinical approaches to improve the efficacy of G12C inhibitors sotorasib and adagrasib (Table 3) [51]. Since G12C mutant lung cancer is an immunologically hot tumor it was evident to start combinations with PD1/PDL1 inhibitors: in case of sotorasib the combination partner is AKG404 (a PD1 inhibitor) in case of adagrasib the partner is Pembrolizumab (also a PD1 inhibitor). Since one of the resistance mechanisms of G12C inhibitors involves the reactivation of EGFR signaling pathway, sotorasib is now clinically tested in combination with afatinib (an EGFR tirozin kinase inhibitor). In case of both G12C inhibitors the efficacy against colorectal cancer is a significant problem therefore combinational trials using anti-EGFR antibodies. Other interesting novel combination involves bevacizumab (anti-VEGF) since this therapy was shown to be inactive in KRAS mutant lung cancer [15]. Furthermore, combinational trials of G12C inhibitors are already initiated with traditional chemotherapies such as carboplatin/pemetrexed. Since acquired resistance to G12C inhibitors may involve reactivation of alternative signaling pathways such as PI3KCA (sotorasib) combination with mTOR inhibitor seems to be a rational approach. It is a completely different approach to increase the KRAS inhibitory efficacy of G12C inhibitors by either SOS1 inhibitors (to block GEF protein activation) or with SHP2 inhibitors (to block reactivation mechanisms) [51]. Since these approaches are pan-RAS targeted, it will be an interesting issue to see that for the prize of increased G12C inhibition what kind of prize can be paid in terms of side effects.

Conclusion

KRAS mutant lung adenocarcinoma is the most frequent molecular subtype of lung cancer but it is still a heterogenous entity since the individual allelic variants are biologically heterogenous. The most frequent allelic variant of KRAS mutant lung cancer is the smoking related G12C which became the focus of the development of mutant-specific irreversible KRAS inhibitors. More importantly, two of the

G12C inhibitors, sotorasib and adagrasib were effective clinically in advanced G12C mutant lung adenocarcinoma patients resulting in conditional approval (linked to annual reporting of the expected clinical efficacy). Meanwhile, similar to other target therapies, upon administration of G12C inhibitors clinical resistance develops which is due to various biological processes predominated by secondary mutations of the KRAS gene. Since the clinical efficacy of G12C inhibitors is not overwhelming, there is a room for improvement which is the bases of development of various combination approaches of G12C inhibitors including immunotherapeutic agents, EGFR inhibitors or RAS signaling modulators. Since mutant KRAS was long considered undruggable, the development and the clinical success of G12C inhibitors pave the way for the development of non-G12C mutant KRAS inhibitors, opening the door for a new era of target therapies aiming at the most frequently mutated human oncogene in various cancers including the lung adenocarcinoma.

Author contributions

JT: writing, original structure, validation. JM: writing, clinical data, validation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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